ASSESSING THE ANTIMICROBIAL, ANTI-QUORUM SENSING AND ANTI-BIOFILM POTENTIAL OF BACTERIA ISOLATED FROM *Pocillopora* and *Acropora* CORALS

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Signature: Date: 3rd December 2021

As the candidate's supervisors, we have approved this thesis/dissertation for submission.

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Title: **ASSESSING THE ANTIMICROBIAL, ANTI-QUORUM SENSING AND ANTI-BIOFILM POTENTIAL OF BACTERIA ISOLATED FROM *Pocillopora* and *Acropora* CORALS**

Date of Submission: 3rd December 2021

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ABSTRACT

Coral bleaching, primarily caused by the rise in seawater temperature, is a major threat to the survival and functionality of coral reef ecosystems in the marine environment. The application of probiotics to corals, particularly bacteria, may be a possible solution to test whether the effects of coral bleaching can be reversed to improve coral health. The present study assessed the anti-biofilm and anti-quorum sensing potential of bacteria isolated from *Pocillopora* and *Acropora* corals. Intertidal zone corals were collected along the KwaZulu-Natal coast and bacteria were isolated. A total of 210 and 132 bacterial isolates were biobanked from *Acropora* and *Pocillopora* spp., respectively. Preliminary characterization of isolated bacteria was carried out, i.e., colony characterization, Gram reaction and cellular morphology. Primary antimicrobial screening was carried against indicator organisms, *Pseudomonas aeruginosa* ATCC 27853, methicillin-resistant *Staphylococcus aureus* using the colony drop assay, as well as quorum sensing inhibition screening using *Chromobacterium violaceum*, *Chromobacterium subtsugae* and *Vibrio harveyi*. Based on preliminary data, 16 *Pocillopora*- and 24 *Acropora*-associated bacteria were selected for further investigation. Antimicrobial potential of extracts against *P. aeruginosa, S. aureus, Shewanella putrefaciens, Vibrio alginolyticus, Vibrio coralliilyticus, Vibrio parahaemolyticus* and *Vibrio shilonii* were carried out, with *Pocillopora*-associated bacterial extracts inhibiting 6.25% (1/16) of clinical indicators and 43.75% (7/16) of marine indicators, while 20.83% (5/24) and 25% (6/24) of *Acropora*-associated bacterial extracts inhibited clinical and marine indicators, respectively. Qualitative and quantitative anti-quorum sensing activity were also assessed against *C. violaceum* and *C. subtsugae* using an agar overlay and quantitative assays where 93.75% (15/16) and 100% of *Pocillopora*-associated bacterial extracts and 25% (6/24) and 29.16% (7/24) of *Acropora*-associated bacterial extracts showed activity against *C. violaceum* and *C. subtsugae*, respectively. Using the qualitative autoinducer-2 agar overlay inhibition assay with *Vibrio harveyi*, 68.75% (11/16) and 25% (6/24) of *Pocillopora*-associated and *Acropora*-associated bacterial extracts demonstrated autoinducer-2 inhibition, respectively. Biofilm inhibition was assessed against *P. aeruginosa* ATCC 27853*, S. aureus* ATCC 43300*, S. putrefaciens* ATCC 8071*, V. coralliilyticus* ATCC_BAA 450*, V. parahaemolyticus* ATCC 17802 and *V. shilonii* ATCC_BAA 91 with selected extracts demonstrating inhibitory potential against initial adhesion and/or mature biofilm reduction. Coral-associated bacteria have demonstrated antimicrobial and/or anti-virulence properties and could be an important source of novel antimicrobial, anti-QS and anti-biofilm compounds, with potential coral probiotic application to overcome coral bleaching.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The prevalent occurrence of coral bleaching events in the marine environment is a major threat leading to the potential destruction and extinction of coral reef ecosystems globally (Ferrier-Pagès *et al*., 2018; Williams *et al.,* 2017). Coral bleaching occurs when the symbiotic relationship between corals and their photosynthetic algae of the genus *Symbiodinium* is lost (Blackstone and Galladay, 2018; Liao *et al*., 2019; Oschenkuhn, 2018; van Oppen and Lough, 2018) due to various environmental stressors such as pollution, strong ultraviolet radiation, ocean acidification as well as the decline and rise in seawater temperature (Bell *et al.,* 2013; Ferrier-Pages *et al.,* 2018; Peixito, 2017; van Hooidonk *et al.,* 2016). However, the primary causes observed in past coral bleaching events are ocean acidification and the rise in seawater temperature (Farag *et al.,* 2018; Grottoli *et al.,* 2018). When seawater temperature rises, healthy corals become stressed and the photosynthetic algae or zooxanthellae are expelled from the coral and the coral appears white, hence the term "bleaching" or "bleached" (Blackstone and Golladay, 2018; Nielsen *et al*., 2018). If stressful conditions decline or are removed, the bleached coral can recover and the zooxanthellae can repopulate it (van Oppen and Blackall, 2019). However, if the stressful conditions continue, the coral may die due to starvation, which may further lead to biodiversity reduction and decline in fish populations as most marine species rely on coral reefs for their survival (Damjanovic *et al.,* 2019). It is estimated that by 2050, 60% of the world's coral reefs will be lost if mitigation strategies to help them acclimatize to the pace of climate change are not developed (Bourne *et al*., 2016; Farag *et al.,* 2018; Fordyce *et al*., 2017; Louis *et al*., 2017; Putnam *et al*., 2017).

The coral microbiome plays a vital role in coral health and extensive research on the diversity and roles of these microbes has been carried out (Lee *et al*., 2016; Morrow *et al*., 2018; van Oppen and Blackall, 2019). The colonization of the coral host by beneficial microbes may lead to the suppression of the host immune response (Ziegler *et al*., 2017). The rise in seawater temperature often leads to a dysbiosis between the coral host and its symbionts because bleaching leads to a shift in coral-associated microbial communities (Bourne *et al*., 2016; Webster and Reusch, 2017). Stressed corals have been observed to have a reduction in beneficial bacteria and an increase in pathogens such as those of the *Vibrio* species, resulting in an increase in virulence factor production (McDevitt *et al*., 2017; Zaneveld *et al*., 2017). Some bacteria such as *Roseofilum reptotaenium* have been observed to cause infectious

diseases in corals and the coral probiotic hypothesis has been proposed to assist corals recover from such diseases through the identification of beneficial bacteria that have a potential to produce antimicrobials, thus, competing with pathogens (Peixoto *et al*., 2017; van Oppen and Blackall, 2019). The availability of nutrients, nutrient cycling of carbon, nitrogen and sulphur and release of antimicrobials by the coral microbiome is vital for the health of the coral microbiome (Morrow *et al*., 2018). Additionally, the production of antimicrobial compounds for growth inhibition of coral pathogens may also find application in treatment of microorganisms associated with human diseases (van Oppen and Blackall, 2019).

1.2 Coral bleaching

For millions of years, corals have formed a symbiotic relationship with photosynthetic algae from the genus *Symbiodinium* which live in their tissues (Peixoto *et al.,* 2017; Nielsen *et al.,* 2018). Both the coral and algae are dependent on each other for survival (Hester, 2018). These algae are a primary source of food to corals and give them their color (Ricaurte *et al*., 2016; Sang *et al*., 2019). When these zooxanthellae are expelled, the coral starves and the tissue disappears due to lack of sufficient algal cells to produce food (Peixoto *et al*., 2021). The coral then loses its colour, turning white (Fig. 1.1), and the skeleton is exposed making it vulnerable and susceptible to fatal diseases (Ochsenkühn *et al.,* 2018; Ushijima *et al*., 2018). This is a result of disrupted photosynthesis in *Symbiodinium* species which prevents the repair of damaged photosystems, leading to the production of more reactive oxygen species that damage thylakoid membranes, thus inhibiting enzymes responsible for carbon fixation (Goulet *et al*., 2017).

Figure 1.1: Example of a healthy and bleached coral, *Acropora* sp. (NOAA Indian Ocean, 2017).

Coral bleaching not only affects the corals but disrupts the whole marine ecosystem because coral reefs provide various services to marine life such as habitats, spawning and nursery grounds (Grottoli *et al.,* 2018; van Oppen *et al*., 2017). Various symbiotic relationships that occur amongst corals and other organisms are also disturbed during coral bleaching thus causing instability in the functionality of the ecosystem (Smith *et al*., 2017). For example, some fish species such as butterfly fish mainly feed on coral polyps and they keep algae from smothering corals (Damjanovic *et al*., 2017). Spiny lobsters are dependent on corals for protection especially during their molting stage and they play an important role of maintaining ecosystem balance (van Oppen *et al*., 2017). Dolphins and whales feed on fish that rely on corals for food, so their population decreases if these fish are not available, and they are considered to have emotional intelligence to assist other organisms (van Oppen *et al*., 2017). Hawkbill sea turtles are highly dependent on coral reefs as their food source and they play a critical role in nutrient cycling and balancing food webs (Chen *et al*., 2017). Therefore, every species lost affects another.

Corals have also formed symbiotic relationships with other beneficial microorganisms such as bacteria (Glasl *et al.,* 2019; Matthews *et al*., 2020), archaea, fungi, endoliths and viruses (van Oppen and Blackall, 2019). The functionality of bacterial symbionts is suggested to significantly contribute to coral health, and most importantly, holobiont resilience to environmental stress (Bourne *et al.,* 2016). However, their role in coral bleaching is less understood (Bourne *et al.,* 2016; Peixoto *et al*., 2017). Therefore, understanding the relationship and roles between corals and their symbionts may assist in mitigating coral bleaching (Levin *et al.,* 2017). The application of probiotics, particularly bacteria to corals, may be a possible solution to test whether the effects of coral bleaching can be manipulated or reversed to improve coral health and resilience to thermal stress (Peixoto *et al.,* 2017).

1.3 The coral holobiont and its response to climate change

Corals are marine invertebrates in the phylum Cnidaria and class Anthozoa (Bindoff *et al*., 2019). They live in colonies and are made up of genetically identical individual polyps which are home to many microorganisms. These colonies grow in multiple shapes (Fig. 1.2) such as laminar, leafy, branching and massive (Liao *et al.,* 2019). The polyp and colony sizes may vary depending on the maturity of the coral (Bernasconi *et al.*, 2019; van Oppen and Blackall, 2019). These polyps secrete skeletons of calcium carbonate to form coral reefs which are also home to many organisms (Bernasconi *et al.*, 2019).

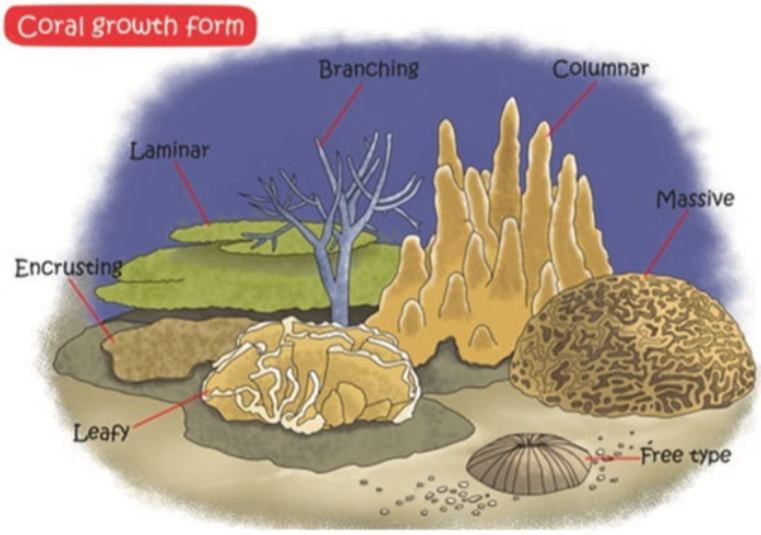

Figure 1.2: Various coral colony shapes found on a typical deep-sea floor ranging from encrusting, leafy, massive, laminar, branching, columnar and free type. The type of colony shape determines the interaction of that coral species with its physical environment and other organisms (Liao *et al*., 2019).

Corals are mostly found in tropical oceans as they grow well in warm, shallow and agitated water where they build reefs (Chen *et al.*, 2017). These organisms are divided into two groups i.e., soft and hard corals (van de Water *et al.,* 2018). Soft corals are without a calcium carbonate skeleton and hard corals secrete calcium carbonate for the formation of their hard skeleton and are responsible for reef-building (Blackstone and Golladay, 2018). For their health and survival, corals have formed symbiotic relationships with microbial symbionts which respond to and assist them to adapt to shifts in their health and survive during environmental stress (Glasl *et al.,* 2016; Ochsenkühn *et al.,* 2018). A holobiont is a group of different organisms from various taxa that coexist and interact (Ochsenkühn *et al.,* 2018). Changes in environmental conditions have an ability to change the physiology of the coral resulting in a change in structure, therefore, altering the arrangement and abundance of microbial populations (Glasl *et al.,* 2016; Ochsenkühn *et al.,* 2018). Furthermore, disturbances due to environmental changes can lead to the occurrence of diseases, which is also a threat to coral reefs (van Oppen *et al.,* 2017).

Climate change is also a major threat to coral reef health as it destabilizes the coral microbiome due to the rise in sea water temperature. This rise in sea water temperature causes oxygen depletion and acidification which threaten coral health (Bindoff *et al*., 2019). Shifts in the coral microbiome composition not only affects the coral host but the entire marine ecosystem because food availability decreases, and organisms spread throughout the sea floor

which is their habitat to look for food around plants and animals growing on the sea ground. (Sweetman *et al*., 2017). Therefore, the response of corals to climate change is suggested to be mitigated by the coral microbiome through the production of bioactive compounds to assist corals acclimatize to the rise in seawater temperature (van Oppen *et al.,* 2017).

1.4 Coral colony microhabitats

The coral colony is made up of different microhabitats (Fig. 1.3) which are the surface mucus layer, tissue layer (Glasl *et al.,* 2016; Neave *et al*., 2017) as well as the gastrovascular cavity and skeleton (Apprill *et al*., 2016). These are occupied by different bacterial communities whose genetic composition and functions are distinct (Bourne *et al.,* 2016). The skeleton and surface mucus layer, which is nutrient-rich (Pollock *et al*., 2018), are habitats of most coral-associated bacteria (CAB). In the early life stages of the corals, a high diversity of bacteria has been observed in the mucus layer of corals, compared to adult colonies (Bernasconi *et al*., 2019). This may be due to environmental factors and the physiology of the species. It may also be because the coral immune system is not yet fully developed (Pollock *et al*., 2018), and the main source of bacteria is the water column. In addition, microbes associated with the mucus layer are suggested to provide antimicrobial protection to the coral host for protection from invasive (non-native species) and disease-causing bacteria (Ushijima *et al.,* 2018). The skeleton, however, possesses the highest diversity of bacteria (Marcelino *et al.*, 2018; Yang *et al.,* 2019). When temperature changes occur, the carbon composition of the mucus also changes, thus altering the structure of the bacterial community (Lee *et al.*, 2016).

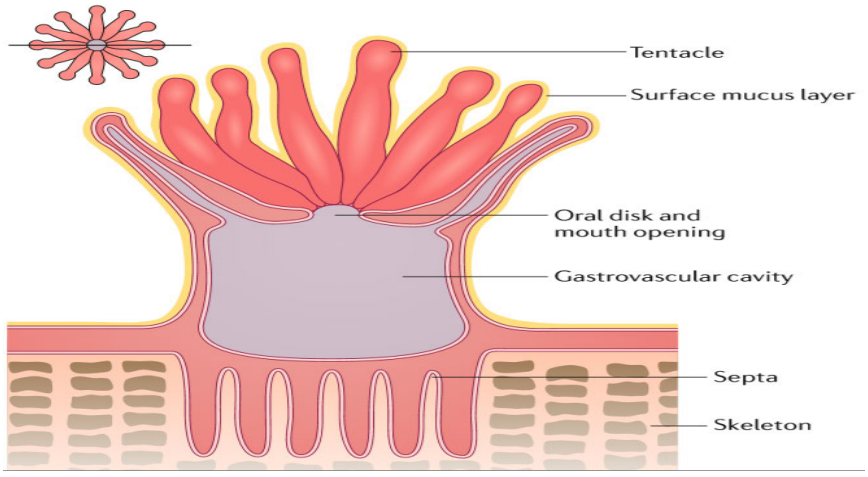

Figure 1.3: Coral host with identical polyps surrounding the oral disc and mouth which serve as both entry and exit to the gactsrovascular cavity. Polyps are made up of two layers, surface mucus layer and the gastroderm. A calcium carbonate – skeleton distinguishes hard corals responsible for reef building, from soft corals that have no skeleton (taken from van Oppen and Blackall, 2019).

1.5 The functional role of microbes within coral holobiont

Healthy corals have formed symbiotic relationships with various symbionts which play an important role in maintaining homeostasis in the holobiont (an assemblage of different species that form a unit) (Bernasconi *et al.,* 2019; Bourne *et al.,* 2016), though the roles of some still need to be discovered (Fig. 1.4). These symbionts include photosynthetic algae *Symbiodinium,* bacteria, archaea, viruses, fungi and endoliths which are autotrophs living inside rocks or corals where they get their nutrients (Hadaidi *et al.,* 2017; Pootakham *et al*., 2018). These organisms have also formed a network of interactions amongst each other for nutrient exchange and transfer of beneficial genes (Glasl *et al*., 2016).

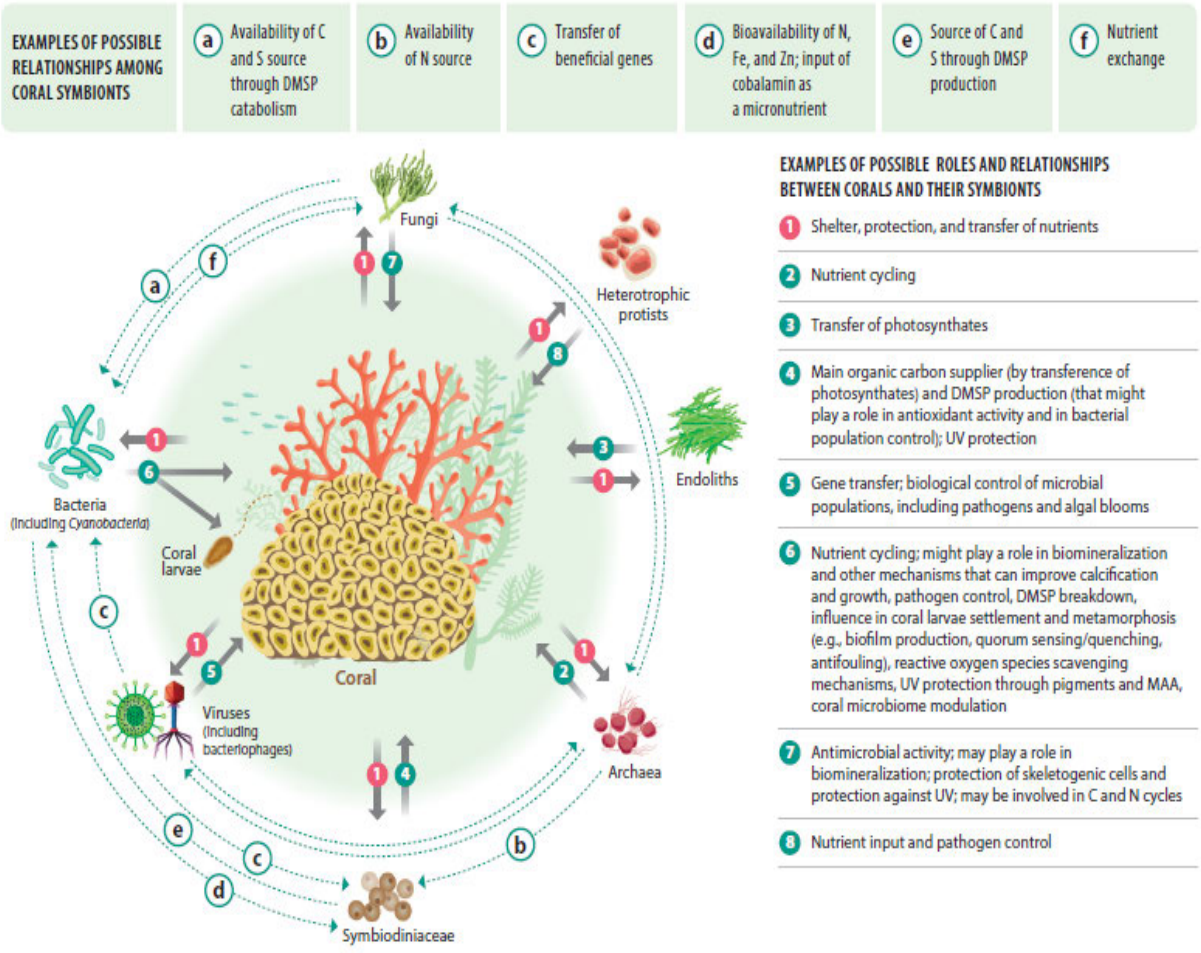

Figure 1.4: Functional roles and relationships between corals, their symbionts and amongst microbial symbionts, mainly responsible for nutrient cycling within the coral host. *Symbiodinium* species and bacteria have formed a mutualistic relationship with the coral, where they both benefit from each other, and some only benefit from the coral host. Some of these interactions and mechanisms have probiotic potential, providing health benefits to the coral, but the role of some of these symbionts is less understood and can be considered for future studies (Peixoto *et al*., 2021).

Symbiodinium species are the main carbon suppliers to the coral and they produce dimethylsulfoniopropionate (DMSP - an organosulphur compound generated in marine environments involved in global sulphur cycling) that might play a role as an antioxidant (Liao *et al*., 2019; Ochsenkühn *et al*., 2018). They also control the bacterial population and protect the coral against UV damage (Ochsenkühn *et al*., 2018). The coral, in turn, provides shelter, protection and plays a vital role in nutrient cycling within the photosynthetic algae.

Coral-associated bacteria have also formed a mutualistic relationship with corals (Peixoto *et al*., 2017). These bacterial populations are diverse and occur in the mucus surface layer of corals including *Acropora* species (Liao *et al*., 2019) and have become predominant residents in the coral holobiont because of the abundance of nutrients in this layer. These bacterial populations secrete antimicrobial peptides to protect the coral host against opportunistic pathogens (Bourne *et al*., 2016). Bacteria play an important role in nutrient cycling of sulphur, carbon, and nitrogen (Damjanovic *et al.,* 2019). They are a biological control of pathogens and degrade DMSP (Hernandez-Agreda *et al*., 2017). They influence the settlement and metamorphosis of coral larvae (Liao *et al*., 2019). It is also suggested that they influence the coral microbiome structure by promoting health and persistence of the coral host (Dunphy *et al.,* 2019; Hernandez-Agreda *et al.,* 2017). In turn, the corals provide shelter, protection and are a source of nutrients to the bacteria (Peixoto *et al*., 2017). The role of archaea is to cycle nitrogen and remove excess ammonium trapped in the mucus layer through nitrification and denitrification. However, other roles are still to be discovered (Bourne *et al*., 2016; Liao *et al*., 2019).

Viruses have also formed an association with corals and play an important role in the coral host structure, although few studies have really focused on their role in corals (van Oppen *et al*., 2017). Within the coral host, viruses are responsible for the transfer of beneficial genetic material and give the coral natural phage therapy by removing bacterial pathogens (Peixoto *et al*., 2017). They also control algal blooms around the coral (Ochsenkühn *et al*., 2018).

Fungi are responsible for antimicrobial activity (Peixoto *et al.,* 2017; Pereira *et al*., 2017), nitrogen cycling, reduction of nitrate to ammonium and assimilation of ammonia for biosynthesis within the coral host (Bourne *et al.,* 2016). Endoliths are responsible for photosynthate transferase (Peixoto *et al.,* 2021), distributing sugars and other compounds to coral tissues. However, when corals bleach, this symbiotic relationship between the coral and its microbes is destabilized.

Figure 1.4 shows a representation of possible relationships between a coral and its symbionts. A tripartite relationship occurs between *Symbiodinium* species, coral and the microbiome, and nutrients are transferred within the coral holobiont (Bourne *et al.,* 2016). Carbon photosynthates as well as other nutrients such as phosphorus and nitrogen from *Symbiodinium* species. are translocated to satisfy the coral's nutritional requirements including those of the coral microbiome (van de Water *et al.,* 2018). In turn, the carbon from the coral can also meet the nutrient requirements of *Symbiodinium* species. and is also useful to the entire microbiome. These microbes contribute to the coral host trace nutrients, metals, and vitamins. The coral and *Symbiodinium* species produce dimethyl sulphide from the secondary metabolite DMSP *via* enzymatic pathways in bacteria, which help give structure the coral microbiome (Bourne *et al.,* 2016). Figure 1.5 illustrates the nutrient pathways and interactions facilitated by this tripartite relationship.

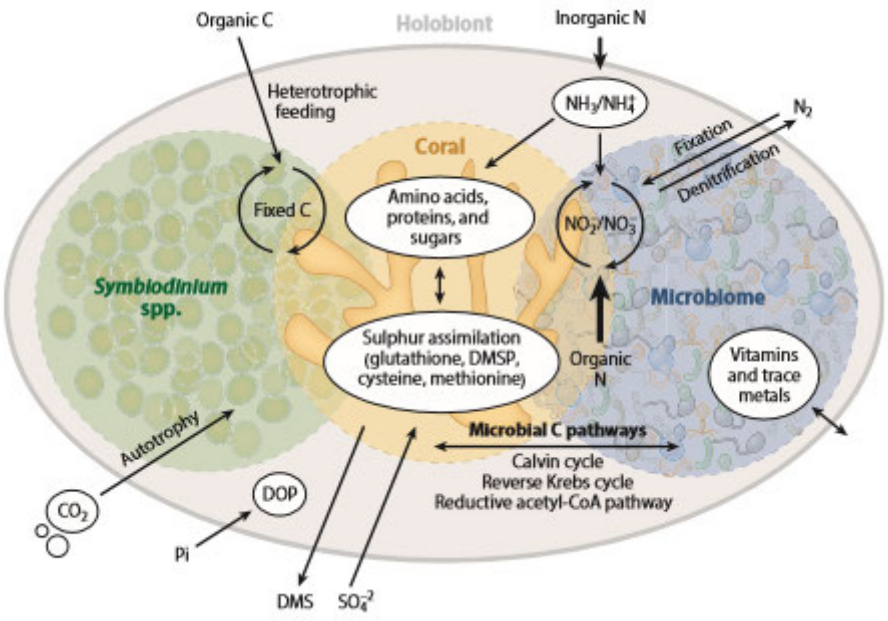

Figure 1.5: A tripartite relationship between *Symbiodinium* species., coral and associated microbiome including nutrient pathways. All three components of the coral holobiont interact and have metabolic pathways. For example, the *Symbiodinium* species supplies fixed carbon to both the coral and its microbiome. Carbon from the coral can also be distributed to meet the *Symbiodinium* species and the microbiome requirements. Trace nutrients, vitamins and metals are also obtained by the holobiont from heterotrophic feeding and further supplemented by those produced by microbiome. *Symbiodinium* species obtain fixed nitrogen from diazotrophs. The coral and *Symbiodinium* spp. produce DMSP for holobiont structure. Abbreviations: DMSP – dimethylsulfoniopropionate; DOP – dissolved organic phosphorus; Pi – orthophosphate (Bourne *et al.,* 2016).

1.6 Effect of climate change on coral microbiome and diseases

The elevated seawater temperature in response to climate change and various environmental factors such as pollution, low salinity and unusual rise and decline of water temperatures (van Oppen and Lough, 2018), has caused a breakdown of the symbiosis between corals and their endosymbionts, particularly of the genus *Symbiodinium,* which supply corals with most of their energy (Levin *et al*., 2017; Olguín-López *et al.*, 2017; Peixoto *et al.,* 2017). This then leads to coral bleaching. The rise in seawater temperature is suggested to be the primary cause of coral bleaching (Huges *et al*., 2017; van Oppen and Blackall, 2019; van Oppen and Lough, 2018) and its role has been extensively investigated (Ferrier-Pagès *et al*., 2018; Gardner *et al*., 2018; Levin *et al*., 2017; Nielsen *et al*., 2018). When the seawater temperature rises, the zooxanthellae are expelled from the coral cells, thereafter, leading to cell death (Gajigan *et al*., 2017). As previously mentioned, bleaching occurs when coral tissue becomes white because the photosynthetic pigment that gives corals their colour is reduced, thus exposing their white calcium carbonate skeleton (van Oppen and Lough, 2018), leaving the corals vulnerable to diseases (Olguín-López *et al.*, 2017). Coral physiology is influenced by its microbial symbionts, therefore, the loss of zooxanthellae results in changes in microbial population structure in the coral host (Levin *et al*., 2017). Physiological processes such as metabolism and antioxidant response, immune defense and calcification are affected when seawater temperature elevates (Grottoli *et al*., 2018). The corals become vulnerable to invasion by pathogens and their cells are damaged when calcification occurs, though some components of immunity are able to cope with environmental change by showing thermal resilience (Stabili *et al.,* 2018). This change in physiology then alters the arrangement and abundance of microbial communities (Glasl *et al*., 2016; Ochsenkühn *et al.,* 2018). A significant genetic shift in the composition of the microbial symbionts occurs, resulting in a widespread occurrence of opportunistic and disease-causing bacterial species from the families *Verrucomicrobiaceae* and *Vibrionaceae* (Glasl *et al*., 2016; Grottoli *et al*., 2018). These bacteria are also suggested to be facilitators of coral bleaching and compromise coral health (Grottoli *et al*., 2018).

1.7 Role of *Vibrio* spp. in coral bleaching

Events of mass mortality in various coral species have not only been associated with the rise in seawater temperature, but also with opportunistic microbial pathogens (Rubio-Portillo, 2016). During bleaching, a shift in the resident microbial community occurs (Bourne *et al.,* 2016; Levin *et al*., 2017) and this community is outcompeted by pathogenic microbes. Several events of coral bleaching and diseases have been reported to be linked with species of the genus

Vibrio (Rajasabapathy *et al*., 2020). Species from this genus are more virulent and transmissible making them more pathogenic (Rosado *et al*., 2019).

Earlier studies have identified two *Vibrio* species as causative agents of coral bleaching and diseases, *Vibrio coralliilyticus* and *Vibrio shiloi* (Grottoli *et al.,* 2018; Liang *et al.,* 2017; Rubio-Portillo *et al.,* 2016; van der Water *et al.,* 2018). The disease outbreaks by these pathogens are triggered by the rise in seawater temperature which resulted in an increase in *Vibrio* abundance (Stabili *et al*., 2018).

Vibrio coralliilyticus mainly has been implicated in disease development in *Paramuricea clavata*, a purple sea-fan (Rubio-Portillo *et al.,* 2016; van der Water *et al*., 2018), as well as bleaching, lysis and tissue loss in *Pocillopora damicornis,* the cauliflower coral (van der Water *et al*., 2018). It has also caused infection in corals such as *Eunicella verrucosa,* the pink sea fan in England, and *Montipora aequituberculata,* an encrusting pore coral (Grottoli *et al.,* 2018). Other species affected include *Montastreae annularis*, a boulder star coral and *Acropora muricata,* a staghorn coral (Grottoli *et al.,* 2018). Infection by *V. coralliilyticus* was manifested by the production of more mucus in the surface mucus layer of these corals in response to increased temperature (Lee *et al*., 2016). This made the corals more vulnerable to diseases even though the coral mucus usually provides nutrients to both beneficial microbes and pathogenic invaders (Rajasabapathy *et al*., 2020; Ushijima *et al*., 2018). When heat stress occurs, a shift in the surface microbial community from healthy to diseased occurs which facilitates pathogen invasion and opportunistic infections (Ricaurte *et al.,* 2016). However, it is uncertain how heat stress facilitates the shift in dominance of pathogens, but a decline in beneficial bacteria and overgrowth of *Vibrio* species preceeds bleaching and disease outbreak (Lee *et al.*, 2016). This leads to the loss of pigmentation and the disappearance of bacterial cells in the coral tissue. The surface mucus layer becomes stressed whereas the abundance of *V. coralliilyticus* increases and signs of bleaching are observed (Ricaurte *et al*., 2016). This pathogen disturbs the symbiotic relationship between the coral and *Symbiodinium* species which is its primary source of food and nutrients (Hadaidi *et al*., 2017). A shift of these photosynthetic algae as a result of increased temperature leads to negative impacts on the yield of *Symbiodinium* species (Blackstone and Golladay, 2018; Pogoreutz *et al.,* 2018).

Some studies demonstrated that the pathogen *V. shiloi* caused bleaching in *Oculina patagonica,* a stangly branched coral (Rubio-Portillo *et al.,* 2016). These pathogens colonize the mucus layer and produce a Toxin P molecule that inhibits algal photosynthesis, thus causing bleaching and tissue loss (Blackstone and Golladay, 2018). The increased activity of various *Vibrio* species virulence genes plays a role in host degradation, motility and antimicrobial

resistance (Pereira *et al*., 2017). Some of the diseases caused by these pathogens include black band disease and Acroporid white syndrome or white band disease (Ushijima *et al.,* 2018). Black band disease occurs when a black band moves across the surface colonies, with pathogens completely degrading the coral tissue, exposing the coral skeleton and leading to the death of the coral (Meyer *et al.,* 2016). White band disease (WBD) largely affects *Acropora* species where a white band is formed across the coral limb, slowly killing it by destroying the coral tissue and changing the coral colour to white (Blackstone and Golladay, 2018; Gignoux-Wolfsohn *et al.,* 2020; Howells *et al.*, 2020). Yellow band disease, characterized by large patches of yellow tissue in corals, and forms a circular ring with a yellow margin, eventually killing the coral (Aeby *et al.,* 2020). White pox disease forms irregular white patches on the coral surface, and coral tissue is lost (Agung *et al.,* 2020). White plague disease is characterized by a white band that forms at the bottom of the coral, which then creeps up the coral colonies resulting in tissue loss thus exposing the coral skeleton (Gignoux-Wolfsohn *et al*., 2020). The occurrence of these diseases results in the segregation of beneficial microbes into isolated patches, disturbing the interactions of these microbes which are responsible for antibiotic production and facilitation of homeostasis within the coral host (Miura *et al*., 2019). Pathogens then dominate resulting in a loss of antibiotic activity by the beneficial microbes (Pereira *et al*., 2017). This then eliminates the ability of corals to defend themselves against diseases, giving pathogens more opportunity to infect and spread within the host, thus increasing the risk of disease and coral bleaching (Miura *et al*., 2019). Therefore, the identification of various beneficial microbes within the coral host is very important in assisting corals to become resistant to the infection and spread of pathogens during thermal stress. This will also help corals acclimatize to the rising seawater temperature, which is currently a major problem affecting the functionality and health of reef ecosystems globally and has potential biotechnological application for human diseases (Mahmoud *et al*., 2016).

1.8 The coral probiotic hypothesis

The coral probiotic hypothesis has been proposed to improve coral health and resistance after the occurrence of bleaching events (Peixoto *et al.,* 2017), where potential probiotics are selected from the coral symbiotic microbes to regulate bleaching events (Miura *et al*., 2019). These live microorganisms are re-seeded on bleached corals with the purpose of restoring their health and observing if the effects of bleaching can be reversed (Fig 1.6), as these probiotics are suggested to provide health benefits to the coral host (Peixoto *et al*. 2021). This hypothesis is thought to be applicable as a remedy for recovery of bleached corals as well as a preventive

measure to protect corals from bleaching (Peixoto *et al.,* 2017). Therefore, this hypothesis proposes that the coral microbiome can be manipulated to improve coral health (Damjanovic *et al*., 2019; van Oppen and Blackall, 2019) and is a possible solution to improve coral adaptation to climate change (Rosado *et al*., 2019). Some of the other health benefits of probiotics include prevention of colonization by pathogens and positive change in the availability of nutrients (Damjanovic *et al*., 2019). Figure 1.6 illustrates the application of probiotics on healthy and bleached corals.

Silva *et al.* (2021) tested the use of probiotics on different coral species and demonstrated coral adaptation to various environmental factors. The development of resistance of corals to pathogens was tested on different coral species challenged with pathogenic *Vibrio* species.Several aquarium experiments were performed to test the effectiveness of the probiotic hypothesis in various corals and appears to be a potential strategy to mitigate coral bleaching (Peixoto *et al*., 2017). For example, CAB from the coral *Galaxea fascicularis* were screened for anti-*V. coralliilyticus* activities, after the selection of potential probiotic bacteria (Peixoto *et al*., 2017). Three potential bacterial strains were identified as *Ruegeria* species and demonstrated inhibitory or protective mechanisms in *G. fascicularis* against *V. coralliilyticus* (Miura *et al.,* 2019). *Ruegeria* species outnumbered populations of *V. coralliilyticus*, and *V. coralliilyticus* later showed a decline, which was interesting because they multiply in increased temperatures (Miura *et al*., 2019). Furthermore, *Ruegeria* species showed resistance to increased temperatures (Miura *et al*., 2019). The presence of *Ruegeria* species has also been observed to play protective roles in other coral species such as *Acropora millepora*, *Porites lutea* and *Platygyra carnosa* (Cai *et al.,* 2018). *Oculina patagonica*- (Rahman *et al.,* 2021) and *Pocillopora damicornis*-associated bacteria from genera *Aeromonas, Edwardsiella, Shewanella* and *Vibrio* species were reported to control virulence genes of pathogenic organisms through the quorum sensing (QS) system (Zhou *et al*., 2020). These bacterial isolates are suggested to produce antimicrobial, adhesion and QS enzymes with a potential to help during coral bleaching (El- Kurdi *et al*., 2021). Subsequently the coral developed resistance against this pathogen, however, the exact mechanisms as to how CAB contribute to coral health are still poorly understood (Rosado *et al*., 2019).

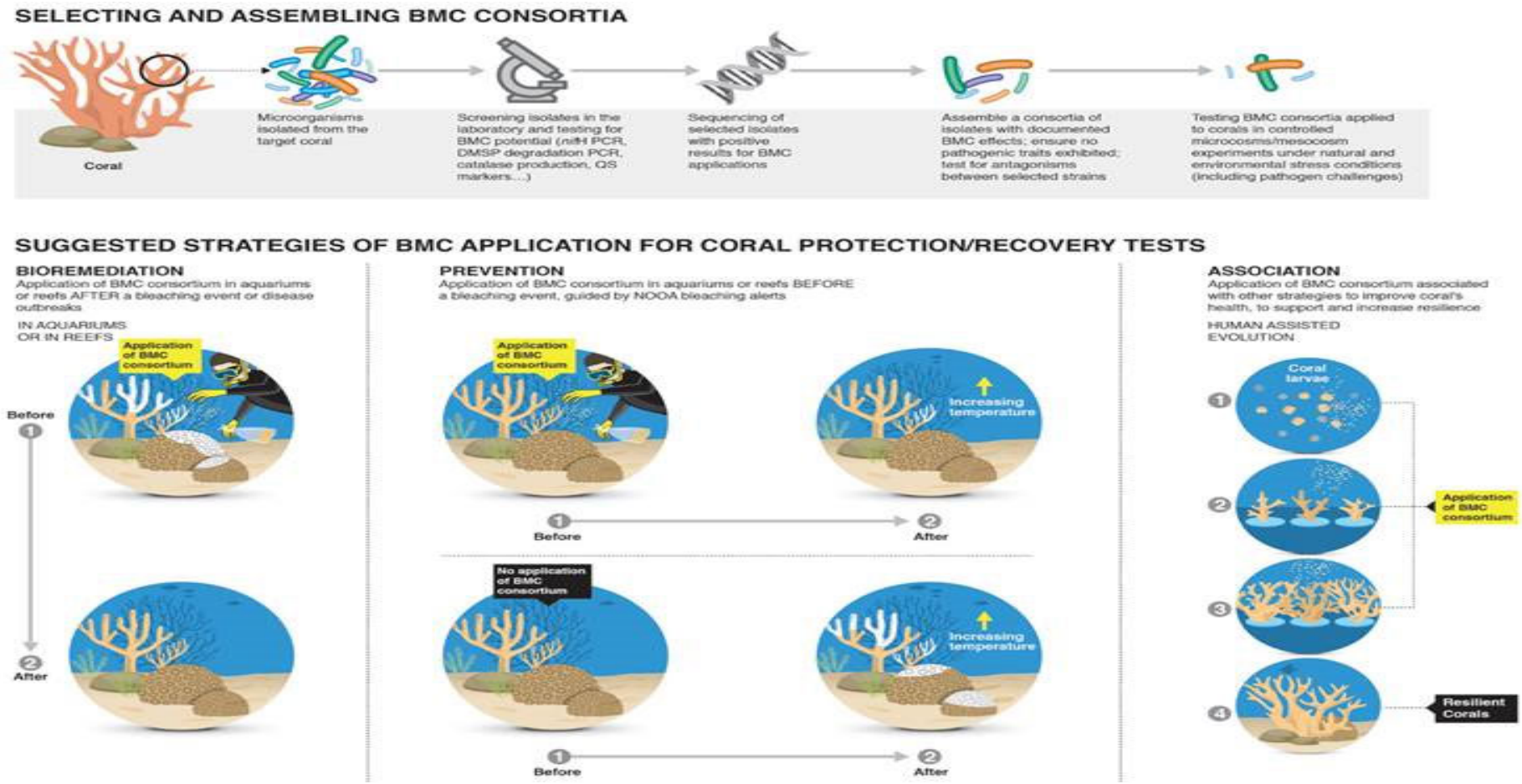

Figure 1.6: Illustration of the selection of probiotics from corals and their application on bleached corals as a remediation measure and on healthy corals as a prevention measure to the increasing temperature. The first step would be the isolation of microorganisms from the surrounding reef water and the target coral species. Then the beneficial bacteria would be identified and screened for beneficial interactions with the coral host through aquarium-based experiments and the mechanisms by which the bacteria confer benefits to the coral host. Extensive screening of beneficial microbes would be undertaken to ensure no pathogenic interactions occurs and investigate potential antagonistic interactions between consortia of selected beneficial microbes. "The final steps would be application of the developed strategy in large mesocosm systems under relevant environmental stress conditions and including bacterial challenges to assess effectiveness of treatments before any field trials can begin" (Peixoto *et al.,* 2017). BMC – beneficial microbes.

1.9 Antimicrobial activity of coral-associated bacteria

The prevalence of antimicrobial- and multidrug-resistant bacteria remains a serious health problem globally (Sang *et al.*, 2019). This has led to an increase in the number of deaths and has economic implications (Sang *et al*., 2019). Many attempts are being made to solve this problem such as modification of the existing drugs but have yielded minimum results as mutation rates of pathogens continue to rise and cannot be overcome (Torres *et al*., 2019). The marine environment has become one of the important habitats to be explored for antimicrobial compounds. Many novel antimicrobial compounds have been isolated from corals and have shown strong activity against various pathogens (Modolon *et al.,* 2020). For example, rare and unknown cembranoids and the aromadendrene sesquiterpernoid palustrol were isolated from the coral *Sarcophyton trochelioporum* and demonstrated activity against *Escherichia coli, Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus pneumoniae* (Rodrigues *et al.,* 2019). Some compounds such as diterpene cembrene and trocheliane isolated from coral *S. trochelioporum* demonstrated antibacterial activity against methicillin-resistant *S. aureus* and *Acinetobacter* species and antifungal activity against *Aspergillus flavus* and *Candida albicans* (Sang *et al*., 2019; Zubair *et al.,* 2016). Therefore, the exploration of coral habitats may be a solution to the current search for antimicrobial drugs.

Coral-associated bacteria play beneficial roles in the coral host to maintain homeostasis, protect the coral from pathogens and cycle essential nutrients (Robbins *et al*., 2019; Rosado *et al*., 2019; Yang *et al*., 2019). Many of these bacteria have antimicrobial and anti-biofilm potentials through their production of secondary metabolites (Nofiani *et al*., 2020). Isolation of bacteria from corals to improve coral health and prevent diseases has been carried out previously (Hou *et al*., 2019; Modolon *et al*., 2020; Peixoto *et al*., 2021) and these bacteria produce chemical defences to inhibit the growth of pathogens (Pernice *et al*., 2020). Kvennefors *et al*. (2012) demonstrated that some of the coral-associated bacteria isolated from an *Acropora* species coral exhibited strong inhibition against *V. corallilyticus* and *Pseudoalteromonass* species Under normal conditions, bacterial isolates from *Acropora* species have demonstrated antimicrobial activity but during bleaching a decrease in antimicrobial activity was observed, allowing pathogens to increase (Raina *et al.,* 2016). Martinez-Luis *et al*. (2011) demonstrated the antimicrobial activity of crude extract and compounds 1 and 2 from *Pseudoalteromonas* spp. against *Bacillus subtilis* and *Vibrio* species (Fig. 1.7). The resident *Pseudoalteromonas* spp. strains demonstrated antagonistic activity against *V. coralliilyticus*, which assisted the *P. damicornis* coral to become resistant to *V. coralliilyticus* (Raina *et al.,* 2016; Rosado *et al.,* 2019). These compounds can be used to

protect corals against potential pathogens to avoid dysbiosis, diseases and bleaching in corals through their chemical properties (Puglisi *et al.*, 2019).

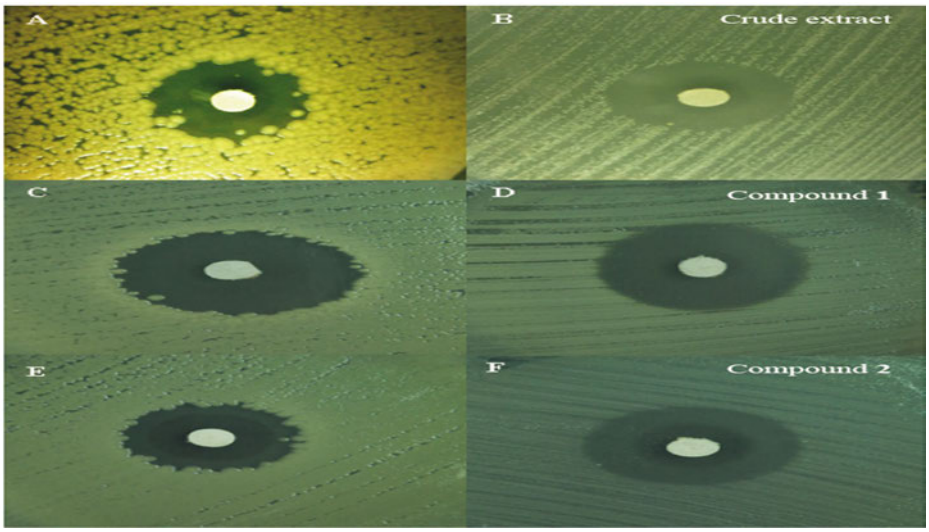

Figure 1.7: Antimicrobial activity of crude extract and compounds 1 and 2 from *Pseudoalteromonas* species isolated from coral *Leptogorgia alba* against *Bacillus subtilis* (A, C and E) and *Vibrio* species (B, D and F) using a disc diffusion assay. Compounds 1 and 2 exhibited greater activity against *B. subtilis* compared to the crude extract, suggesting that the compounds are promising potential producers of bioactive compounds (Martinez-Luis *et al*., 2011).

Coral-associated bacteria and actinobacteria continue to be an extraordinary source of antimicrobial and anti-biofilm compounds for biomedicine and biotechnology applications (Sarmiento-Vizcaíno *et al*., 2017). The compounds produced include ant-inflammatory, cytotoxic, antimicrobial, antivirus and antifouling compounds (Modolon *et al.,* 2020). These chemically diverse compounds include lipopeptides such as massetolide A, antibacterial lactones and phenazines which exhibit a variety of activities against bacteria and fungi pathogenic to humans and plants as well as leukemic cells (Modolon *et al.,* 2020). The production of these compounds may be a result of drastic changes in environmental conditions. Such antibiotics are also useful in combating human pathogens, with probiotic potential (Gomma *et al.,* 2016; Ma *et al.,* 2018; Martinez-Luis *et al*., 2011; Reina *et al*., 2019).

Some studies have shown CAB such as *Pseudoalteromonas* and *Bacillus* species to produce antimicrobial and anti-biofilm compounds against clinical multi-drug resistant strains (Modolon *et al*., 2020). Coral-associated bacteria have a potential in biotechnological applications through production of secondary metabolites, antimicrobial and anti-biofilm compounds. Sarmiento-Vizcaíno *et al.* (2017) assessed 18 bioactive cultivable bacteria isolated from different coral phyla including *Actinobacteria*. These isolates belonged to various genera

including *Micromonospora, Myceligenerans, Pseudonocardia* and *Streptomyces*, however, most of these isolates belonged to the *Streptomyces* genus. These bacterial isolates demonstrated diverse antibiotic activities against Gram-positive *Micococcus luteus* and Gram-negative *E. coli.* These isolates also demonstrated antimicrobial activity against other important human pathogens such as *Klebsiella pneumoniae*, *P. aeruginosa* and *S. aureus.* Atencio *et al.* (2018) isolated 39 *Pseudoalteromonas* species strains isolated from the octocoral *Leptogorgia cofrini*. These strains demonstrated antimicrobial activity against Gram-positive target bacteria such as *Bacillus pumilus, B. subtilis* and *S. aureus*. A number of secondary metabolites with antimicrobial and antifungal activities have also been identified from members of *Pseudoalteromonas, Streptomyces, Bacillus, Pseudomonas, Erythrobacter, Shewanella, Psychrobacter, Labrenzia* and *Vibrio* species (Atencio *et al*., 2018; ElAhwany *et al*., 2015; Pham *et al*., 2016; Sharma *et al*., 2019). Sang *et al*. (2019) reported that several current drugs and potential drugs in clinical trials have been developed from marine natural products, hence the discovery of novel pharmaceutical compounds from CAB is a promising strategy.

1.10 Quorum sensing and its inhibition in corals

Microbes have developed an advanced system of regulating their own population densities (Bhedi *et al.,* 2017). This is achieved by a cell-to-cell communication mechanism referred to as QS, which allows regulation of gene expression when cell density changes in both Gram-negative and Gram-positive bacteria using internal signal molecules which allow communication (Certner and Vollmer, 2018). This communication is made possible through the production of small chemical signal molecules called autoinducers (AI) or bacterial quoromones which allow alterations in their transcription through the adjustment of population needs (Grandclément *et al*., 2016).

Quorum sensing was first discovered in a marine bacterium, *Vibrio fischeri*, a Gram-negative bacterial QS system where two regulatory proteins, LuxI and LuxR facilitated QS (Figure 1.8). LuxI serves as an AI synthase enzyme responsible for production of homoserine lactone (HSL) and N-(3-oxohexanoyl) homoserine lactone (3-oxo-HSL) whereas the LuxR binds the AI and activated transcription of the *luxL CDABE* operon (Santhakumari and Ravi, 2019). Many pathogens use the acyl-homoserine lactone (AHL) regulated QS communication and these include bacteria in the genera *Chromobacterium, Citrobacter, Enterobacter, Klebsiella, Pseudomonas, Serratia,* and *Vibrio* (Santhakumari and Ravi, 2019).

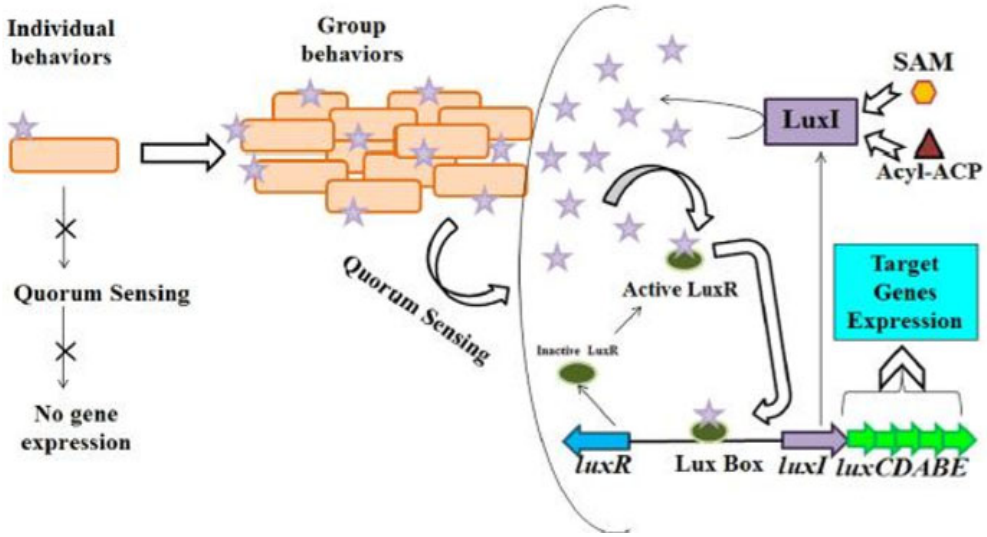

Figure 1.8: Mechanism of QS in Gram-negative bacteria where two regulatory proteins, LuxI and LuxR facilitated QS. LuxI serves as an AI synthase enzyme responsible for the production of homoserine lactone (HSL) and N-(3-oxohexanoyl) homoserine lactone (3-oxo-HSL) whereas the LuxR binds the AI and activated transcription of the *luxL CDABE* operon (Santhakuman and Ravi, 2019). SAM – *S*-adenosylmethionine; ACP – acyl carrier protein.

Bacteria produce many types of autoinducers, specific (AI-1) and universal (AI-2) autoinducers which influence signalling in Gram-negative and Gram-positive bacteria. The primary autoinducer in Gram-positive bacteria are the autoinducing peptides (AIP). Gram-negative bacteria which carry out QS produce *N*-acyl homoserine lactone (AHL) signalling molecules (Zhang *et al*., 2017) which they use to coordinate communication in different environments although some factors such as temperature affect QS regulation. However, AI-2 is a major signal molecule used by bacteria for QS as it is responsible for the regulation of gene expression and their physiological behaviour in stress response (Zhao *et al.*, 2018). Its receptors are mainly found inside the cell and rarely found on the cell surface. AI-2 is used for conversations between unrelated bacteria as it promotes interactions of microbes within the host (Lorenz *et al.,* 2017). Coral microbes use AI-2 molecules for interspecies communication which allows biofilm formation and gene regulation in other microbial communities, thus leading to shifts in microbial species in the environment (Zhao *et al*., 2018) These autoinducers control bacterial behaviours which include host colonization, virulence factor production, biofilm formation, antibiotic and enzyme production and sporulation (Certner and Vollmer, 2018). They achieve this by use of antibiotic resistance and virulence genes to activate and inhibit responses of bacterial QS (Certner and Vollmer, 2018). Both Gram-negative and Gram-positive bacteria can make use of AI-2 although the individual signalling involves different classes of QS molecules. The global increase of antibiotic-resistant pathogens has led to a

search of alternative development of new antibacterial drug treatment strategies (Ma *et al*., 2018; Reina *et al.,* 2019). CAB especially pathogens use chemical signaling molecules to communicate between individual bacteria (Zhou *et al*.,2020).

Coral-associated bacteria have been reported to contain bioactive compounds with anti-QS potential (Golberg *et al.,* 2013; Pham *et al*., 2016). Several studies have developed strategies on inhibiting QS of bacterial pathogens including biofilm formation without being bactericidal or bacteriostatic (Pham *et al.,* 2016). El-Kurdi *et al.* (2020) screened marine bacteria including CAB for anti-QS and biofilm formation and reported CAB to exhibit the highest percentage of bacteria with anti-QS activity. This might be due to the dominance of quorum quenching (QQ) acylase enzymes, with lactonase enzymes being abundant (Muras *et al.,* 2018; Reina *et al.,* 2019). Furthermore, the QQ CAB exhibited strong biofilm activity against *P. aeruginosa* and *Vibrio alginolyticus*, inducing biofilm formation by *V. alginolyticus* and inhibiting biofilm of *P. aeruginosa* (El-Kurdi *et al.*,2020). These results demonstrate that CAB can be probiotics in pharmaceutical and environmental industries, although the individual signaling involves different classes of QS molecules.

Quorum sensing of *P. aeruginosa* is known to be a major regulator of virulence factors in biofilm formation. Bakkiyaraj *et al*. (2013) reported CAB bioactive compounds to have inhibited QS of *P. aeruginosa* as they inhibited the expression of different virulence factors including biofilm formation and the production of extracellular enzymes such as protease and elastase. Inhibition of QS is a novel strategy to combat drug resistant pathogens, since traditional antibiotics increase the development of resistance. Bakkiyaraj *et al*. (2013) also reported the inhibition of QS and biofilm formation by *S. marcescens* by CAB. These bacteria demonstrated inhibition of QS virulence factors including the production of protease and lipase.

Though there is limited knowledge about QS in coral health, studies to detect the production of QS molecules by coral-associated bacteria and pathogens have been undertaken (Certner and Vollmer, 2018). Quorum sensing signalling molecules of three coral bacterial genera, *Pseudoalteromonas, Roseobacter* and *Vibrio* species were reported to facilitate QS (Cude and Buchan, 2013). The QS of *Roseobacter* species was found to regulate motility, biofilm formation and the degradation of particulate organic carbon. Papenfort and Bassler (2016) suggested that *Vibrio* species use three kinds of QS signalling molecules AHL, AI-2 and cholerae autoinducer – 1 (CAI-1), in the expression and regulation of up to 715 genes. Quorum sensing of *Pseudoalteromonas* species is involved in the regulation of the production of antimicrobial compounds, and CAB belonging to *Erythrobacter, Pseudoalteromonas*, *Ruegeria* and *Vibrio* were found to produce AHLs (Li *et al*., 2016, 2017). Furthermore, it was

found that AHL and AI-2 molecules were commonly found in microbes isolated from black band disease coral samples (Zimmer *et al*., 2014). These above studies suggest that QS microbes are present in coral reef ecosystems. Zhou *et al.* (2020) also reported the presence of AHL producers among CAB belonging to different genera known to be opportunistic pathogens including *Aeromonas*, *Cobetia, Edwardsiella*, *Marinomonas Shewanella* and *Vibrio* species Certner and Vollmer (2018) suggested that AHLs may cause a shift in the microbial community structure, while McDevitt-Irwin *et al.* (2017) reported that opportunistic bacteria can increase in abundance in the coral host through QS, thus disrupting microbial stability and inducing coral bleaching.

In *Vibrio* species QS is used to consolidate information with cell density, including environmental limitations (Casillo *et al.,* 2017; Santhakumari and Ravi, 2019). They use QS for their metabolism and infection processes (Certner and Vollmer, 2018). QS allows opportunistic bacteria to take advantage of the rise in seawater temperature, allowing infection by these pathogens, thus compromising coral health (Zhou *et al*., 2020). At increased temperatures, *Vibrio* species can produce different types of QS autoinducers (Lorenz *et al.,* 2017) which can convert a healthy coral holobiont into a pathogen.

Interference with QS within the coral holobiont may be a novel strategy to regulate the microbiome (Bhedi *et al.,* 2017) because CAB use QS to regulate gene expression when shifts occur in the availability of cells due to factors such as increased temperatures (Ma *et al*., 2018). This will then stimulate and/or inhibit the growth of beneficial and pathogenic microbes in the coral host (van de Water *et al*., 2018). Gignoux-Wolfsohn *et al*. (2020) explored the possibility of a quorum sensing inhibitor (QSI) strategy to inhibit the activity of disease-associated bacteria on corals. Healthy *Acropora cervicornis,* was dosed with a QS inhibitor or an autoinducer antagonist, (Z-)-4-bromo-5-(bromomethylene)-2(5H)-furanone. The QSI showed inhibition of WBD development because the addition of QSI prevented the activation of WBD-causing bacteria virulence genes, thus hindering infection by pathogens and preventing their overgrowth (Certner and Vollmer, 2018; Torres *et al*., 2019). In contrast, *Acropora* species infected with WBD without QSI treatment showed signs of infection. Meyer *et al.* (2016) suggested the application of an identified naturally occurring QSI, cyanobacterial metabolite acid, for controlling interactions within black band disease bacteria especially *Vibrio* species Their study further suggested that QSI can be used to prevent the production of virulence factors by pathogens such as *Vibrio* species These results showed that the development of black band disease (BBD) can be somewhat be regulated by QS. Therefore, the ability to disrupt QS may be a novel approach to target pathogens and prevent communication as opposed to

inhibiting their growth, as many pathogens use QS (Zhou *et al*., 2020). Moreover, studies of the exact mechanisms of how QS and QSI aids coral health are essential as they will assist in the development of resistance to diseases and bleaching events in the coral microbiome. This will also show how QSIs may prevent the initiation of infection by pathogenic bacteria.

Multidrug resistance is a major problem in the human health care sector. Bakkiyaraj *et al.* (2013) suggested that CAB have the potential to inhibit the expression of various virulence genes that are involved in *P. aeruginosa* biofilm formation and/or infection of patients by cystic fibrosis, wounds and burns by the opportunistic pathogen. With QS facilitating virulence and broad-spectrum resistance in *P. aeruginosa*, its inhibition is vital in the treatment of these infections. Song *et al.* (2018) also reported CAB as a source for developing QS inhibition and antifouling agents. Their study screened CAB for their ability to inhibit QS and demonstrated that a *V. alginolyticus* extract inhibited biofilm formation of *P. aeruginosa*. It inhibited virulence traits by down-regulating motility ability and elastase activity. Ma *et al*. (2018) screened bacterial isolates from *P. damicornis* for their ability to inhibit QS using *Chromobacterium violaceum* ATCC 12472. Among these was Gram-positive *Staphylococcus homonis* which exhibited anti-QS activity and inhibition of biofilm formation by clinical isolate *P. aeruginosa* in a dose-dependent pattern. This study showed that the QQ bacteria associated with corals have a strong ability to inhibit the biofilm formation. Coral-associated bacteria are thus an ideal source from which QS inhibitory products can be developed as several studies have highlighted coral microbes as a novel source for identifying and developing QS inhibitors and antifouling agents (Sun *et al*., 2018).

1.11 Biofilm formation

Biofilm formation occurs when microorganisms attach and grow on natural and artificial surfaces, whose attachment cannot be reversed without mechanical or chemical intervention (Benneche *et al.,* 2016). This colonization occurs on all kinds of materials and biofilm formation on biotic and abiotic surfaces is a serious problem in the medical field as it leads to multiple infections and serious morbidity and/or mortality (Cordeiro and Werner, 2011). The accumulation of these microorganisms on surfaces also negatively affects various other fields including industrial and marine environments (Gozoua *et al*., 2019). In the marine environment, attachment of bacteria and diatoms influence colonization on the surfaces by facilitating or inhibiting settlement on the submerged areas of marine vessels, thus affecting pipelines, filtration systems and fish nets (Gozoua *et al*., 2019). Clinically, biofilms play a significant role in public health as biofilm-associated microorganisms are resistant to and

exhibit minimum susceptibility to commonly used antimicrobial agents (Gozoua *et al*., 2019). Microbial biofilms are suggested to assist in complete attachment and metamorphosis of corals on appropriate substrata (Benneche *et al*., 2016).

Biofilm formation occurs in four stages starting with the attachment of bacteria on the surface, the formation of microcolonies, biofilm maturation and dispersal (Fig. 1.9). Microbial biofilms also consist of bacterial cells enclosed into an exopolysaccharide matrix (EPS). The EPS contains extracellular polymers which partake in the formation of microbial aggregates (Sangsawang, *et al*., 2017). Quorum sensing regulates the development of mature biofilm (Passos da Silva *et al*., 2017; Vogel and Quax, 2019).

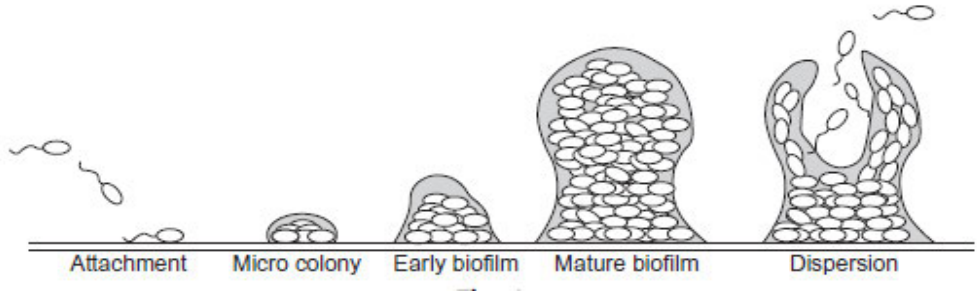

Figure 1.9: Schematic diagram of biofilm formation from attachment stage to dispersion stage. During stage 1, the bacteria attach on the surface and form small colonies, followed by the production of eDNA and EPS compounds denoted by the grey colour. The colonies then develop from an early biofilm to a mature biofilm. At the latest stage, biofilm dispersion occurs and single cells segregate from the biofilm (Vogel and Quax, 2019).

Currently, anti-adhesive hydrogels are used to control medical biofilm formation. Alternatively, attached cells can be killed using antibiotics, inhibited by QSIs, or dispersed using surfactants or enzymes (Vogel and Quax, 2019). The marine environment is considered to be rich in the production of natural products with anti-biofilm properties and corals are among organisms that produce active compounds (Song *et al*., 2018). Coral-associated bacteria including *Vibro* species have shown to form biofilms in the marine environment and the identification of CAB that have an ability to inhibit QS against pathogens is vital (Benneche *et al*., 2016). Coral-associated bacteria have been recognized to play an important role in biofilm formation, producing compounds with inhibition properties (Song *et al*., 2018). Antifouling compounds have been identified in CAB, QQ enzymes and small molecule compounds (Bzdrenga, *et al*., 2017). These studies provide evidence that CAB and their metabolic compounds have the potential to offer anti-biofouling resources in the marine environment.

These QSI compounds have the ability to reduce and inhibit biofilm formation (Camesi *et al.,* 2016). *Bacillus*, *Pseudomonas*, *Pseudoalteromonas*, and *Vibrio* species have been

identified as prevalent sources that produce QSI compounds (Casillo *et al.,* 2017). Dang *et al.* (2016) reported QS inhibition by *Vibrio* species against CAB and identified a compound, rhodamine isothiocyanate which degraded QS signalling molecules in CAB thus disrupting biofilm formation (Fig. 1.10). It further suggested that QS signalling molecules may have been responsible for the disruption of the AI system in *P. aeruginosa*. The use of quorum QQ to reduce signalling molecules or disrupt biofilm formation could assist pathogens become more susceptible to antibiotics as well as the host immune system.

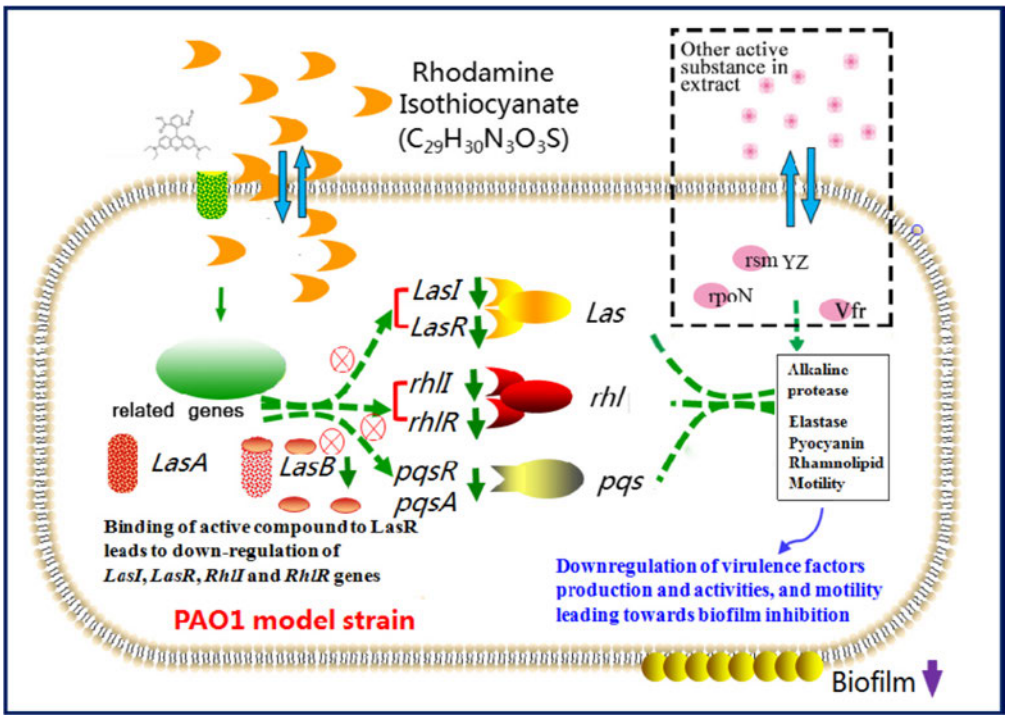

Figure 1.10: The possible mechanism (transcriptional regulation) of QSI activity in *Pseudomonas aeruginosa* PAO 1. QSI activity identified on a *Vibrio* species isolated from coral; with rhodamine isothiocyanate identified as a compound responsible for this effect. The rhodamine isothiocyanate molecules may have disrupted the AI system in *P. aeruginosa*; microscopy confirmed that its biofilm featured a reduced thickness and biomass (Song *et al*., 2018).

Rationale for the study

The increase in antibiotic resistant organisms has become a major problem in human and animal health (Song *et al*., 2018). The search for alternative strategies in the discovery of natural bioactive compounds from the marine environment has taken a rise (Ma *et al*., 2018). Bioactive compounds isolated from corals are suggested to exhibit a wide range of activities such as antimicrobial, anti-QS, anti-viral and anti-biofilm (Tan *et al.,* 2017). Previous studies reported bacteria to coordinate their virulence through QS and biofilm formation (Singh *et al.,*

2016). Biofilms are a serious threat to human health, with the rise in antibiotic resistance causing more infections in humans (Song *et al*., 2018). There is a great need for the development of eco-friendly compounds with the potential to inhibit development of marine biofilms (Song *et al*., 2018) for the development of coral probiotics as well as those with potential bioactivity against clinical pathogens. Inhibition of QS is a promising strategy to inhibit virulence of pathogenic bacteria through the production of QS inhibitors which regulate the virulence of pathogens and interfere with their QS (Camesi *et al.,* 2016). The marine environment is considered to be rich in the production of natural products with the antimicrobial, anti-QS and anti-biofilm properties and corals are among organisms that produce these compounds, however, less information is available on them (Song *et al*., 2018). This study aimed to identify CAB with the ability to produce bioactive compounds with antimicrobial, anti-biofilm and anti-QS abilities to be used as an alternative strategy to combat antibiotic resistance in humans and animals.

1.12 Hypothesis

It is hypothesized that coral-associated bacteria isolated from *Acropora* and *Pocillopora* corals have the ability to produce antimicrobial, anti-quorum sensing and anti-biofilm compounds for possible application as coral probiotics.

1.13 Aim

The following aims were established:

1.13.1 To isolate and identify coral-associated bacteria and assess their antimicrobial, anti-quorum sensing and anti-biofilm potential.

1.14 Objectives

The following objectives were pursued:

1.14.1 To collect two different coral species at different locations from the intertidal habitat on the KwaZulu-Natal coast;

1.14.2 To isolate cultivable bacteria from corals in order to assess coral diversity

1.14.3 To screen isolated bacteria for antimicrobial activity using a colony drop assay and indicator bacteria;

1.14.4 To assess the quorum sensing inhibitory potential of isolated bacteria using an agar overlay assay with *Chromobacterium violaceum* ATCC 12472;

1.14.5 To carry out fermentation of selected bacteria and obtain extracts for downstream testing;

1.14.5 To undertake secondary screening of isolated bacterial extracts for antimicrobial activity using the disk diffusion assay;

1.14.6 To test bacterial extracts for anti-QS activity using qualitative agar diffusion and quantitative violacein inhibition assays and;

1.14.7 To investigate the anti-biofilm potential of isolated bacterial extracts using microtitre plate assays.

1.15 Key questions to be answered

1.15.1 What are the different culturable symbiotic bacteria that can be isolated from corals?

1.15.2 Do coral bacterial strains produce antimicrobial compounds?

1.15.3 Do coral-associated bacteria have an anti-quorum sensing potential?

1.15.4 Do coral-associated bacteria have inhibitory effects on microbial adhesion or mature biofilm formation?

1.16 References

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CHAPTER 2

PRIMARY ANTIMICROBIAL AND QUORUM SENSING INHIBITION SCREENING OF BACTERIA ISOLATED FROM *Acropora* AND *Pocillopora* CORAL SPECIES

Abstract

Coral-associated bacteria are a potential source for production of secondary metabolites and their discovery is a promising strategy for the probiotics and biopharmaceutical industries. Bacteria were isolated from corals belonging to the genera *Acropora* (n=210) and *Pocillopora* (n=132), and preliminary characterization (colony characteristics, pigmentation, Gram reaction and cellular morphology) was undertaken. Primary screening for antimicrobial activity of bacterial isolates was assessed using agar overlay assays against common indicator organisms, *Pseudomonas aeruginosa* ATTC 27853 and methicillin-resistant *Staphylococcus aureus* ATTC 43300 using the colony picking technique. Quorum sensing (QS) inhibition was assessed using qualitative violacein inhibition assays against *Chromobacterium violaceum* ATCC 12472 and *Chromobacterium subtsugae* CV017. Bioluminescence inhibition was assessed against *Vibrio harveyi* BB120 using agar overlay assays. Antimicrobial activity was demonstrated against *P. aeruginosa* by 2.85% (6/210) and 11.36% (15/132) of *Acropora* and *Pocillopora* isolates, respectively. Against *S. aureus*, antimicrobial activity was demonstrated by 20.47% (43/210) and 37.12% (49/132) of *Acropora* and *Pocillopora* isolates. Antimicrobial activity against both clinical strains was observed with 1.90% (4/210) and 10.60% (14/132) of *Acropora* and *Pocillopora* isolates, respectively. Quorum sensing inhibition activity against *C. violaceum* ATCC 12472 was demonstrated by 3.80% (8/210) and 0.75% (1/132) of *Acropora* and *Pocillopora* isolates. Quorum sensing inhibition against both biosensors was observed with 2.38% (5/210) of *Acropora* and 0% (0/132) *Pocillopora* isolates, respectively. Bioluminescence inhibition against *V. harveyi* was demonstrated by 16.66% (35/210) and 6.81% (9/132) of *Acropora* and *Pocillopora* isolates, respectively. These results corroborate various studies of coral-associated bacteria exhibiting antimicrobial and QS inhibition against clinical and/or marine indicator organisms. Therefore, coral-associated bacteria could be an important source of secondary metabolites that can be applied as probiotics in clinical and environmental settings.

2.1 Introduction

Corals harbor a huge diversity of beneficial microbes which occupy different habitats within the host, such as the surface mucus layer, tissue layer, gastric cavity and skeleton (Boilard *et al*., 2020; Bourne *et al.,* 2016). Coral-associated bacteria (CAB) are known to be a potential source of novel compounds with antimicrobial properties and quorum sensing inhibition through production of secondary metabolites (Ferrier-Pagès *et al*., 2018; Peixito *et al*., 2017; Pham *et al*., 2016). The production of compounds by CAB is made possible through symbiotic interactions between the coral and the beneficial microbes which play an important role in the maintenance of coral health as they protect and supply nutrients within the coral holobiont (Peixoto *et al*., 2017; Pernice *et al*., 2019). These CAB help corals increase resistance to diseases and thermal environmental stress threatening corals (Assis *et al.,* 2020; Robbins *et al.,* 2019). Bacterial diversity in corals have demonstrated to be beneficial in both the marine environment and pharmaceutical industry through their bioactivity potential against marine and clinical pathogens (Rosado *et al*., 2019). Pereira *et al*. (2017) demonstrated the diversity of bacteria and the antimicrobial activity of cultivable bacteria isolated from different corals. Proteobacteria, Actinobacteria and Firmicutes were isolated from the mucus layer and surrounding environment and an abundance of *Acinetobacter*, *Alcanivorax, Aurantimonas* and *Erythrobacter* bacteria were identified. Over 80% of the CAB demonstrated antimicrobial activity against *Bacillus cereus*, *Escherichia coli*, *Serratia marcescens*, *Staphylococcus aureus* and *Vibrio parahaemolyticus*. Majority of the CAB exhibiting positive antimicrobial activity were identified as *Bacillus*, *Erythrobacter* and *Paracoccus* species Pham *et al.* (2016) reported that cultivable bacterial diversity from the soft coral *Alcyonium digitatum* exhibited antimicrobial activity. They demonstrated that 48.6% of 251 isolated bacteria exhibited antimicrobial activity against marine and clinical pathogens. These isolates were identified to be *Bacillus methylotrophicus* and *Bacillus amyloliquefaciens.* This study also suggested that these CAB could be involved in cycling of sulfur in corals as well as coral health. This suggests that CAB have antimicrobial activity with a broad range of activity. Bioactive compounds produced play an important defensive role in corals against opportunistic pathogens and in the maintenance of coral health. This is achieved through the production of antimicrobial compounds and can be an important strategy used by bacteria against other competitor microorganisms in the coral environment.

However, the antimicrobial activity demonstrated by CAB bacteria is not only important in ecological interactions within the coral microbiome (Marston *et al.,* 2016), but can also be a novel source of new compounds targeting antibiotic resistance in human bacterial pathogens

(Marston *et al.,* 2016). Wiese *et al.* (2008) reported 13 bacteria isolated from different coral species which possessed genes responsible for the production of secondary metabolites (polyketides and peptides) with antimicrobial properties. Moreira *et al.* (2014) reported *Bacillus*, *Pseudovibrio*, *Shewanella, Staphylococcus* and *Vibrio* species among genera identified from different corals and their surrounding environments. Several studies have reported these genera to be cultivable bacteria associated with corals which exhibit antimicrobial and quorum sensing potentials (Ma *et al.,* 2018; Wiese *et al.,* 2008, Zhao *et al.*, 2018). Raina *et al.* (2016) reported the antimicrobial activity of *Pseudovibrio* sp. P12 isolated from the coral *Pocillopora damicornis.* This bacterium metabolized dimethylsulfoniopropionate (DMSP) and played a significant role in structuring bacterial communities in corals. This strain also produced tropodithietic acid which inhibited the growth of coral pathogens *Vibrio coralliilyticus* and *Vibrio owensii*. Sang *et al*. (2019) reported that compounds, sarcotrocheliol acetate and sarcotrocheliol, from CAB isolated from coral *Sarcophyton trocheliophorum*, exhibited strong activity against methicillin-resistant *Staphylococcus aureus* and *Acinetobacter* species. Another compound, trocheliane displayed strong activity against multiple microorganisms including *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. Modolon *et al.* (2020) reported CAB as producers of bioactive compounds with a wide range of bioactivities including antimicrobial activity. *Streptomyces* and members of genus *Micromonospora* isolated from soft corals were reported to produce secondary metabolites such as thiocoraline which exhibited antimicrobial activity. Fang *et al.* (2020) reported that nucleoside antibiotics from *Pseudonocardia* species isolated from coral *Galaxea fascicularis* exhibited antimicrobial activity against methicillin-resistant *S. aureus* and *Acinetobacter baumannii*.

Gram-negative bacteria produce diverse *N*-acyl-homoserine lactones (AHL) signalling molecules (Certner and Vollmer, 2015; Tait *et al.,* 2010), which they use to coordinate intraspecies communication in different environments. Coral-associated bacteria use quorum sensing (QS) to regulate gene expression when shifts occur in the availability of cells due to factors such as increased temperatures (Charlesworth *et al*., 2019; Tait *et al.,* 2010). Several studies have reported the presence of AHL-producing CAB belonging to different genera such as *Aeromonas, Cobetia*, *Edwardsiella, Shewanella*, and *Vibrio* (Ma *et al.,* 2016; Zhou *et al.,* 2020). Coral-associated bacteria also use the signalling molecule, autoinducer-2 (AI-2) for gene expression regulation and their physiological behaviour in stress response (Zhao *et al.*, 2018). It is used for interspecies communication as it promotes interactions of microbes within the host (Certner and Vollmer, 2018). Autoinducer-2 is implicated in black band disease and

surface mucus layer as both Gram-positive and Gram-negative bacteria produce *(S-)*-4,5-dihydroxy-2,3-pentanedione (DPD) (Zimmer *et al*., 2014). Some *Vibrio* species are pathogenic and known to produce AHLs and DPD (Başaran, *et al*., 2020).

Coral microbial communities are rich sources for pharmacologically important natural products with anti-quorum sensing activities (Bhedi *et al.,* 2017; Ma *et al.,* 2020). Several studies have reported QS inhibition (QSI) by CAB isolated from *P. damicornis* against Gram-negative AHL-based reporter strains *Chromobacterium violaceum* ATCC 12472, and *C. violaceum* CV026 (El-Kurdi *et al.,* 2021; Ma *et al.,* 2018). These isolates demonstrated minimal QSI while some yielded high QSI against biosensor strain *C. violaceum* ATCC 12472, which was observed by the reduction of purple pigment by the formation of opaque zones of inhibition. These studies suggest that CAB may be promising sources of novel quorum quenching compounds which can also be used in medical and agricultural industries. Previous studies have shown corals *Oculina patagonica* and *P. damicornis* are infected by pathogens *Vibrio shiloi* and *Vibrio coralliilyticus* (Zhou *et al*., 2020). Coral-associated bacteria isolated from different corals demonstrated QSI and were able to disrupt QS of these pathogens moving them away from the coral surface (Certner and Vollmer, 2018; Torres *et al.,* 2019). Experimental studies have attempted to detect QS by coral-associated bacteria and pathogens (Certner and Vollmer, 2018) and they suggest that the interference with QS within the coral holobiont may be an important strategy to regulate the coral microbiome (Bhedi *et al.,* 2017; Ransome *et al.,* 2014). When the coral pathogen *Serratia marcescens* PDL100 was targeted against a coral polyp of *Aipstasia pallida* (Tait *et al.,* 2010), bacterial isolates inside the polyp were able to inhibit QS and prevented the infection by the pathogen. The ability of bacteria to inhibit QS helps control the behaviour and virulence of the bacteria that may affect the health of the coral host and may even hinder the spread of diseases, by Gram-positive and Gram-negative bacterial pathogens (Barragan *et al.,* 2018; Ma *et al.,* 2018).

Various antifouling and antibacterial compounds have been isolated from corals against pathogens (Hou *et al*., 2019; Modolon *et al*., 2020). It is therefore vital to identify potentially beneficial bacteria in corals and to assess their bioactivity potential. Two South African coral species were thus selected for isolation of their associated bacteria to identify their antimicrobial, anti-QS and anti-biofilm potential.

2.2 Materials and Methods

2.2.1 Coral collection and bacteria isolation

Two coral species (*Acropora* and *Pocillopora* spp.) were collected from the intertidal zone along the intertidal zone of KwaZulu-Natal coast (29.4833° S, 31.2333° E) (Fig. 2.1). Coral fragments were collected in separate ziplock bags, stored on ice and transported to the University of KwaZulu-Natal Microbiology lab for processing within 3 h of collection. Coral samples were thoroughly rinsed with natural filter-sterilized seawater to remove all loosely attached bacteria, aseptically dissected using a sterile scalpel and homogenized using a sterilized commercial blender (HGB600). The blender was filled with a diluted sanitizer based on the manufacturer's instructions. Serial dilutions were made using autoclaved seawater and 100 µl aliquots were spread in duplicate (undiluted, 10^{-2} and 10^{-4}) onto selective media: Actinomycetes Isolation Agar (AIA), Casein agar (CA), Enriched Anacker & Ordal's Agar (EAOA), Glycerol Asparagine Agar (GAA), Luria Bertani (LB), Marine Agar (MA), Seawater Yeast Extract (SWYE) and Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) (Pfeffer and Oliver, 2003). All media were prepared using filtered, autoclaved seawater. Three antibiotics were added: cycloheximide (0.02 g/l) and nystatin (0.05 g/l) to inhibit fungal growth, and nalidixic acid (0.02 g/l) to inhibit rapid bacterial growth. Plates were incubated at 30 °C for 5 - 30 d and were observed at regular intervals. Single colonies were purified to obtain pure cultures. Cultures were maintained on agar plates at 4 °C as short-term stocks. Glycerol stocks (20%v/v) were prepared using pure cultures and stored at -80 °C for long-term storage.

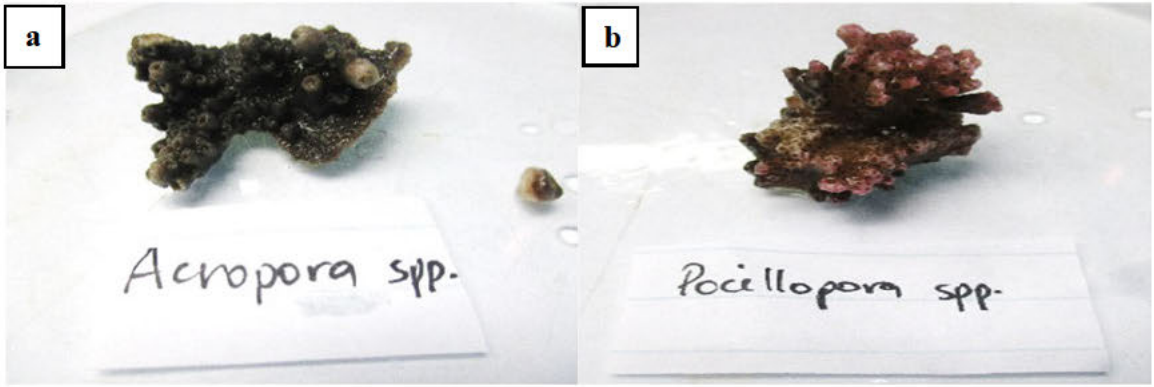


Figure 2.1: Coral samples collected along the KwaZulu-Natal Coast (29.4833° S, 31.2333° E). (a) *Acropora* species and (b) *Pocillopora* species.

2.2.2 Preliminary characterization of bacterial isolates from *Acropora* and *Pocillopora* species

Preliminary characterization was carried out and isolates were differentiated according to colony characteristics, Gram reaction and cellular morphology. Colonies were described based

on their shape, size and pigmentation. To distinguish between Gram-positive and Gram-negative bacteria and their cellular morphology, Gram staining was carried out (Barile, 2012). Results were observed under oil immersion using a Zeiss-Primo star compound light microscope, Plan ACHROMAT. Slides were viewed up to 100x Gram-positive bacteria stain blue or purple due to the presence of a thick layer of peptidoglycan on their cell walls and were either rod-shaped or cocci and Gram-negative bacteria stain pink or red due to their thin layer of peptidoglycan on their cell walls (Barile, 2012).

2.2.3 Primary screening of bacterial isolates of *Acropora* and *Pocillopora* species - Antimicrobial screening using colony picking method

Antimicrobial screening of 210 *Acropora* and 132 *Pocillopora* species bacterial isolates was performed against common indicator organisms, *Pseudomonas aeruginosa* ATTC 27853 and methicillin-resistant *Staphylococcus aureus* ATTC 43300. Screening for antimicrobial activity was carried out using a colony picking technique (Hettiarachchi *et al*., 2017) where pure culture colonies of target coral-associated bacteria and indicator bacteria were prepared by streaking a loopful of stock onto marine-seawater agar plates. Indicator bacteria were spread on LB-seawater agar plates by swabbing, then a pure single target bacterium colony was picked and placed on the inoculated agar plate culture, followed by incubation at 30 °C for 16 - 24 h. In this study, 12 target bacterial isolates were dropped on a single agar plate with a single indicator organism to observe zones of inhibition. Antimicrobial activity was interpreted by observing the presence of zones of inhibition surrounding the target bacteria (Hettiarachchi *et al*., 2017).

2.2.4 Primary screening for AHL-based quorum sensing inhibition (QSI)

The two biosensors used were: *Chromobacterium subtsugae* CV017 to detect short chain (C4-C6) AHL inhibition (Chernin *et al.,* 1998) and *Chromobacterium violaceum* ATCC 12472 to detect long chain (C10+) AHL inhibition (Morohoshi *et al*., 2008). *Chromobacterium violaceum* ATCC 12472 is a long chain *N*-acyl-homoserine lactone (AHL) producer and *C. subtsugae* CV017 is a short chain AHL producer and these AHLs are responsible for the production of the purple pigment, violacein.

Twelve single colonies of target CAB were spotted onto each LB-seawater agar plate and incubated overnight at 30 °C. This assay is a modification of colony picking technique by Hettiarachchi *et al*. (2017). *Chromobacterium violaceum* ATCC 12472 and *C. subtsugae* CV017 were inoculated into LB broth and incubated with 150 rpm agitation on a rotary shaker at 30 °C for 24 h. Thereafter, 150 µl of the respective cultures were inoculated into 5 ml of LB

soft agar and poured over the LB-seawater agar plates with target bacterial isolates and incubated at 30 °C overnight. Plates were viewed using a Porta-Trace/Gagne light box for clear appearance of the QSI zones. Quorum sensing inhibition was observed by loss of purple pigmentation surrounding the target bacteria i.e., the appearance of colorless, opaque, but viable zones around the target bacteria. An observation of growth inhibition or clear zones was indicative of killing by the target bacteria. Quorum quenching *Bacillus cereus* ATCC 14579 was used as a QSI-positive control (Chenia, 2013).

2.2.5 Primary screening for autoinducer-2 inhibition

An autoinducer-2 inhibition assay was also carried against the indicator *Vibrio harveyi* BB120 (Teasdale *et al.,* 2011) using a modification of the Hettiarachchi *et al*. (2017) colony picking technique. *V. harveyi* is a wild-type bioluminescent strain producing the furanosyl borate diester AI-2, which is a universal QS molecule responsible for the regulation of bioluminescence and controlling various physiological pathways (Bassler, 1997; Zhao, 2018).

Twelve single colonies of target bacteria were dropped onto LB-seawater agar plates and incubated at 30 °C for 24 h. *Vibrio harveyi* was inoculated into Marine broth and incubated with 150 rpm agitation on a rotary shaker at 30 °C for 24 h. Thereafter, 150 µl of the *V. harveyi* culture, equivalent to 0.5 McFarland standard was inoculated into Marine soft agar (0.5% w/v) and poured over the LB-seawater agar plates with target bacterial isolates, followed by incubation at 30 °C overnight. Zones of growth inhibition were observed visually, while bioluminescence inhibition was observed by using GeneSys image analyzer (Syngene). AI-2 inhibition was identified by the appearance of dark zones and lack of bioluminescence around the bacterial colonies. Zones of inhibition were measured in mm and recorded. Cinnamaldehyde (10 µg/ml) was used as positive AI-2 inhibitor control (Brackman *et al*., 2008).

2.3 Results

2.3.1 Collection of corals and preliminary screening of bacterial isolates

Two coral specimens, *Acropora* and *Pocillopora* species were collected, weighed and used for bacterial culture isolation. A sum of 342 bacterial isolates were obtained and stocked according to their growth on eight selective media (Tables 2.1 - 2.2).

Table 2.1: The amount (mg) of sample used for isolation of coral-associated bacteria and the number of pure culture bacterial isolates stocked.

Coral species	Weight (mg)	Bacterial isolates stocked
Acropora	42.43	210
Pocillopora	80.26	132

Table 2.2: Number of coral-associated bacteria isolates obtained based on growth on selective media.

Source	AIA	CA	EAOA	GAA	LB	MA	SWYE	TCBS
Acropora	23	8	37	33	47	27	15	20
Pocillopora	20	0	19	16	24	44	9	12

AIA – Actinomycetes Isolation Agar**; CA** – Casein agar**; EAOA** - Enriched Anacker & Ordal's Agar**; GAA** – Glycerol Asparagine Agar**; LB** – Luria Bertani**; MA** – Marine Agar**; SWYE** – Seawater Yeast Extract and **TCBS** – Thiosulfate-Citrate-Bile Salts-Sucrose. All media were prepared using filtered, autoclaved seawater.

2.3.2 Colony morphology, Gram stains and cellular morphology results of the *Acropora* and *Pocillopora* isolates

Bacterial isolates obtained from the *Acropora* and *Pocillopora* species showed different colony characteristics on the different media used for isolation. Some of the colony surfaces appeared glossy and smooth, whilst some were wrinkled, characteristic of actinomycetes that are spore-forming in nature (Suppl. Tables S2.1-S2.2). They differed in colony pigmentation as some appeared brown, cream, orange, pink, white, yellow and were either translucent, opaque or dark as can be seen in Fig. 2.2.

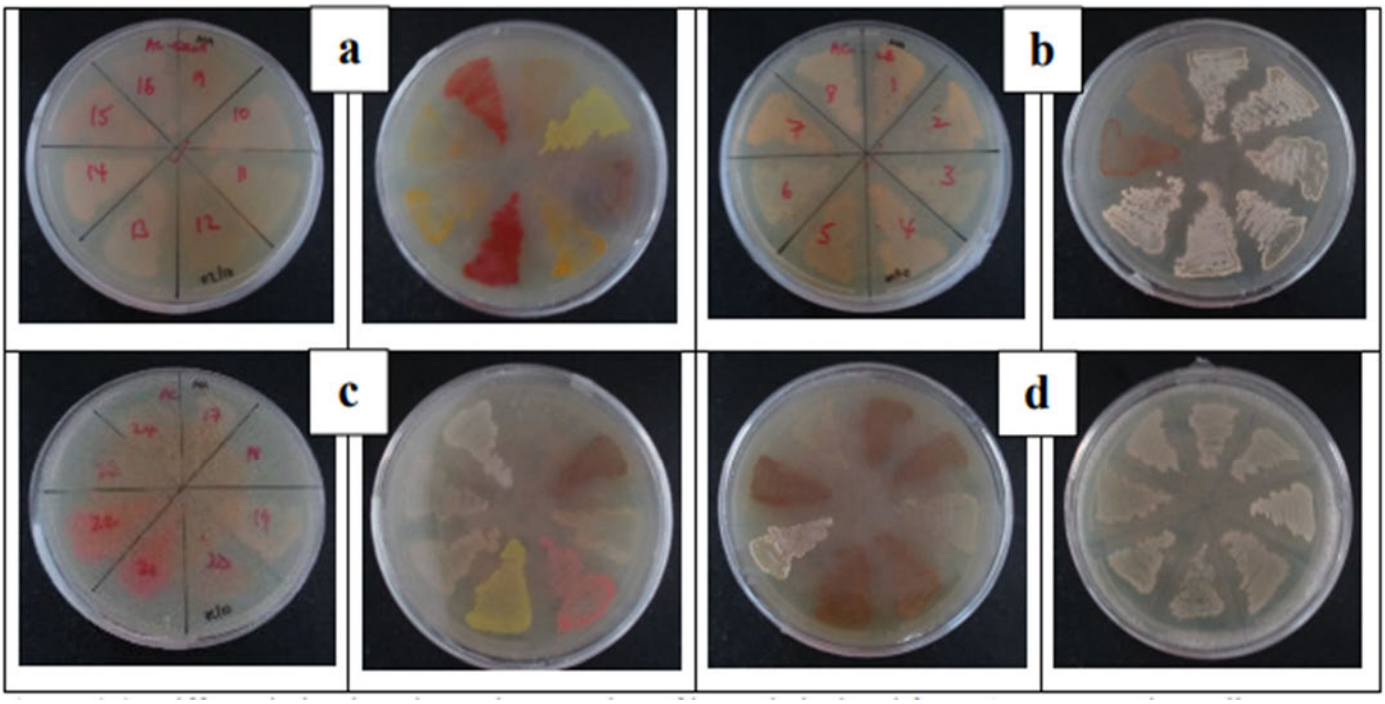


Figure 2.2: Differentiation in colony pigmentation of bacteria isolated from *Acropora* and *Pocillopora* species ranging from (a) – (d) brown, cream, orange, pink, white, yellow and were either translucent, opaque or dark. Some of the colony surfaces appeared glossy and smooth, whereas some were wrinkled characteristic of (b) spore-forming actinomycetes.

A total of 210 *Acropora* spp. bacterial isolates were characterized by Gram staining and light microscopy. Gram stain results of *Acropora* species bacterial isolates showed that 33.80% (71/221) were Gram-negative (Fig. 2.3; Suppl. Table S1 and Suppl. Fig. S1**)** and 71.42% (150/221) were Gram-positive. Moreover, a total of 92.85% (195/221) were rod-shaped, either in chains or scattered, and 8% (16/221) were cocci arranged in clusters or singly.

A total of 132 *Pocillopora* spp. bacterial isolates were characterized by Gram staining and light microscopy. Gram stain results of *Pocillopora* isolates showed that 49.24% (65/134) were Gram-negative (Fig. 2.4, Suppl. Table S2 and Fig. S2**)** and 52,27% (69/134) were Gram-positive. Moreover, a total of 77% (102/134) were filamentous and rod-shaped, either in chains or scattered, and 23% (30/134) were cocci arranged in clusters or singly.

Table 2.3 summarises the Gram-stain and cellular morphology characteristics of isolates obtained from the *Acropora* and *Pocillopora* species Isolates from both *Acropora* and *Pocillopora* were predominantly Gram-positive rather than Gram-negative and rod-shaped bacteria were more prevalent compared to cocci.

Table 2.3: Summary of Gram stain and cell morphology of bacteria isolated from *Acropora* and *Pocillopora* species.

Gram stain and cell morphology	*Acropora* bacteria	*Pocillopora* bacteria
Gram-negative rods	51	44
Gram-negative cocci	8	21
Gram-positive rods	153	65
Gram-positive cocci	9	4
Total	**221**	**134**

2.3.3 Primary screening of *Acropora* and *Pocillopora* bacterial isolates against clinical indicator organisms.

2.3.3.1 Antimicrobial screening using picking technique.

Primary screening for antimicrobial activity of *Acropora* and *Pocillopora* bacterial isolates was carried out against *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 43300. Antimicrobial activity against test organisms *P. aeruginosa* and *S. aureus* was observed by the presence of a clear zone around the target bacteria, suggesting their potential as antimicrobial compound producers. For both *Acropora* and *Pocillopora* bacterial isolates, marked antimicrobial activity was shown against *S. aureus* particularly by isolates EAOA, LB and GAA media (Figs. 2.5-2.6).

For *Acropora*-associated bacteria, 2.85% (6/221) of isolates had activity against *P. aeruginosa,* while 20.47% (43/221) had activity against *S. aureus*, with only 1.90% (4/210) demonstrating activity against both strains (Table 2.4; Suppl. Fig. S2.3). For *Pocillopora*-associated isolates, 10.60% (14/134) showed antimicrobial activity against *P. aeruginosa*, 37.12% (49/134) showed activity against *S. aureus*, while 4.09% (14/134) showed activity against both indicator strains (Table 2.5; Suppl. Fig. S2.4).

2.3.3.2 Primary screening for Gram-negative quorum sensing inhibition

Primary screening of Gram-negative quorum sensing inhibition (Suppl. Figs. S5-S6) was carried out using *C. violaceum* 12472 (long chain AHL-producing strain) and *C. subtsugae* CV017 (short chain AHL-producing strain). Quorum sensing inhibition against test organism *C. violaceum* ATCC 12472 and *C. subtsugae* CV017 was observed by the presence of an opaque halo around the target bacteria as a result of loss of the purple pigmentation, violacein. Killing was also observed by the appearance of a clear zone around the target bacteria.

Acropora species bacterial isolates showed QSI and killing against both *C. subtsugae* CV017 and *C. violaceum* ATCC 12472 in comparison to *Pocillopora* bacterial isolates which only showed killing and no QSI (Figs. 2.7-2.8; Suppl. Figs. S5-S6). For *Acropora* bacterial isolates, 3.81% (8/221) of isolates showed QSI and 3.81% (8/221) showed killing against short-chain AHL-producing *C. subtsugae* CV017*,* 2.86% (6/210) showed QSI and 3.33% (6/221) showed killing against long chain AHL-producing *C. violaceum* ATCC 12472.

For *Pocillopora* species bacterial isolates, 6.06% (8/132) showed killing against *C. subtsugae* CV017 and 6.06% (8/134) killing against *C. violaceum* ATCC 12472 and 0.75% (1/134) had QSI activity against *C. violaceum* ATCC 12472. Isolates (L17-L25) showed killing and no QSI against both *C. subtsugae* CV017 and *C. violaceum* ATCC 12472 (Table 2.5). Against both strains, 3 isolates demonstrated QSI activity (Table 2.4).

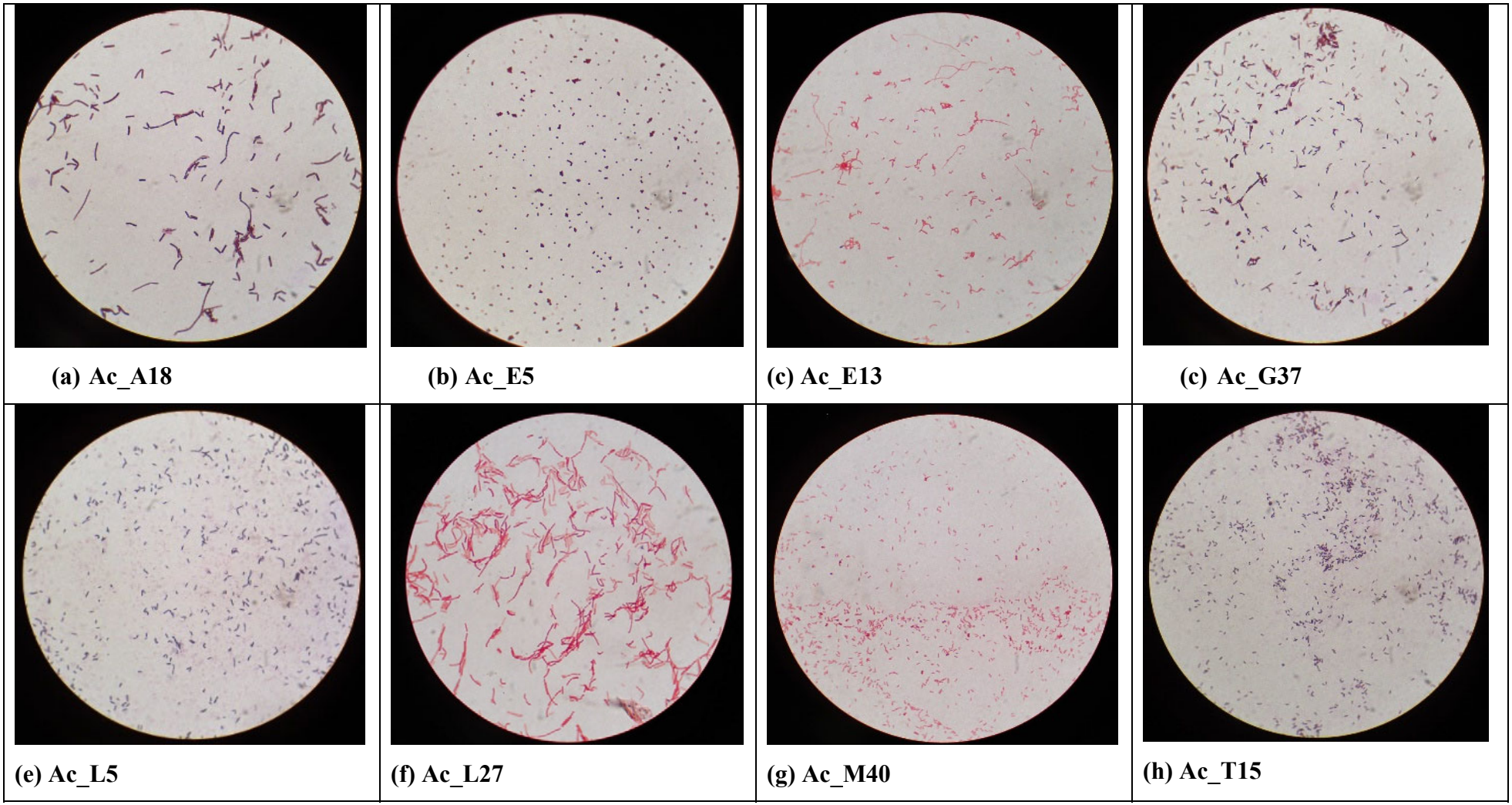


Figure 2.3: Gram stain results of selected *Acropora* bacterial isolates; (a) Ac_A18, (b) Ac_E5, (c) Ac_E13, (d) Ac_G37, (e) Ac_L5, (f) Ac_L27, (g) Ac_M40 and (h) Ac_T15.

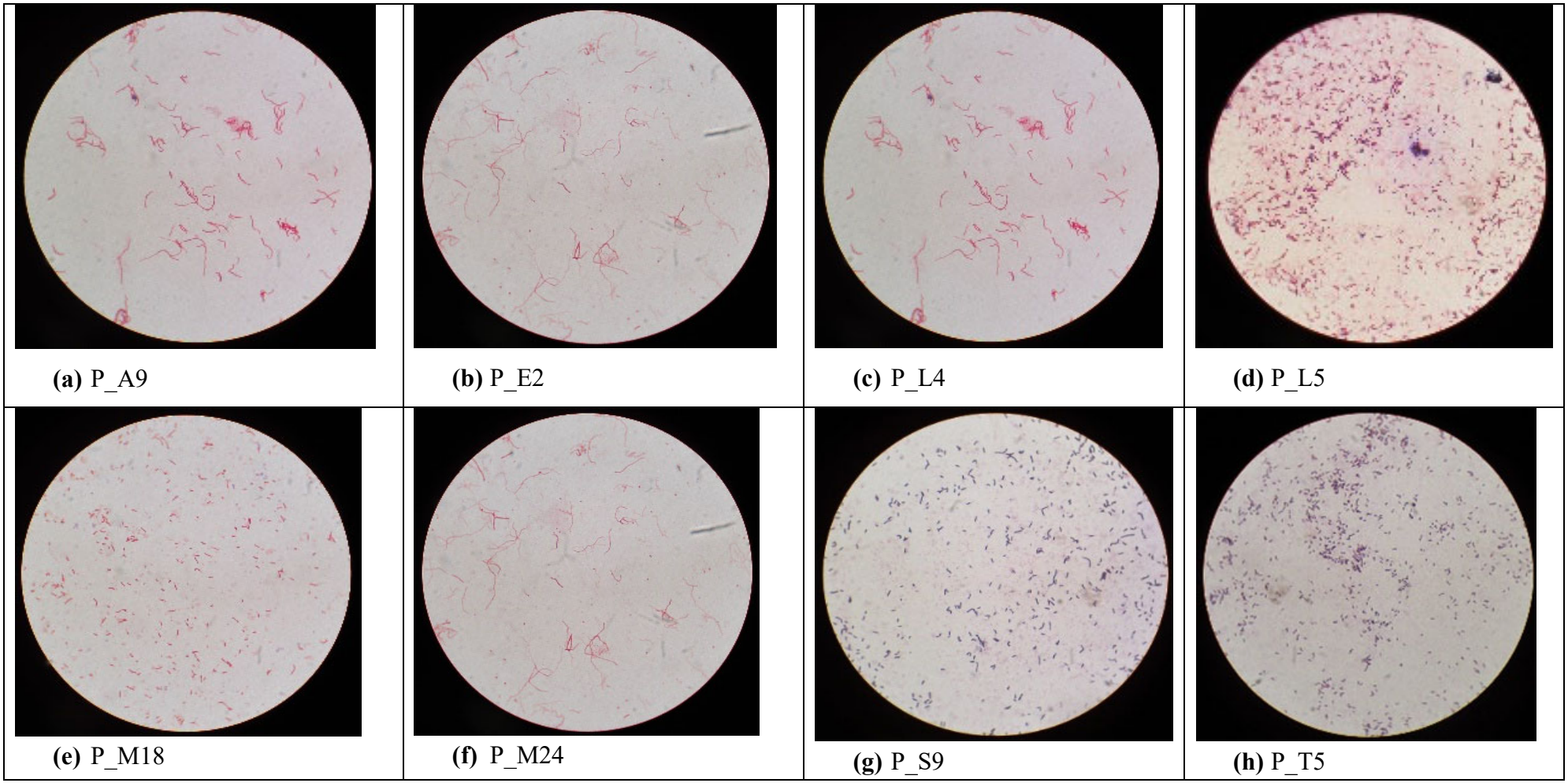
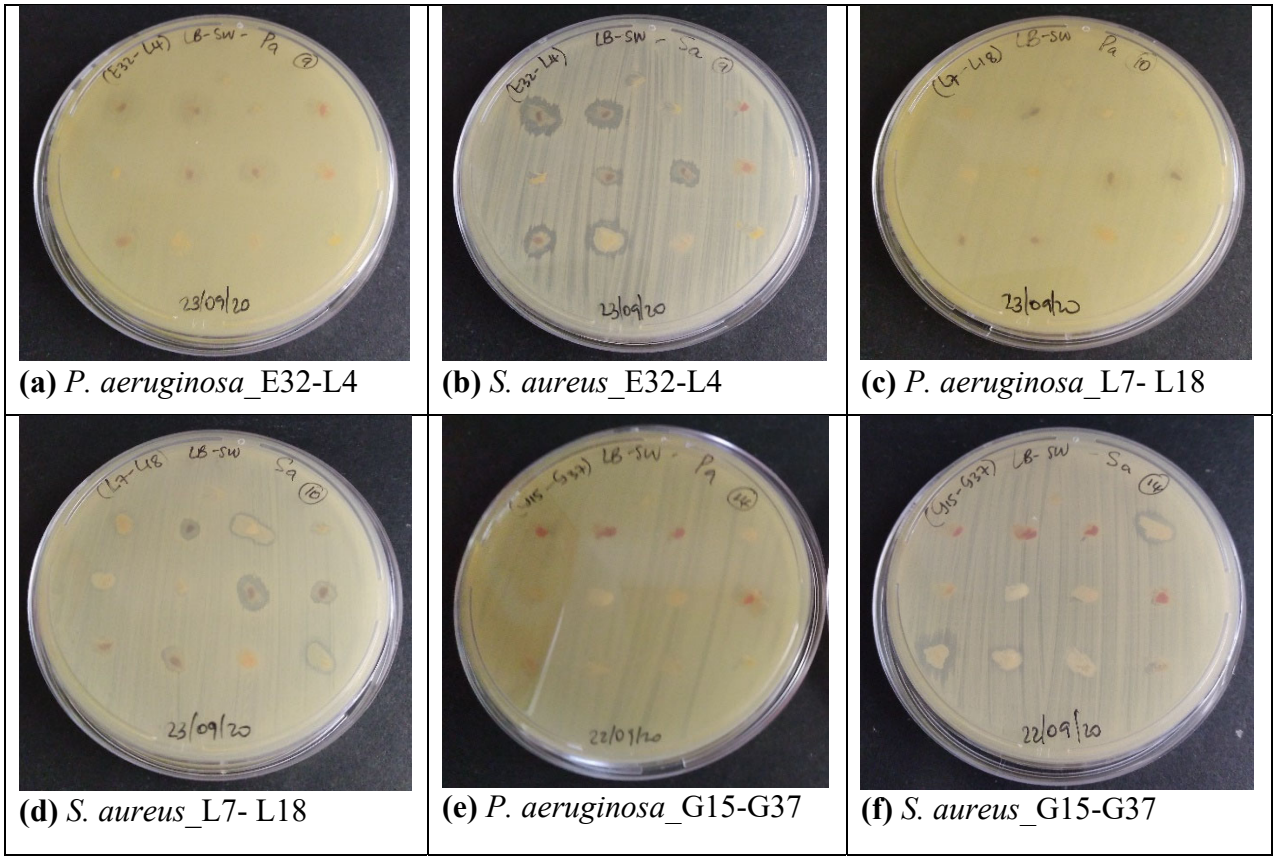


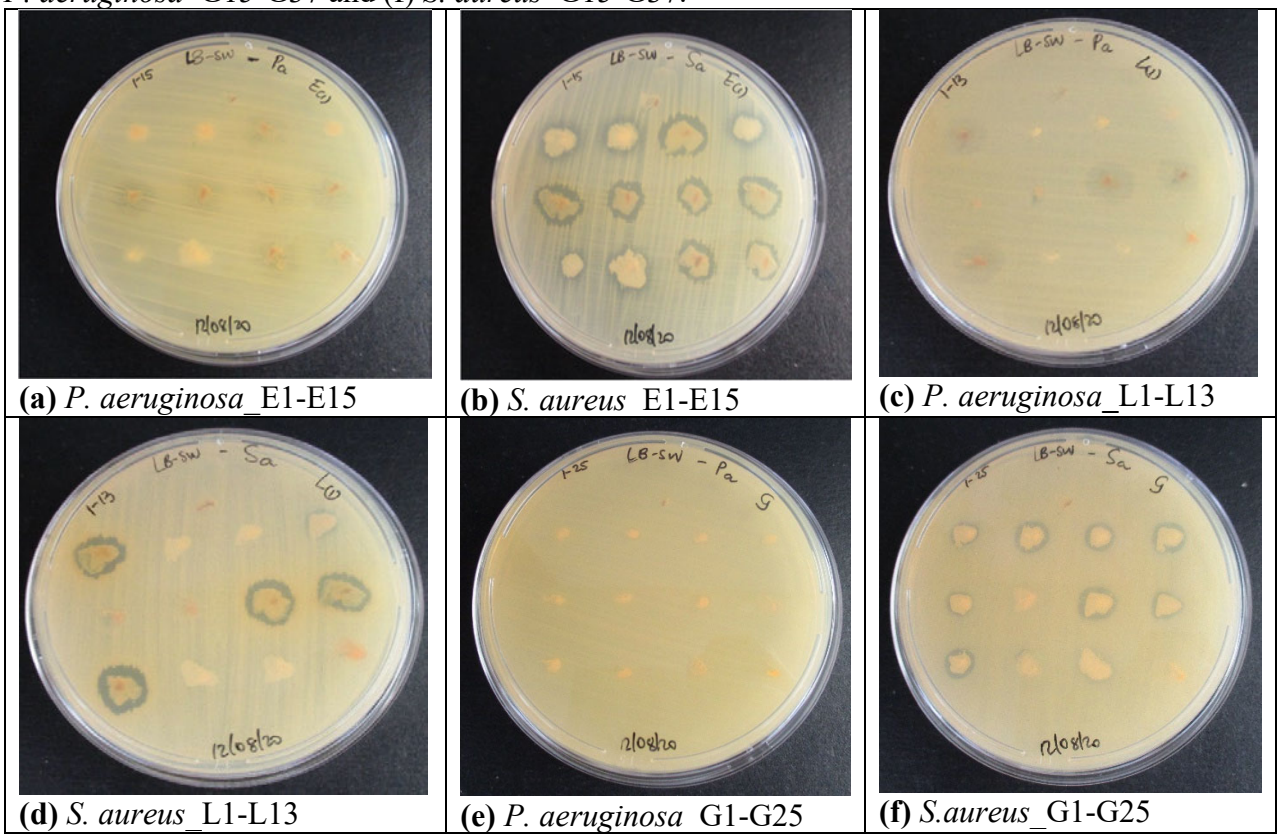
Figure 2.4: Gram stain results of selected *Pocillopora* bacterial isolates; (a) P_A9, (b) P_E2, (c) P_L4, (d) P_L5, (e) P_M18, (f) P_M24, (g) P_S9 and (h) P_T.



(a) *P. aeruginosa*_E32-L4 **(b)** *S. aureus*_E32-L4 **(c)** *P. aeruginosa*_L7- L18

(d) *S. aureus*_L7- L18 **(e)** *P. aeruginosa*_G15-G37 **(f)** *S. aureus*_G15-G37

Figure 2.5: Primary antimicrobial screening of *Acropora* bacterial isolates: (a) *P. aeruginosa*_E32-L4, (b) *S. aureus*_E32-L4, (c) *P. aeruginosa*_L7-L18, (d) *S. aureus*_L7-L18, (e) *P. aeruginosa* G15-G37 and (f) *S. aureus* G15-G37.



(a) *P. aeruginosa*_E1-E15 **(b)** *S. aureus* E1-E15 **(c)** *P. aeruginosa*_L1-L13

(d) *S. aureus*_L1-L13 **(e)** *P. aeruginosa* G1-G25 **(f)** *S.aureus*_G1-G25

Figure 2.6: Primary antimicrobial screening of *Pocillopora* bacterial isolates: (a) *P. aeruginosa*_E1-E15, (b) *S. aureus*_E1-E15, (c) *P. aeruginosa*_L1-L13, (d) *S. aureus*_L1-L13, (e) *P. aeruginosa*_G1-G25 and (f) *S. aureus*_G1-G25.

Table 2.4: Primary antimicrobial screening, Gram-negative quorum sensing inhibition and autoinducer-2 inhibition by *Acropora* bacterial isolates against indicator organisms.

Isolate Code	Inhibition and killing zone diameter (mm)				
	Pseudomonas aeruginosa ATCC 27853	***Staphylococcus aureus*** ATTC 43300	***Chromobacterium subtsugae*** CV017	***Chromobacterium violaceum*** ATCC 12472	***Vibrio harveyi*** BB120
Ac_A3	0	6	0	0	0
Ac_A4	0	5	0	0	0
Ac_A5	0	9	0	0	10
Ac_A6	0	9	0	0	0
Ac_A12	0	9	0	0	0
Ac_A15	0	10	0	0	0
Ac_A17	0	10	0	0	0
Ac_A18	0	12	0	0	0
Ac_A19	0	11	0	0	0
Ac_A20	0	15	0	0	0
Ac_A21	0	14	0	0	0
Ac_E2	0	9	0	0	0
Ac_E3	0	0	0	0	0
Ac_E5	0	13	0	0	10
Ac_E13	0	0	14	10	0
Ac_E14	0	10	0	0	0
Ac_E17	0	13	0	0	0
Ac_E19	0	17	0	0	0
Ac_E21	0	0	2*	1*	0
Ac_E23	0	0	2*	1*	9
Ac_E26	0	0	0	0	8
Ac_E28	0	0	0	0	8
Ac-E32	0	0	0	0	8
Ac_E34	0	0	0	0	8
Ac_G36	0	0	2*	0	9
Ac_G37	0	13	13	0	9
Ac_G38	0	10	0	0	0
Ac_G39	0	8	0	0	8
Ac_G56	0	13	0	0	0
Ac_G57	0	12	0	0	10
Ac_L4	0	10	0	0	0
Ac_L5	10	8	0	0	0
Ac_L7	0	10	0	0	9
Ac_L8	0	14	0	0	0
Ac_L9	6	6	0	0	0
Ac_L10	0	5	0	0	0
Ac_L12	10	10	0	0	0
Ac_L15	0	8	3	4	8
Ac_L17	0	8	0	0	0
Ac_L23	0	0	0	0	9
Ac_L26	0	0	0	0	8
Ac_L27	0	10	0	0	0
Ac_L2	0	6	0	0	8
Ac_L29	0	0	0	0	9

Ac_L3	0	0	0	0	11
Ac_L34	0	0	0	0	10
Ac_L35	0	7	0	0	0
Ac_L36	0	10	0	0	9
Ac_L38	0	0	7	0	12
Ac_L39	12	0	1	0	0
Ac_L41	9	14	0	0	0
Ac_L4	19	0	0	2	0
Ac_M7	0	0	0	0	10
Ac_M25	0	0	0	0	9
Ac_M40	0	8	0	0	0
Ac_M42	0	0	0	0	8
Ac_M62	0	0	0	0	9
Ac_M64	0	0	0	0	9
Ac_M6	0	9	0	0	0
Ac_M73	0	0	0	0	10
Ac_M74	0	0	0	0	11
Ac_S5	0	0	1*	1*	0
Ac_S6	0	0	1*	1*	0
Ac_S8	0	0	1*	1*	0
Ac_S9	0	0	1*	1*	0
Ac_S12	0	0	0	0	0
Ac_S13	0	0	1*	1*	0
Ac_S14	0	8	0	0	0
Ac_S15	0	7	0	0	0
Ac_S16	0	7	0	0	0
Ac_T1	0	0	0	0	9
Ac_T4	0	7	0	0	13
Ac-T8	0	0	0	0	13
Ac_T10	0	0	0	0	8
Ac-T10	0	0	0	0	9
Ac_T11	0	0	0	0	9
Ac_T13	0	6	0	0	13
Ac_T15	0	0	5	6	0
Ac_T16	0	9	2	4	0
Ac_T19	0	0	1	2	0

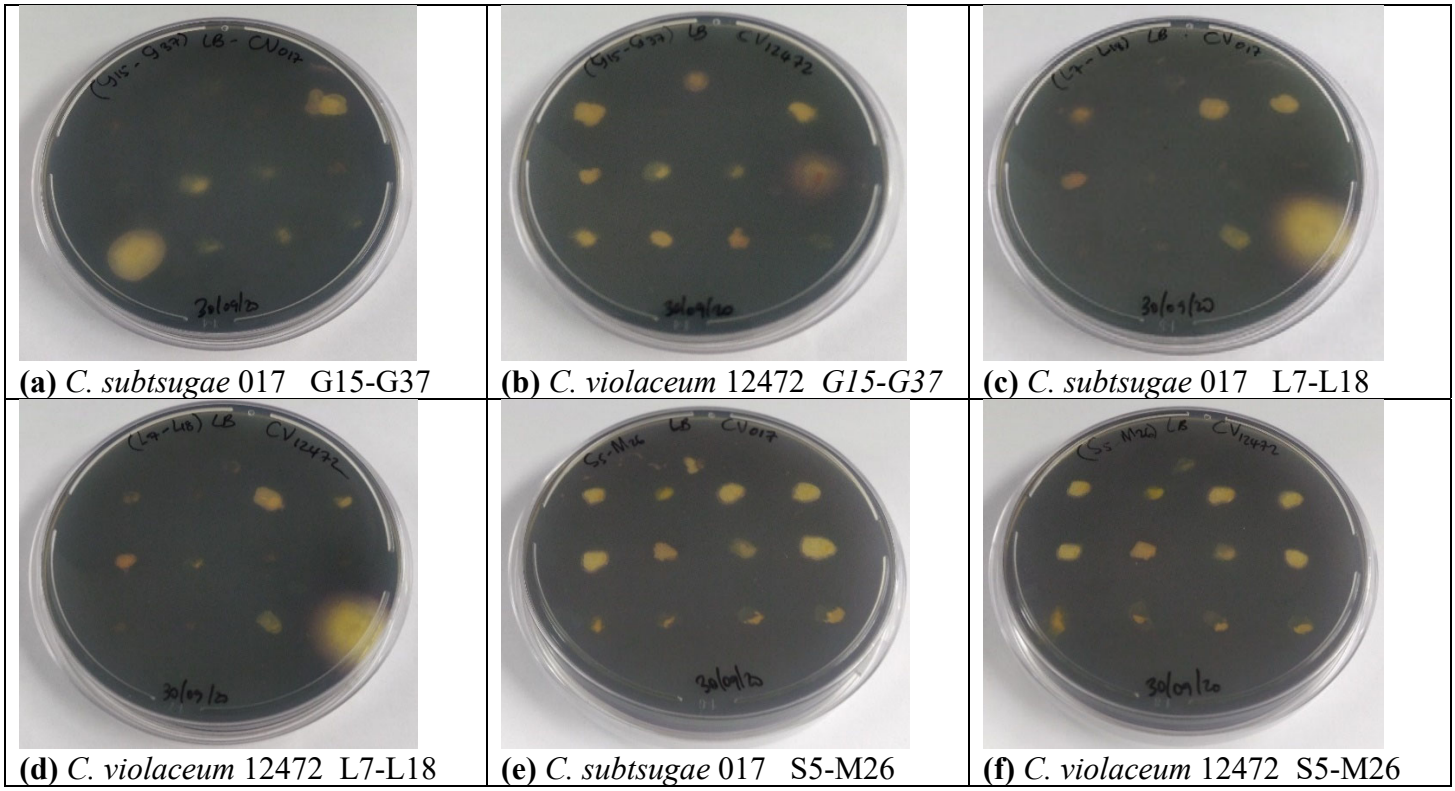
*Killing – transluscent inhibition zone
**P. aeruginosa: S. aureus* – inhibition zone diameters
**C. subtsugae: C. violaceum* – QS inhibition – opaque halos zone diameter
**V. harveyi* – bioluminescence inhibition zones diameter

Table 2.5: Primary antimicrobial screening, Gram-negative quorum sensing inhibition and autoinducer-2 inhibition by *Pocillopora* bacterial isolates against indicator organisms.

Isolate Code	Inhibition and killing zone diameter (mm)				
	Pseudomonas aeruginosa ATCC 27853	***Staphylococcus aureus*** ATTC 43300	***Chromobacterium subtsugae*** CV017	***Chromobacterium violaceum*** ATCC 12472	***Vibrio harveyi*** BB120
P_A1	0	6	0	0	0
P_A2	0	8	0	0	0
P_A6	0	9	0	0	0
P_A7	0	7	0	0	0
P_A8	0	9	0	0	0
P_A9	0	11	0	0	0
P_A10	0	7	0	0	0
P_E1	0	9	0	0	0
P_E2	12	14	0	0	0
P_E3	0	10	0	0	0
P_E5	0	10	0	7	7
P_E6	12	12	0	0	0
P_E7	13	11	0	0	0
P_E8	11	11	0	0	0
P_E9	10	13	0	0	0
P_E10	11	9	0	0	0
P_E12	14	11	0	0	0
P_E13	0	11	0	0	0
P_E15	0	7	0	0	0
P_E20	0	10	0	0	0
P_E22	0	9	0	0	0
P_G1	0	9	0	0	0
P_G3	0	8	0	0	0
P_G4	0	9	0	0	0
P_G10	0	9	0	0	0
P_G11	0	8	0	0	0
P_G13	0	10	0	0	0
P_G16	0	8	0	0	0
P_L4	11	13	0	0	0
P_L5	9	13	0	0	0
P_L6	11	0	0	0	0
P_L12	12	12	0	0	0
P_L14	0	10	0	0	0
P_L17	0	0	3*	3*	3
P_L18	0	0	2*	2*	2
P_L19	0	0	2*	2*	2
P_L20	0	0	3*	2*	2
P_L21	0	0	4*	5*	5
P_L22	0	0	0	2*	2
P_L23	0	0	4*	2*	2
P_L24	0	0	2*	2*	2
P_L25	0	0	2*	0	0
P_M18	11	10	0	0	0
P_M19	13	11	0	0	0

P_M23	9	11	0	0	0
P_M24	13	14	0	0	0
P_M45	0	5	0	0	0
P_M50	0	5	0	0	0
P_M51	0	6	0	0	0
P_S8	0	6	0	0	0
P_S9	0	9	0	0	0
P_T1	0	18	0	0	0
P_T2	0	16	0	0	0
P_T3	0	22	0	0	0
P_T4	0	11	0	0	0
P_T5	0	23	0	0	0
P_T6	0	13	0	0	0
P_T7	0	22	0	0	0
P_T8	0	14	0	0	0

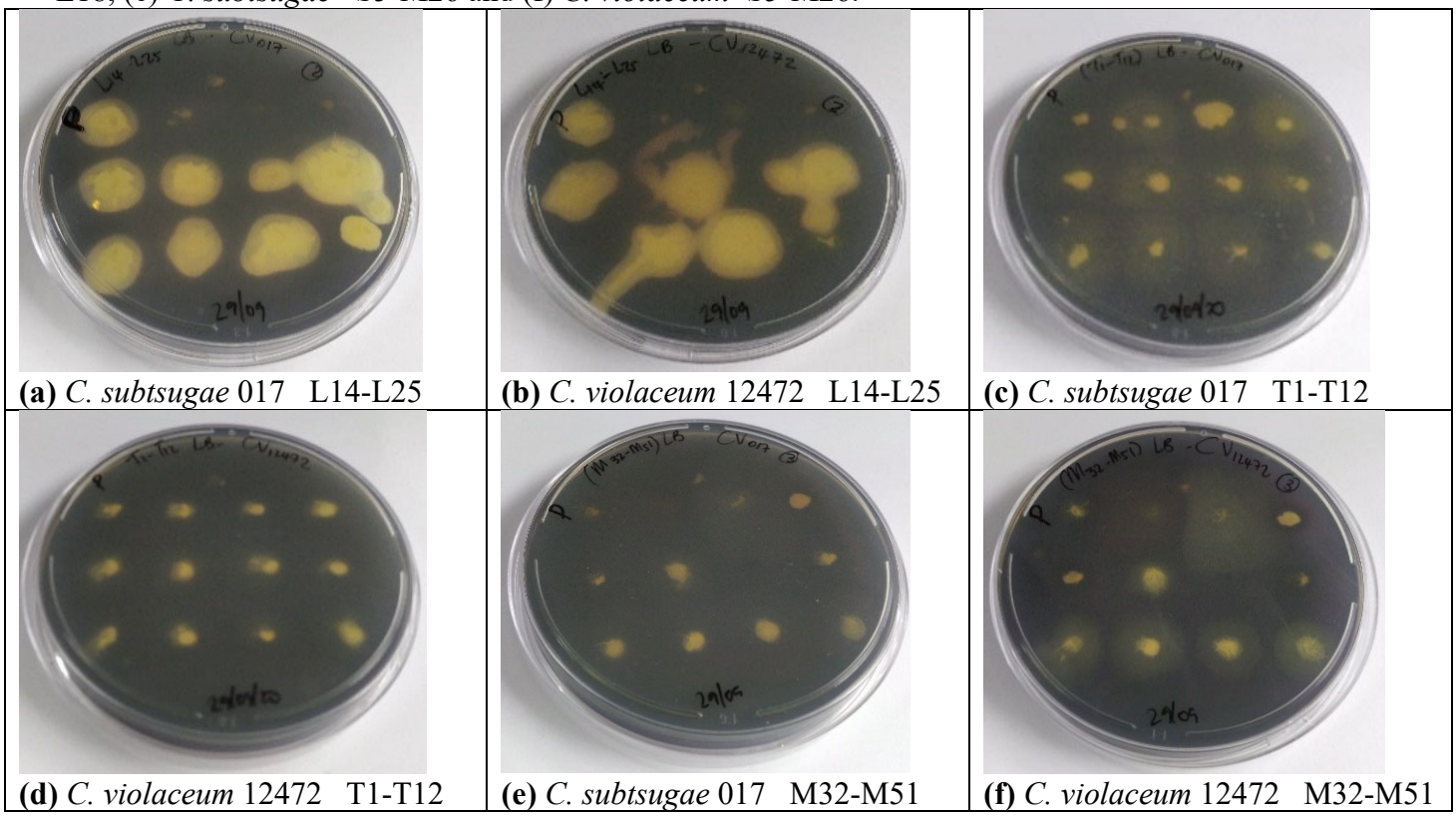
*Killing – transluscent inhibition zone
**P. aeruginosa: S. aureus* – inhibition zone diameters
**C. subtsugae: C. violaceum* – QS inhibition – opaque halos zone diameter
**V. harveyi* – bioluminescence inhibition zones diameter



(a) *C. subtsugae* 017 G15-G37 **(b)** *C. violaceum* 12472 *G15-G37* **(c)** *C. subtsugae* 017 L7-L18

(d) *C. violaceum* 12472 L7-L18 **(e)** *C. subtsugae* 017 S5-M26 **(f)** *C. violaceum* 12472 S5-M26

Figure 2.7: Gram-negative QS inhibition screening of *Acropora* bacterial isolates against *Chromobacterium violaceum* ATCC 12472 and *Chromobacterium subtsugae* CV017: (a) *C. subtsugae*_G15-G37 (b) *C. violaceum*_G15-G37, (c) *C. subtsugae*_L7-L18, (d) *C. violaceum*_L7-L18, (e) C. *subtsugae* S5-M26 and (f) *C. violaceum* S5-M26.



(a) *C. subtsugae* 017 L14-L25 **(b)** *C. violaceum* 12472 L14-L25 **(c)** *C. subtsugae* 017 T1-T12

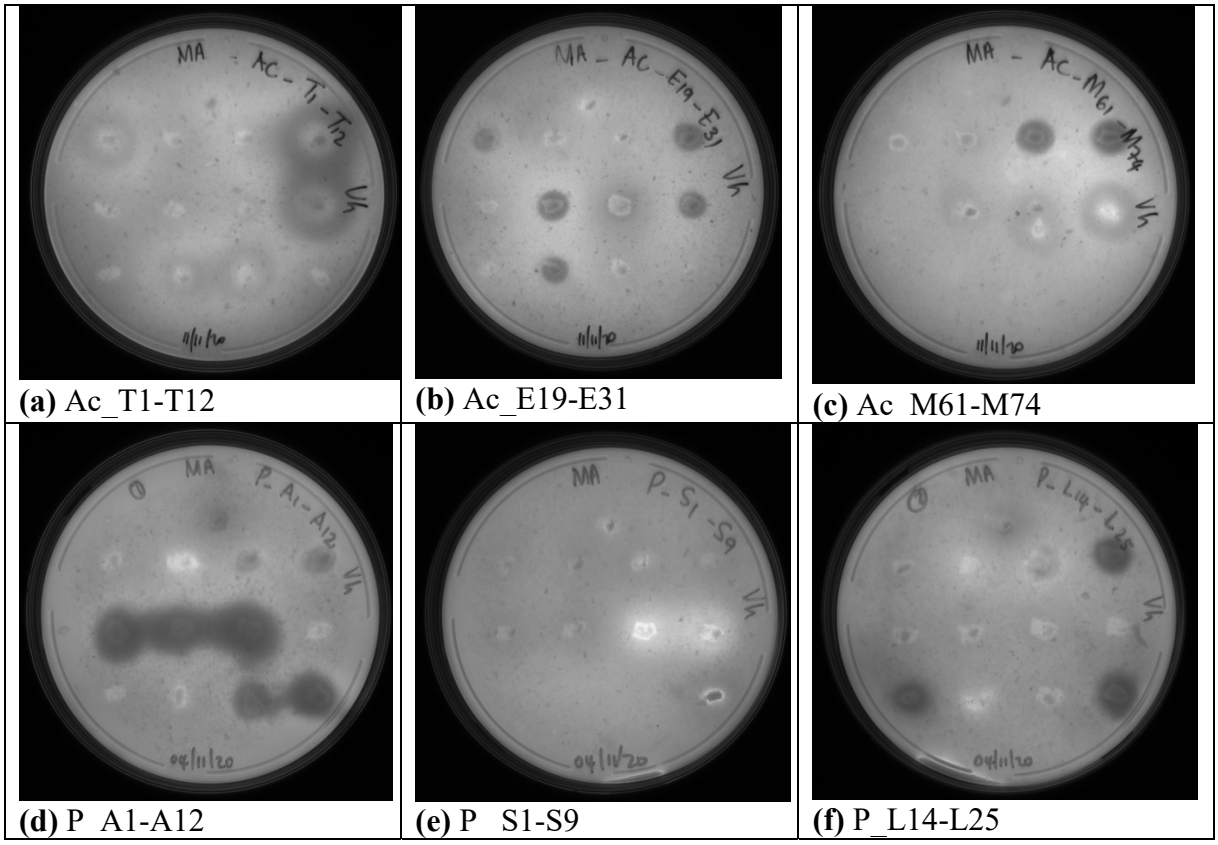
(d) *C. violaceum* 12472 T1-T12 **(e)** *C. subtsugae* 017 M32-M51 **(f)** *C. violaceum* 12472 M32-M51

Figure 2.8: Gram-negative QS inhibition screening of *Pocillopora* bacterial isolates against *Chromobacterium violaceum* ATCC 12472 and C*hromobacterium subtsugae* CV017: (a) *C. subtsugae*_L14-L25 (b) *C. violaceum*_L14-L25; (c) *C. subtsugae* _T1-T12, (d) *C. violaceum*T1-T12, (e) C. *subtsugae*_M32-M51and (f) *C. violaceum*_M32-M51.

2.3.3.3 Primary screening for autoinducer-2 inhibition

The autoinducer-2 inhibition assay was carried out against the indicator *V. harveyi* BB120 for the detection of bioluminescence inhibition. Zones of AI-2 inhibition were indicated by a dark halo signifying absence of bioluminescence, while growth inhibition was indicated by translucent zones around bacterial colonies.

Bioluminescence inhibition was observed for 16.66% (35/221) of *Acropora* bacterial isolates (Fig. 2.9; Table 2.4; Suppl. Fig. S2.7), while 6.81% (9/134) of *Pocillopora* bacterial isolates demonstrated AI-2 inhibition. However, an interesting feature for *Pocillopora* isolates such as P_S7 and P_S8 (Fig. 2.9 (e); Table 2.5; Suppl. Fig. S2.8**)** formed a white halo around the isolates and bioluminescence appeared to be stimulated rather than inhibited. *Acropora* isolates demonstrated less inhibition activity on clinical isolates *P. aeruginosa* and *S. aureus* compared to *Pocillopora* isolates (Table 2.6). Against *C. subtsugae* and *C. violaceum. Acropora* spp. isolates exhibited more QSI activity compared to *Pocillopora* isolates. *Acropora* isolates exhibited more AI-2 inhibition compared to *Pocilloropa* isolates (Table 2.6).



(a) Ac_T1-T12 **(b)** Ac_E19-E31 **(c)** Ac M61-M74

(d) P A1-A12 **(e)** P S1-S9 **(f)** P_L14-L25

Figure 2.9: Screening for AI-2 inhibition of *Acropora* and *Pocillopora* bacterial isolates as indicated by inhibition of *Vibrio harveyi* BB120 bioluminescence. (a) Ac_T1-T12, (b) Ac_E19-E31, (c) Ac_M61-M74 and (d) P_A1-A12, (e) P_ S1-S9, (f) P_L14-L25.

Table 2.6: Comparison of inhibitory activity between *Acropora* and *Pocillopora* isolates exhibiting bioactivity against indicator organisms *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 43300, *Chromobacterium violaceum* ATCC 12472, *Chromobacterium subtsugae* CV017 and *Vibrio harveyi* BB120.

Indicator organism	*Acropora* isolates	*Pocillopora* isolates
***Pseudomonas aeruginosa* ATCC 27853 inhibition**	2.85% (6/221)	11.36% (15/134)
***Staphylococcus aureus* ATCC 43300 inhibition**	20.47% (43/221)	37.12% (49/134)
***Chromobacterium subtsugae* CV017 QSI**	3.81% (8/221)	0% (0/134)
***Chromobacterium violaceum* ATCC 12472 QSI**	2.86% (6/221)	0.75% (1/134)
***Vibrio harveyi* BB120 AI-2 inhibition**	16.66% (35/221)	6.81% (9/134)

2.4 Discussion

The marine environment has received great attention recently as microorganisms associated with marine invertebrates, particularly corals, with CAB exhibiting bioactive compounds (Hou *et al*., 2019). These invertebrates are suggested to be a novel source of new natural compounds with pharmaceutical and environmental importance (Peixoto *et al*., 2021). Coral-associated bacteria are known as an excellent source of secondary metabolite production due to their ability to produce a wide range of bioactive compounds with antimicrobial, antiviral, anti-quorum sensing and anti-biofilm potential (Miura *et al*., 2019; Sang *et al*., 2019). These compounds, therefore, are suggested to be a promising strategy to combat the current resistance of bacterial communities against traditional antibiotics (Glasl *et al.,* 2019). This study aimed to isolate associated with two intertidal zone corals, i.e., *Acropora* and *Pocillopora* species and their antimicrobial, anti-quorum sensing and AI-2 inhibitory activities.

Preliminary characterization of the two coral specimens collected, *Acropora* and *Pocillopora* spp., following the bacterial isolation was carried out. Different media were used to culture these isolates and they displayed a variety of characteristics including colony color, morphology and texture. The results demonstrated that different bacteria are media-dependent with *Acropora* species isolates yielding the highest on Luria-Bertani media and *Pocillopora* spp. isolates yielding the highest growth on Marine agar (Table 2.2). This is in line with a study by Mahmoud and Kalendar (2016) who reported the culturability of CAB from different genera such as *Arthrobacter, Microbacterium, Micrococcus, Micromonospora, Nocardia, Renibacterium, Rhodococcus* and *Streptomyces* species. This study by Mahmoud and Kalendar (2016) compared bacterial isolates obtained from three coral hosts, *Coscinaraea columna*, *Platygyra daedalea* and *Porites harrisoni*. These isolates were cultured on three different media M4, R2A and SCA. M4 medium yielded the highest numbers and diversity of isolates, whereas the R2A medium produced the second-highest numbers and diversity, and

the SCA medium yielded the lowest numbers. Nithyanand *et al*. (2011) reported the isolation of actinomycetes from *Acropora digitifera* and that Actinobacteria members can be isolated using various isolation media as well as low nutrient media.

Gram stains of the cultured bacterial isolates were obtained and a trend of Gram-positive culturable bacteria being predominant was observed in both *Acropora* and *Pocillopora* species bacterial isolates (Figs. 2.2-2.3; Suppl. Tables S2.1-S2.2) with less Gram-negative bacteria. This could be due to the isolation media used, growth, etc., for example excluding nalidixic acid may have resulted in more Gram-negative isolates, as well as incubating at 37°C could have also changed the type of isolated obtained. For both *Acropora* and *Pocillopora* bacterial isolates, Gram-positive rods appeared to predominate, followed by Gram-negative rods and Gram-positive and Gram-negative cocci (Suppl. Tables S2.1-S2.2). This is in line with studies by Li *et al*. (2014) and Mahmoud and Kalendar (2016) who reported Gram-positive actinobacteria to be predominant residents in coral hosts. In contrast, Raina *et al.* (2016) isolated culturable bacteria from coral species *Pocillopora damicornis, Acropora millepora* and *Montipora aequituberculata* and found Gram-negative genera, *Pseudoalteromonas, Pseudomonas*, and the *Roseobacter* clade to have dominant association with these corals compared to Gram-positive species. This may be as a result of their role of fixing dissolved nitrogen, which is a vital process in coral reefs (Lema *et al*., 2012). The *Roseobacter*, *Pseudomonas* and *Oceanospirillales* are also suspected to play a role in structuring coral-associated bacterial communities or brcause culturability is also influenced by the isolation process and procedures (Raina *et al*., 2016).

Primary screening for antimicrobial activity of *Acropora* and *Pocillopora* isolates was carried out against *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 43300 using a colony picking technique (Hettiarachchi *et al*., 2017). Both *Acropora* and *Pocillopora* bacterial isolates exhibited activity against *S. aureus* particularly by organisms cultured from EAOA, LB and GAA media (Fig. 2.2). Ritchie (2006) reported on bacterial isolates from *Acropora palmata* demonstrated that 20% (155/776) of these isolates had antibiotic activity against different strains including coral pathogens *Vibrio* and *Serratia* species under normal conditions. These CAB demonstrated more antimicrobial activity against *S. aureus* compared to *P. aeruginosa* for both *Acropora* and *Pocillopora* bacterial isolates. This could be due to the direct interaction between the indicator and target bacteria which may have enhanced the activation of cryptic genes which are responsible for producing metabolites (Ahila *et al*., 2017; Hettiarachchi *et al*., 2017; Sang *et., al.,* 2019; Shnit-Orland and Kushmaro, 2012). This could also be because *S. aureus* is Gram-positive and Gram-positive bacteria have

a thick peptidoglycan cell wall and lack an outer membrane which makes them more susceptible to antibiotics, whereas, Gram-negative bacteria contain a thin peptidoglycan cell wall and have an outer membrane which makes them less susceptible to antibiotics (Shnit-Orland *et al.,* 2012).

Coral-associated bacteria use QS to regulate gene expression when shifts occur in the availability of cells due to factors such as increased temperatures (Charlesworth *et al*., 2019; Tait *et al.,* 2010). Primary screening of *Acropora* and *Pocillopora* bacterial isolates was carried out against *C. violaceum* ATCC 12472 and *C. subtsugae* CV017 for quorum sensing inhibition. A low number of isolates demonstrated a QSI potential by inhibiting the production of violacein around the bacteria. This is in line with a study by Ma *et al.* (2018) which screened CAB isolated from *P. damicornis* for their ability to inhibit QS of biosensor strain *C. violaceum* ATCC 12472. Their results demonstrated 15% (30 isolates) of the isolated bacteria exhibited anti-QS activity, against the indicator strain by the formation opaque zones of inhibition through the reduction of purple pigment. Bussetti *et al.* (2015) reported that 26 CAB were screened for the production of QSIs against the Gram-negative AHL-based reporter strains *C. violaceum* ATCC 12472, and *C. violaceum* CV026. Nine isolates (34.61%) strongly inhibited QS-dependent violacein production by both biosensors. However, a study by El-Kurdi *et al.* (2021) screened CAB for anti-quorum sensing potential and reported a greater yield of 66.2% of quorum quenching CAB. Bacterial isolates demonstrated QSI against both long and short chain AHLs for both corals, however, *Acropora* isolates demonstrated more QSI against short chain AHL *C. subtsugae* CV017 compared to long chain AHL *C. violaceum* ATCC 12472. Whereas, *Pocillopora* isolates demonstrated more QSI against long chain AHL *C. violaceum* ATCC 12472 compared to short chain AHL *C. subtsugae* CV017. Ma *et al.* (2016) suggested that the production of long-chain AHL molecules may be an ecological strategy to escape degradation and a possible regulator of CAB to adapt to the changing environmental conditions. Therefore, results from the current and previous studies suggest that CAB may be promising sources of novel quorum quenching compounds which can also be used in medical and agricultural industries.

Autoinducer-2 is a molecule that induces bioluminescence in the marine bacterium *Vibrio harveyi* BB120 (Teasdale *et al*., 2011). It has been detected in both Gram-negative and Gram-positive bacteria (Certner and Vollmer, 2018). It is used for conversations between unrelated bacteria (interspecies) as it promotes interactions of microbes within the host (Certner and Vollmer, 2018; Peixoto *et al.,* 2017). The AI-2 inhibition assay was carried out against bioluminescent *V. harveyi* BB120 for the inhibition of interspecies bacterial quorum sensing.

A low number of bacterial isolates from both *Acropora* and *Pocillopora* demonstrated AI-2 inhibition against *V. harveyi* and bioluminescence inhibition was observed and no signs of killing were observed. Isolates demonstrated more AI-2 inhibition than AHL inhibition. This might be because the current study demonstrated Gram-positive bacteria to be more prevalent than Gram-negative, and AHLs are commonly used by Gram-negative bacteria as QS autoinducers (LaSarre *et al.,* 2013). LaSarre *et al.* (2013) further reported that many bacterial species respond to AI-2 and AI-2 receptors have been identified in *V. harveyi* as it detects different forms of AI-2. Therefore, AI-2 can be a better strategy for discovering bacteria with compounds that interfere with QS. The present study is in line with a study by Teasdale *et al*. (2011) which demonstrated that marine organisms such as *Bacillus* and *Halobacillus* species can interfere with QS through the production of secondary metabolites by either inhibiting bioluminescence or the production of violacein in the case of *C. violaceum*.

Understanding the interactions between CAB and other microbes, and their ability to produce secondary metabolites is important in identifying potential antibacterial and antibiotic producers which could be used in clinical applications or as coral probiotics. Both *Acropora* and *Pocillopora* bacterial isolates have shown positive bioactivity against selected indicator microorganisms, indicating that these isolates may be antimicrobial compound producers with an inhibitory potential under favourable conditions. Additionally, some of these CAB may be able to disrupt QS in pathogens and inhibit AI-2, which suggests potential application as probiotics in both clinical and environmental settings.

2.5 Conclusion

Antimicrobial activity and QS inhibition results of both *Acropora* and *Pocillopora* bacterial isolates through primary screening assays clearly indicate that some bacteria within the coral host may be producers of inhibitory compounds and other secondary metabolites under specific conditions and the production of these compounds may be driven by environmental factors or symbiotic interactions within the coral host. More studies are required to clarify the exact role these microbes play in the production of these antimicrobial compounds. It is necessary to screen selected CAB extracts to identify those CAB with novel inhibitory potential to improve coral health regardless of environmental stressors that threaten their health and/or be used in treatment of diseases or infections caused by multidrug-resistant human clinical pathogens.

2.6 References

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2.7 Supplementary Information

2.7.1 Bacterial isolate morphology, pigmentation, Gram stain and cellular morphology.

Table S2.1: Colony pigmentation, Gram stain results and colony shape of 210 *Acropora* bacterial isolates.

Isolate Code	Colony Pigmentation	Gram reaction	Cellular morphology
Ac_A1	Pink	Positive	Cocci
Ac_A2	Light yellow	Positive	Rods
Ac_A3	Cream white	Negative	Rods
Ac_A4	Light yellow	Positive	Rods
Ac_A5	White	Positive	Rods
Ac_A6	Cream yellow	Positive	Rods
Ac_A7	White	Positive	Rods
Ac_A8	White	Positive	Rods
Ac_A9	Yellow	Positive	Rods
Ac_A10	Pink	Positive	Rods
Ac_A11	Cream white	Positive	Rods
Ac_A12	Cream white	Positive	Rods
Ac_A13	Yellow (Actinomycete)	Positive	Rods
Ac_A14	Cream white	Positive	Rods
Ac_A15	Cream white	Positive	Rods
Ac_A16	Cream white	Positive	Cocci
Ac_A17	Yellow (Actinomycete)	Positive	Rods
Ac_A18	Yellow (Actinomycete)	Positive	Filamentous Rods
Ac_A19	Yellow (Actinomycete)	Positive	Rods
Ac_A20	Pink	Positive	Rods
Ac_A21	Yellow	Positive	Rods
Ac_A22	Yellow	Positive	Rods
Ac_A24	Cream white	Negative	Rods
Ac_C1	Orange	Positive	Rods
Ac_C2	Pink	Positive	Rods
Ac_C3	Pink	Positive	Rods
Ac_C4	Lighy yellow	Positive	Rods
Ac_C5	Cream white	Positive	Rods
Ac_C6	Orange	Positive	Rods
Ac_C7	Lighy yellow	Positive	Rods
Ac_C8	Cream white	Positive	Rods
Ac_E1	Cream white	Positive	Rods
Ac_E2	Pink	Positive	Rods
Ac_E3	Yellow	Negative	Rods
Ac_E4	Yellow	Negative	Rods
Ac_E5	Yellow	Positive	Rods
Ac_E6	Tan/light brown	Positive	Rods
Ac_E7	Tan/light brown	Positive	Rods

Ac_E8	Pink / tan	Positive	Rods
Ac_E9	Brown	Positive	Rods
Ac_E10	Tan/light brown	Positive	Rods
Ac_E11	Tan/light brown	Positive	Rods
Ac_E12	Brown	Positive	Rods
Ac_E13	Yellow	Negative	Rods
Ac_E14	Pink	Negative	Rods
Ac_E15	Orange	Negative	Rods
Ac_E16	Pink	Negative	Rods
Ac_E17	Cream white	Negative	Rods
Ac_E18	Yellow	Negative	Rods
Ac_E19	Brown	Negative	Rods
Ac_E20	Cream white	Negative	Rods
Ac_E21	Cream white	Negative	Rods
Ac_E23	Brown	Negative	Rods
Ac_E24	Brown	Negative	Rods
Ac_E25	Cream white	Negative	Rods
Ac_E26	Brown	Negative	Rods
Ac_E27	Light orange	Negative	Rods
Ac_E28	Light orange	Negative	Rods
Ac_E29	Light yellow	Negative	Rods
Ac_E30	Brown	Positive	Rods
Ac_E31	Pink	Positive	Rods
Ac_E32	Orange	Positive	Cocci
Ac_E34	Yellow (Actinomycete)	Positive	Rods
Ac_E35	Brown	Positive	Rods
Ac_E37	Brown	Positive	Rods
Ac_E38	Orange	Positive	Rods
Ac_E39	Brown	Positive	Rods
Ac_E40	Brown	Positive	Rods
Ac_G1	Cream/light yellow	Positive	Rods
Ac_G3	Orange	Positive	Rods
Ac_G5	Orange	Positive	Rods
Ac_G7	Dark orange	Positive	Rods
Ac_G12	Dark orange	Positive	Rods
Ac_G14	Dark orange	Positive	Rods
Ac_G15	Dark orange	Positive	Rods
Ac_G18	Dark orange	Positive	Rods
Ac_G19	Dark orange	Positive	Rods
Ac_G20	Orange	Negative	Rods
Ac_G23	Orange	Positive	Rods
Ac_G25	Light yellow	Negative	Rods
Ac_G26	Cream white	Negative	Cocci
Ac_G33	Light yellow	Negative	Rods
Ac_G34	Yellow (Actinomycete)	Positive	Rods
Ac_G35	Yellow (Actinomycete)	Positive	Rods

Ac_G37	Yellow	Positive	Filamentous Rods
Ac_G38	Yellow (Actinomycete)	Positive	Rods
Ac_G39	Yellow (Actinomycete)	Positive	Rods
Ac_G40	Yellow (Actinomycete)	Positive	Rods
Ac_G41	Yellow (Actinomycete)	Positive	Rods
Ac_G42	Yellow (Actinomycete)	Positive	Rods
Ac_G43	Yellow (Actinomycete)	Positive	Rods
Ac_G44	Yellow (Actinomycete)	Positive	Rods
Ac_G45	Yellow (Actinomycete)	Positive	Rods
Ac_G48	Yellow (Actinomycete)	Positive	Rods
Ac_G50	Yellow (Actinomycete)	Positive	Rods
Ac_G51	Yellow (Actinomycete)	Positive	Rods
Ac_G52	Yellow (Actinomycete)	Positive	Rods
Ac_G55	Yellow (Actinomycete)	Positive	Rods
Ac_G56	Orange	Negative	Filamentous Rods
Ac_G57	Orange	Negative	Rods
Ac_L1	Yellow (Actinomycete)	Positive	Rods
Ac_L2	Yellow (Actinomycete)	Positive	Rods
Ac_L3	Light yellow	Positive	Rods
Ac_L4	Yellow	Positive	Rods
Ac_L5	Brown	Negative	Rods
Ac_L7	Brown	Negative	Rods
Ac_L8	Cream white	Positive	Filamentous Rods
Ac_L9	Brown	Positive	Rods
Ac_L10	Light yellow	Positive	Rods
Ac_L11	Brown	Positive	Rods
Ac_L12	Brown	Negative	Filamentous Rods
Ac_L13	Yellow (Actinomycete)	Negative	Rods
Ac_L14	Yellow (Actinomycete)	Negative	Rods
Ac_L15	Light yellow	Positive	Filamentous Rods
Ac_L16	Orange	Positive	Rods
Ac_L17	Brown	Positive	Rods
Ac_L18	Brown	Positive	Rods
Ac_L19	Orange	Positive	Rods
Ac_L21	Brown	Negative	Rods
Ac_L22	Brown	Negative	Rods
Ac_L23	Brown	Negative	Rods
Ac_L24	Yellow	Negative	Rods
Ac_L25	Yellow translucent	Negative	Rods
Ac_L26	Brown	Negative	Rods
Ac_L27	Cream white	Positive	Rods
Ac_L28	Brown	Negative	Rods
Ac_L29	Brown	Negative	Rods
Ac_L30	Cream white	Negative	Rods
Ac_L31	Brown	Negative	Rods
Ac_L33	Orange	Positive	Rods

Ac_L34	Yellow	Positive	Rods
Ac_L35	Brown	Positive	Rods
Ac_L36	Yellow (Actinomycete)	Positive	Rods
Ac_L37	Yellow	Positive	Rods
Ac_L38	Yellow	Positive	Rods
Ac_L39	Cream white	Positive	Rods
Ac_L40	Cream white	Negative	Rods
Ac_L41	Yellow (Actinomycete)	Positive	Rods
Ac_L42	Yellow (Actinomycete)	Positive	Rods
Ac_L43	Light Yellow	Negative	Rods
Ac_L45	Light yellow	Negative	Rods
Ac_L46	Yellow	Negative	Rods
Ac_L47	Orange	Negative	Rods
Ac_L48	Yellow	Negative	Rods
Ac_L49	Brown	Negative	Rods
Ac_L50	Yellow (Actinomycete)	Positive	Rods
Ac_M3	Cream	Negative	Rods
Ac_M7	Cream white	Negative	Cocci
Ac_M13	Cream white	Positive	Rods
Ac_M25	Light yellow	Negative	Cocci
Ac_M30	Cream white	Positive	Rods
Ac_M33	Orange	Positive	Rods
Ac_M35	Orange	Positive	Rods
Ac_M36	Cream white	Positive	Rods
Ac_M38	Pink/tan	Negative	Rods
Ac_M39	Orange	Negative	Rods
Ac_M40	Yellow	Positive	Rods
Ac_M41	Cream	Negative	Rods
Ac_M42	Cream	Positive	Cocci
Ac_M46	Orange	Positive	Rods
Ac_M47	Light yellow	Negative	Rods
Ac_M53	Brown	Positive	Rods
Ac_M54	Tan translucent	Negative	Rods
Ac_M55	Orange	Negative	Rods
Ac_M57	Orange	Positive	Rods
Ac_M58	Brown	Positive	Rods
Ac_M61	Brown	Negative	Rods
Ac_M62	Brown	Negative	Cocci
Ac_M64	Tan	Negative	Cocci
Ac_M65	Neon Yellow	Negative	Cocci
Ac_M69	Yellow	Positive	Rods
Ac_M73	Orange	Positive	Rods
Ac_M74	Orange	Negative	Cocci
Ac_S1	Orange	Positive	Rods
Ac_S3	Cream white	Positive	Cocci
Ac_S4	Orange	Positive	Rods

Ac_S5	Orange	Positive	Rods
Ac_S6	Orange	Positive	Rods
Ac_S7	Yellow	Positive	Rods
Ac_S8	Cream orange (Actinomycete)	Positive	Rods
Ac_S9	Cream orange (Actinomycete)	Positive	Rods
Ac_S10	Light orange	Positive	Rods
Ac_S12	Cream white	Negative	Rods
Ac_S13	Cream white	Negative	Rods
Ac_S14	Yellow (Actinomycete)	Positive	Rods
Ac_S15	Pink (Actinomycete)	Positive	Cocci
Ac_S16	Pink (Actinomycete)	Positive	Cocci
Ac_T1	Cream white	Positive	Rods
Ac_T2	Cream white	Positive	Rods
Ac_T3	Cream white	Positive	Rods
Ac_T4	Cream white	Positive	Rods
Ac_T5	Cream white	Positive	Rods
Ac_T6	Cream white	Positive	Rods
Ac_T7	Cream white	Positive	Rods
Ac_T8	Cream white	Positive	Rods
Ac_T9	Cream white	Positive	Rods
Ac_T10	Light yellow	Negative	Rods
Ac_T11	Cream white	Positive	Rods
Ac_T12	Cream white	Negative	Rods
Ac_T13	Cream white	Negative	Rods
Ac_T14	Light yellow	Positive	Rods
Ac_T15	Cream white	Positive	Rods
Ac_T16	Cream white	Positive	Rods
Ac_T17	Cream white	Positive	Rods
Ac_T18	Light yellow	Positive	Rods
Ac_T19	Light yellow	Positive	Rods
Ac_T20	Light yellow	Positive	Rods

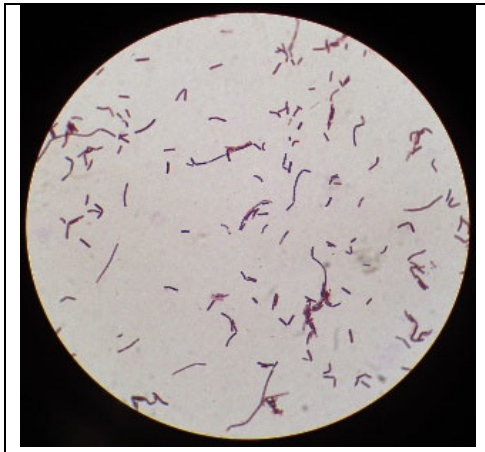
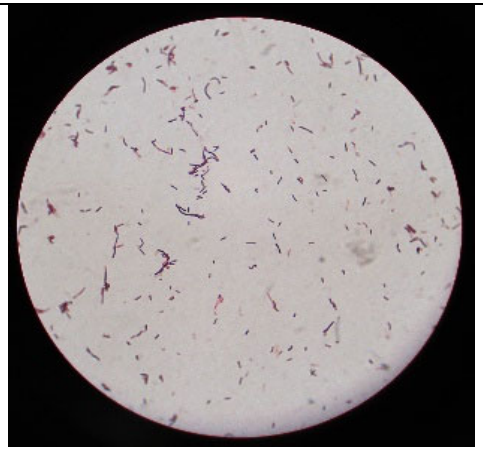
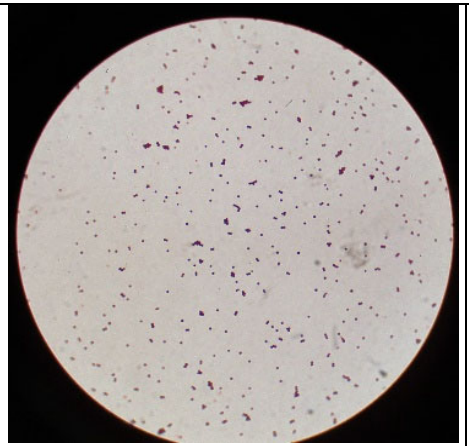
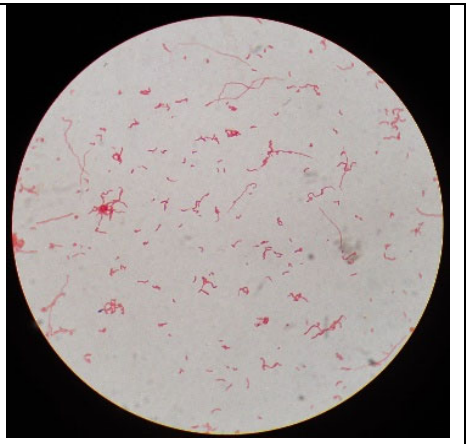
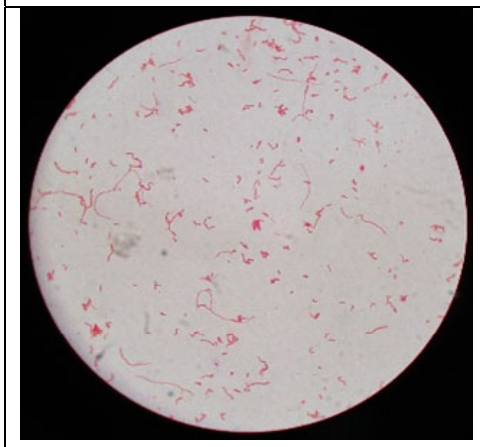
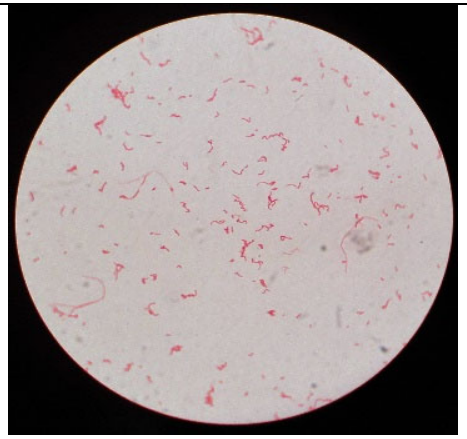
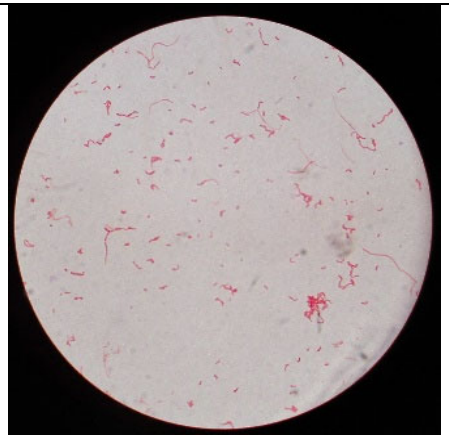
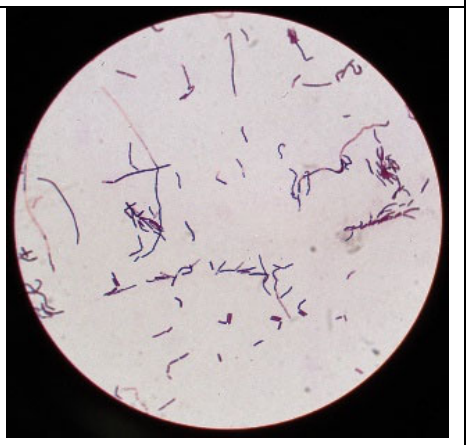
Table S2.2: Colony pigmentation, Gram stain results and colony shape of 132 *Pocillopora* bacterial isolates.

Isolate Code	Colony Pigmentation	Gram reaction	Cellular Morphology
P_A1	Cream white glossy	Positive	Rods
P_A2	Cream white glossy	Positive	Rods
P_A3	Yellow	Positive	Rods
P_A4	Yellow translucent	Positive	Rods
P_A5	Yellow translucent	Positive	Rods
P_A6	Cream white	Negative	Rods
P_A7	Cream white	Negative	Rods
P_A8	Cream white	Negative	Rods
P_A9	Cream white	Positive	Rods
P_A10	Cream white	Negative	Rods
P_A11	Light yellow	Positive	Cocci
P_A12	White	Negative	Cocci
P_A13	White	Positive	Rods
P_A14	Light yellow	Negative	Rods
P_A15	Cream white	Negative	Rods
P_A16	Yellow	Negative	Rods
P_A17	Cream white	Negative	Cocci
P_A18	Cream white	Negative	Cocci
P_A19	Cream white	Negative	Cocci
P_A20	Cream white	Negative	Cocci
P_E1	White glossy	Negative	Rods
P_E2	Brown	Negative	Filamentous Rods
P_E3	Cream white	Negative	Rods
P_E5	Cream white glossy	Positive	Rods
P_E6	Brown	Negative	Cocci
P_E7	Brown	Negative	Cocci
P_E8	Brown	Negative	Rods
P_E9	Brown	Negative	Rods
P_E10	Brown	Negative	Rods
P_E12	Brown	Negative	Rods
P_E13	Pink	Negative	Rods
P_E15	Cream white	Negative	Cocci
P_E18	Cream white	Positive	Rods
P_E19	Cream white	Positive	Cocci
P_E20	Cream white	Negative	Rods
P_E21	Yellow	Positive	Cocci
P_E22	Cream white	Negative	Cocci
P_G1	Cream white glossy	Positive	Filamentous Rods
P_G3	Cream white glossy	Positive	Rods
P_G4	Cream white glossy	Positive	Rods
P_G10	Cream white	Positive	Rods
P_G13	Cream white	Positive	Rods
P_G16	Cream white	Negative	Cocci

P_G17	Light yellow	Negative	Cocci
P_G21	White (Actinomycete)	Positive	Cocci
P_G23	Cream white	Positive	Rods
P_G24	Cream white	Positive	Rods
P_G25	Cream white	Positive	Rods
P_L1	Cream white	Negative	Rods
P_L2	Yellow	Negative	Cocci
P_L3	Cream white	Negative	Cocci
P_L4	Brown	Negative	Rods
P_L5	Brown	Negative	Rods
P_L6	Brown	Negative	Rods
P_L8	Brown	Negative	Rods
P_L9	Brown	Negative	Rods
P_L10	Orange	Negative	Rods
P_L11	Cream white	Negative	Cocci
P_L12	Cream white	Positive	Cocci
P_L13	Brown	Negative	Rods
P_L14	Light brown	Negative	Rods
P_L15	Yellow translucent	Negative	Rods
P_L17	Cream white	Positive	Rods
P_L18	Cream white	Positive	Rods
P_L19	Cream white	Positive	Rods
P_L20	Cream white	Positive	Rods
P_L21	Cream white	Positive	Rods
P_L22	Cream white	Positive	Rods
P_L23	Light yellow	Positive	Rods
P_L24	Light yellow	Positive	Rods
P_L25	Light yellow	Positive	Rods
P_M2	Cream white	Negative	Cocci
P_M3	light orange/tan	Negative	Rods
P_M4	Yellow	Negative	Rods
P_M5	Light yellow	Negative	Rods
P_M7	Yellow	Negative	Rods
P_M8	Yellow translucent	Negative	Rods
P_M9	Tan/ light brown	Negative	Rods
P_M13	Cream white	Negative	Cocci
P_M14	Yellow	Positive	Rods
P_M15	Yellow	Positive	Rods
P_M16	Yellow	Negative	Cocci
P_M17	Yellow	Positive	Rods
P_M18	Brown	Negative	Rods
P_M19	Brown	Negative	Rods
P_M20	Orange	Negative	Rods
P_M21	Yellow translucent	Negative	Rods
P_M23	Brown	Negative	Rods
P_M24	Brown	Negative	Rods

P_M25	White	Negative	Cocci
P_M26	Orange	Negative	Rods
P_M28	Orange	Negative	Rods
P_M30	Light yellow glossy	Positive	Rods
P_M31	Light yellow glossy	Positive	Rods
P_M32	Cream white	Positive	Rods
P_M33	Cream white	Positive	Rods
P_M35	Cream white	Negative	Rods
P_M37	Light yellow	Positive	Rods
P_M38	Cream white	Positive	Rods
P_M44	Tan/light yellow	Negative	Rods
P_M45	Cream white	Positive	Rods
P_M46	Cream white	Positive	Rods
P_M48	Cream white	Positive	Rods
P_M49	Cream white glossy	Positive	Rods
P_M50	Cream white glossy	Positive	Rods
P_M51	Cream white glossy	Positive	Rods
P_M53	Brown	Negative	Rods
P_M54	Cream white glossy	Positive	Rods
P_M55	Cream white glossy	Positive	Rods
P_S1	White	Positive	Rods
P_S2	White (Actinomycete)	Positive	Rods
P_S3	White (Actinomycete)	Positive	Rods
P_S4	White (Actinomycete)	Positive	Rods
P_S5	Light yellow	Positive	Rods
P_S6	White (Actinomycete)	Positive	Rods
P_S7	Cream white	Positive	Cocci
P_S8	White (Actinomycete)	Positive	Rods
P_S9	Yellow-blue (Actinomycete)	Positive	Rods
P_T1	Cream white	Positive	Rods
P_T2	Cream white	Positive	Rods
P_T3	Cream white	Positive	Rods
P_T4	Cream white	Positive	Rods
P_T5	Cream white	Positive	Rods
P_T6	Cream white	Positive	Rods
P_T7	Cream white	Positive	Rods
P_T8	Cream white	Positive	Rods
P_T9	Cream white	Positive	Rods
P_T10	Cream white	Positive	Rods
P_T11	Cream white	Positive	Rods
P_T12	Cream white	Positive	Rods

Gram staining

			
Ac_A18	**Ac_A20**	**Ac_E5**	**Ac_E13**
			
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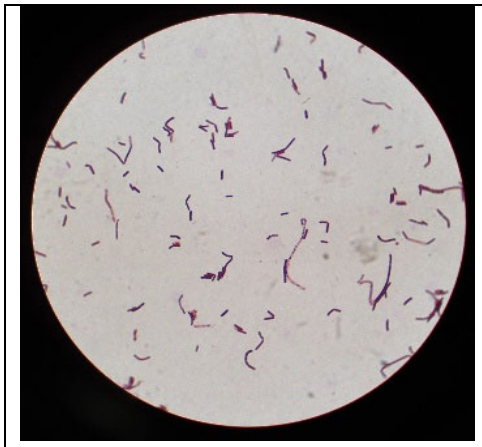
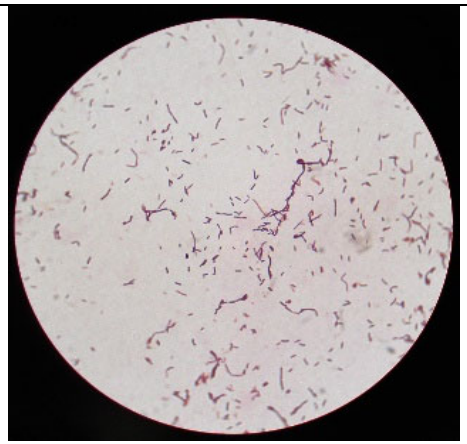
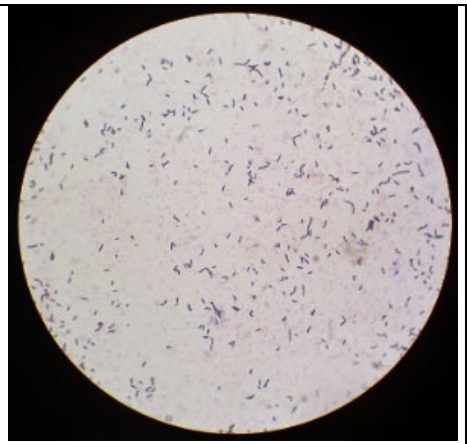
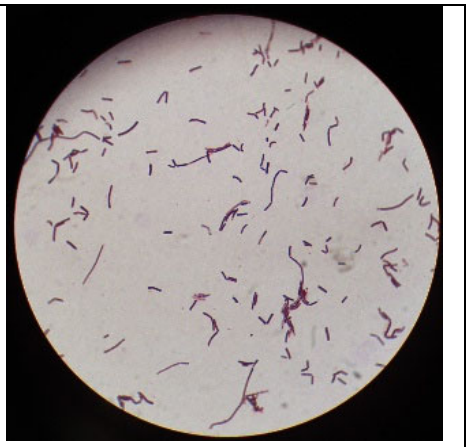
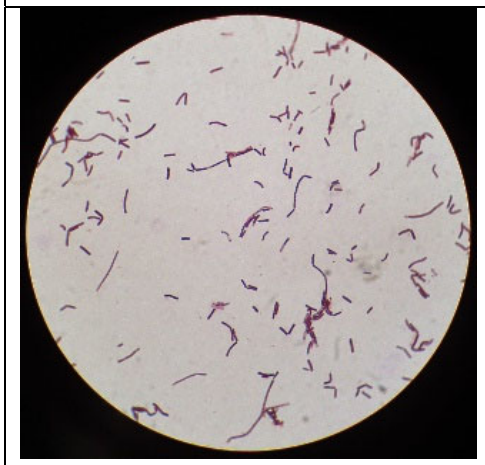
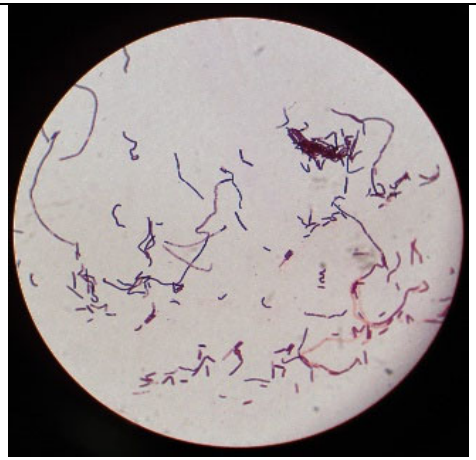
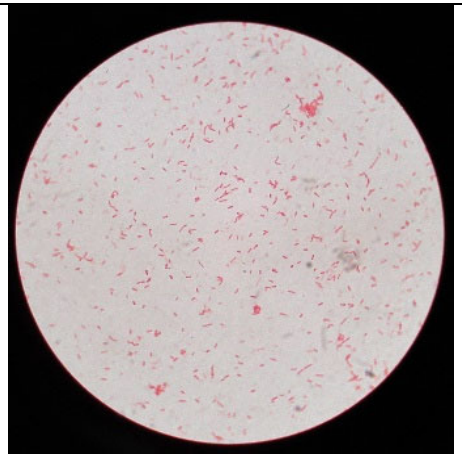
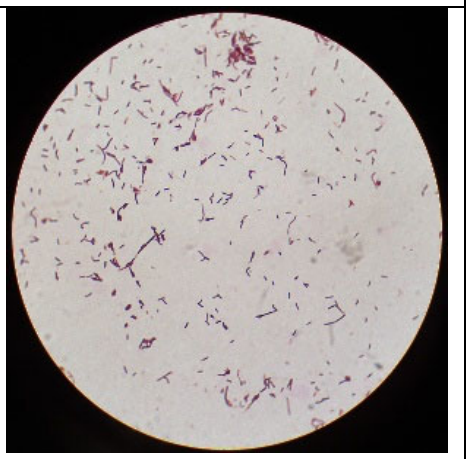
			
Ac_G56	**Ac_G57**	**Ac_L5**	**Ac_L8**
			
Ac_L12	**Ac_L15**	**Ac_L27**	**Ac_L38**

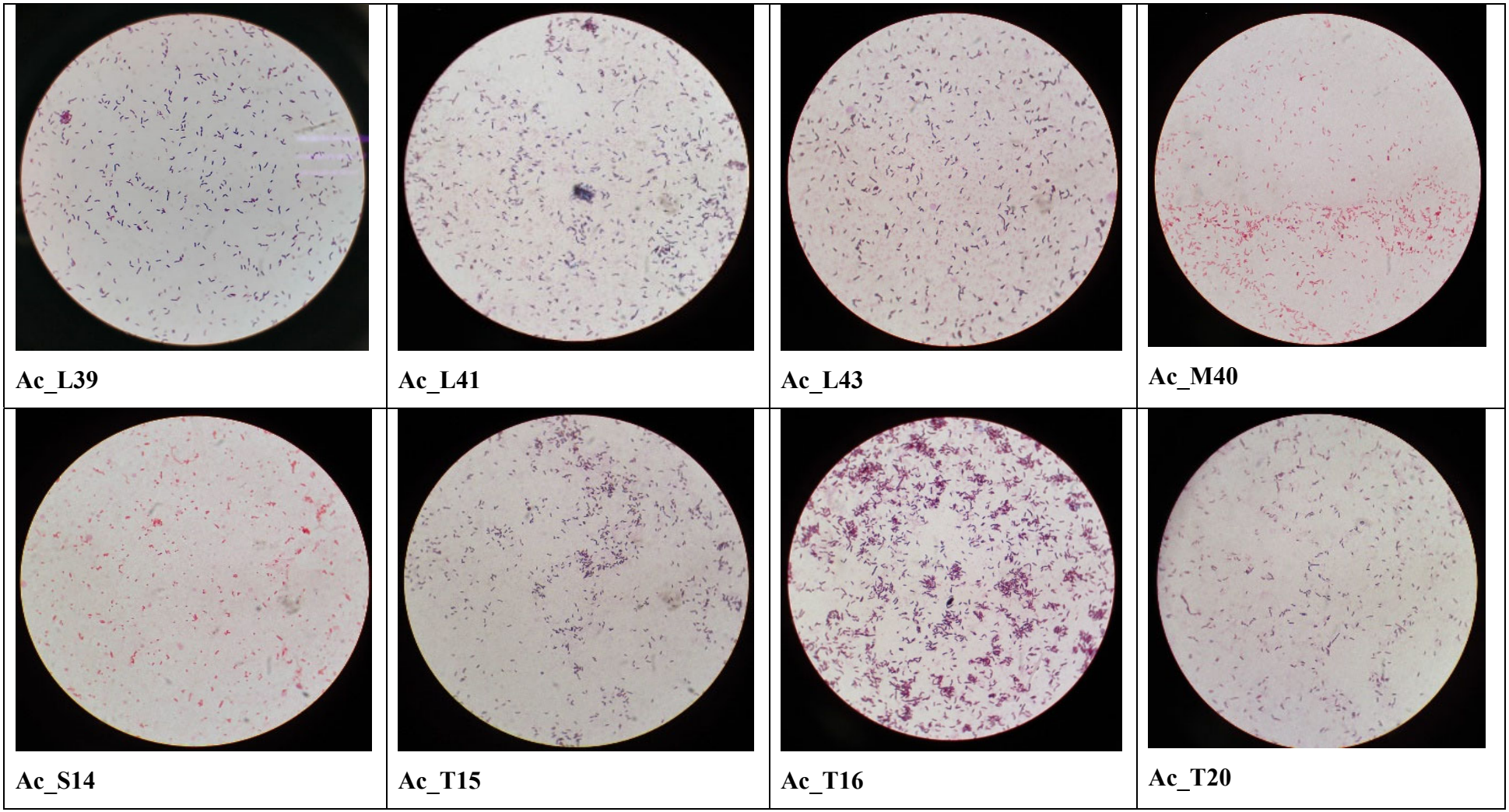
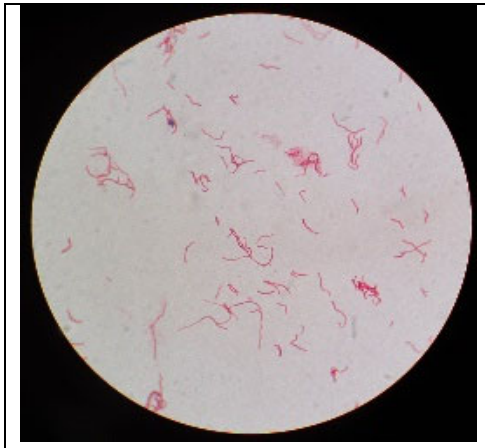
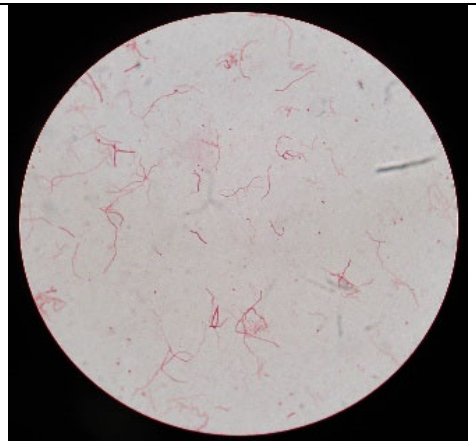
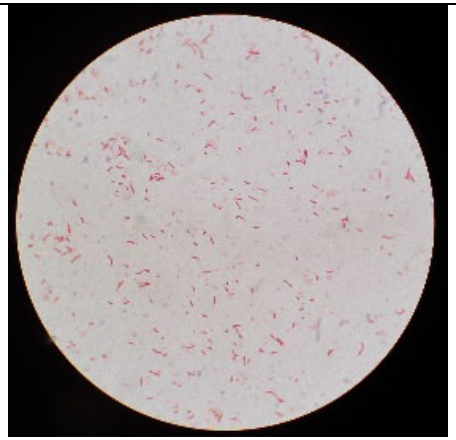
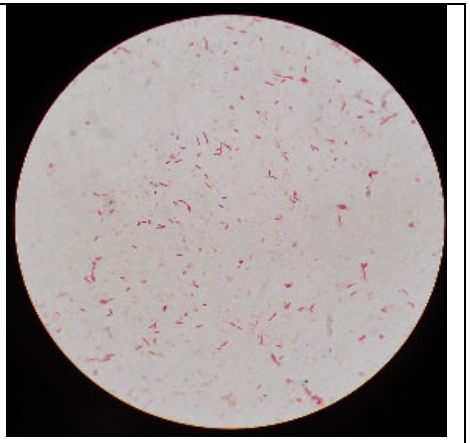
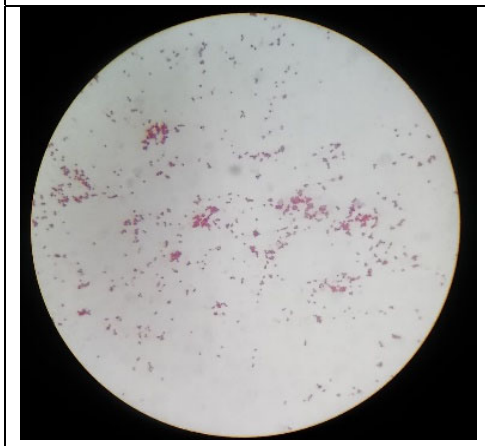
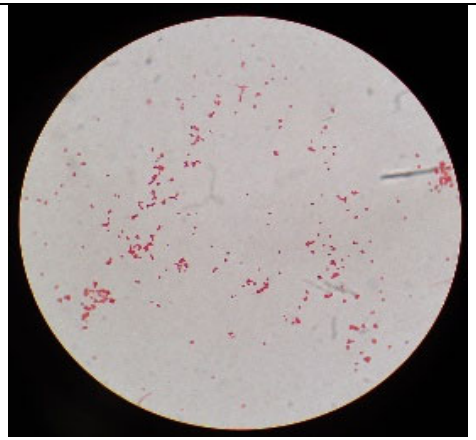
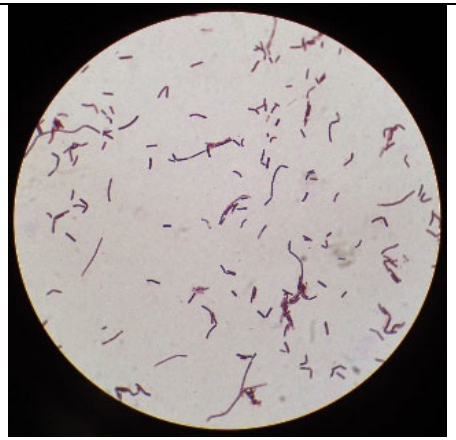
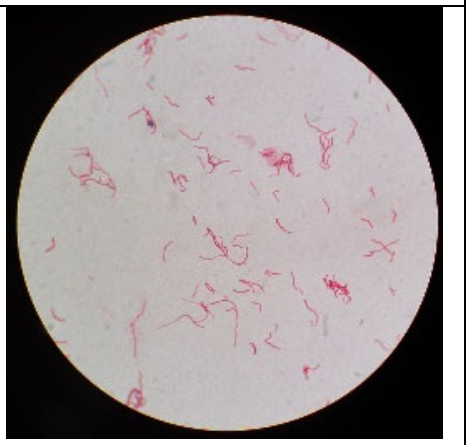


Figure S2.1: Gram stain results of 24 selected *Acropora* isolates showing Gram-negative (pink) and Gram-positive (purple) reactions and cellular morphology (rod-like or cocci) of bacterial isolates.

			
P_A9	**P_E2**	**P_E5**	**P_E12**
			
P_E13	**P_E20**	**P_G1**	**P_L4**

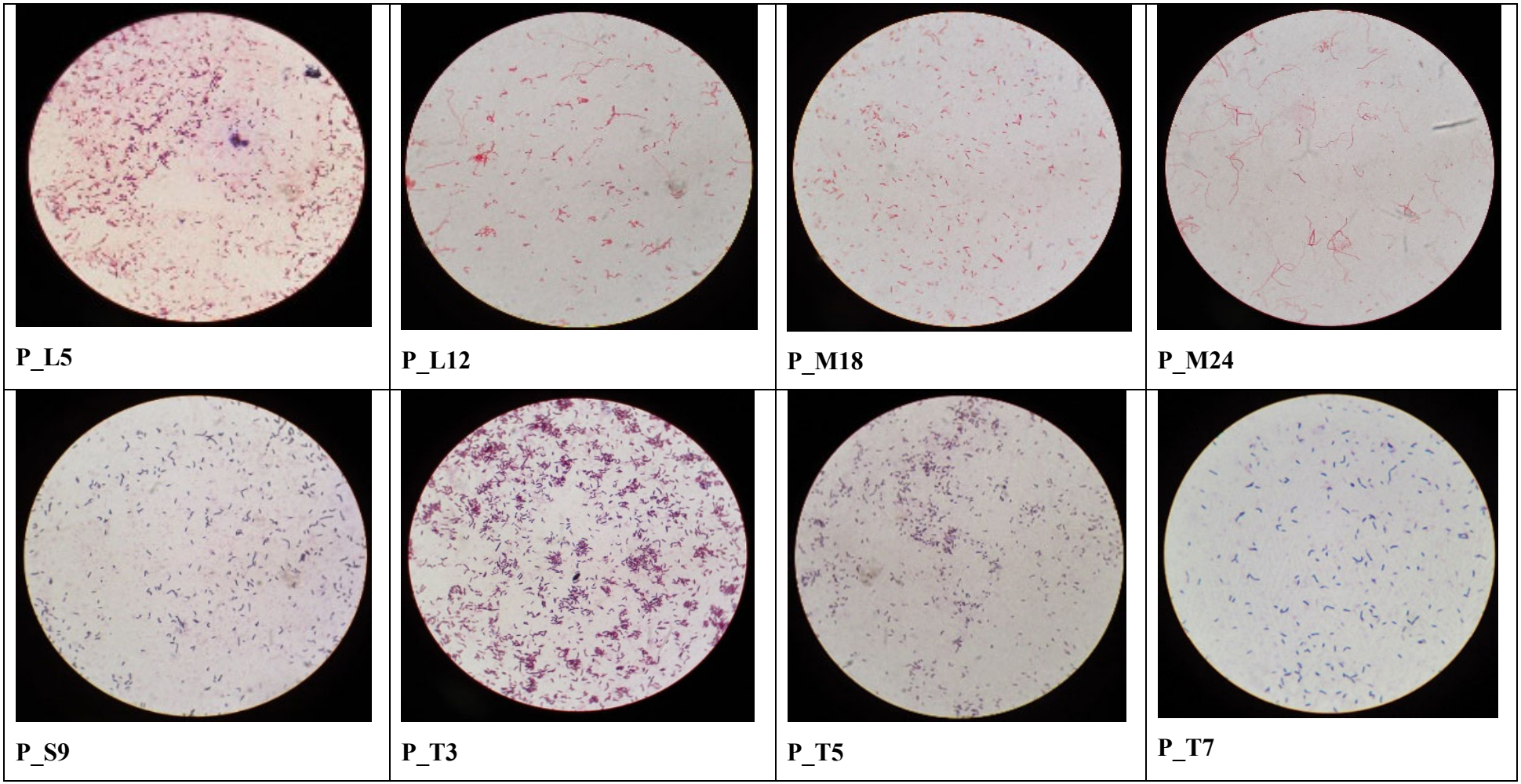
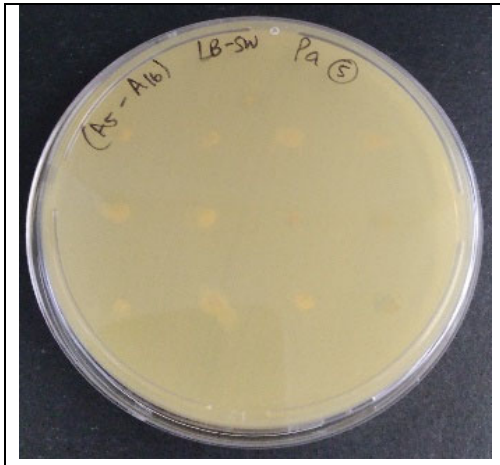
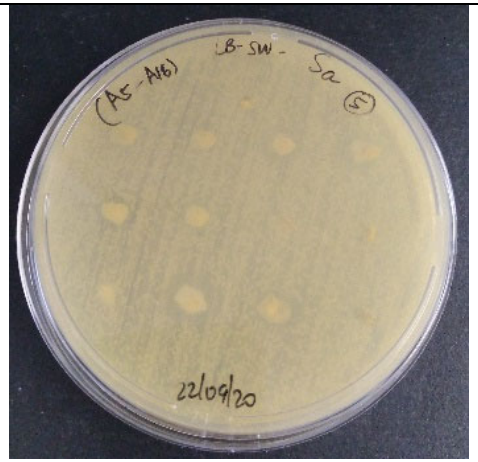


Figure S2.2: Gram stain results of 16 *Pocillopora* isolates showing Gram-negative (pink) and Gram-positive (purple) reactions and cell morphology (rod-like or cocci) of selected bacterial isolates.

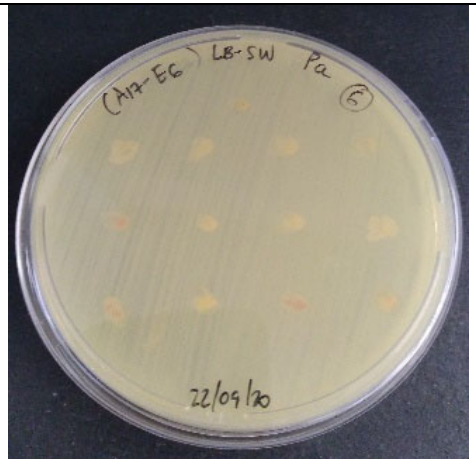
Antimicrobial activity



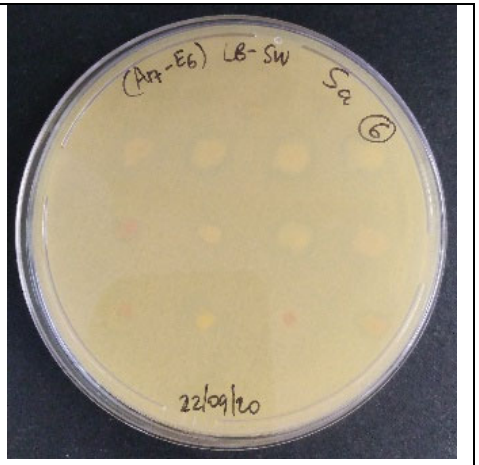
Ac_*P. aeruginosa*_A5-A16



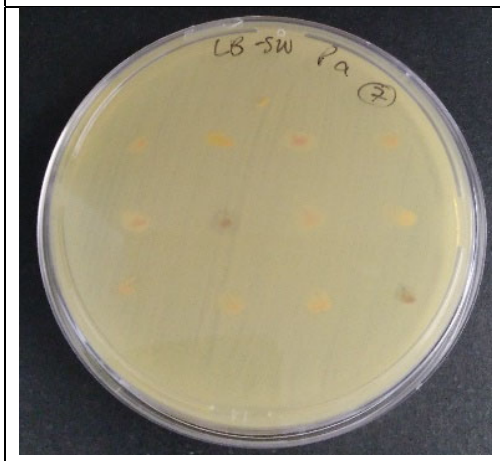
Ac_*S. aureus*_A5-A16



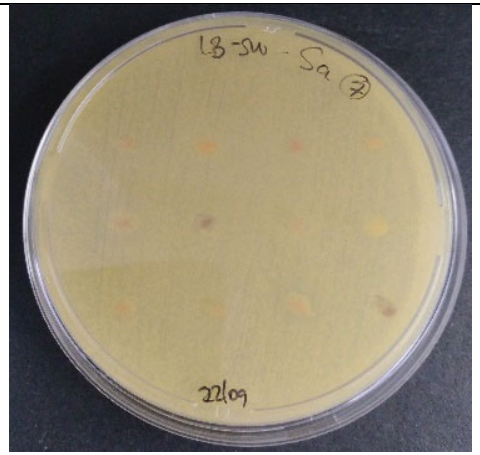
Ac_*P. aeruginosa*_A17-E6



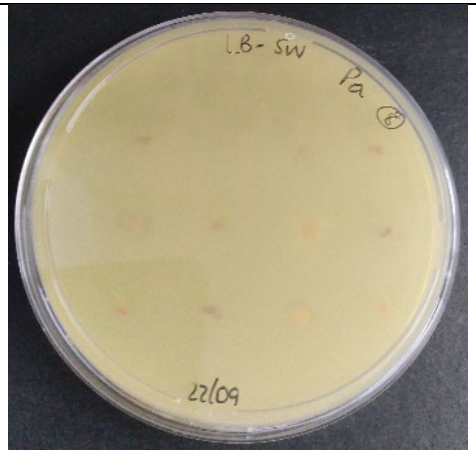
Ac_*S. aureus*_A17-E6



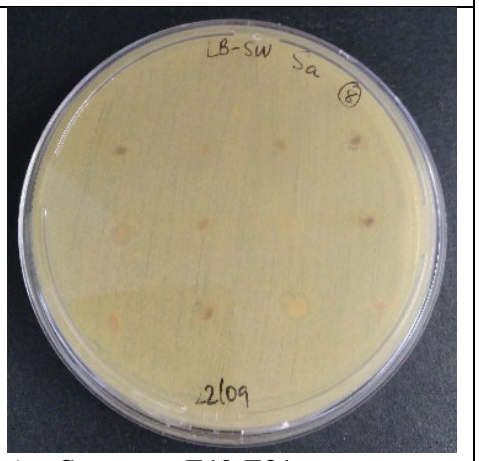
Ac_*P. aeruginosa*_E7-E18



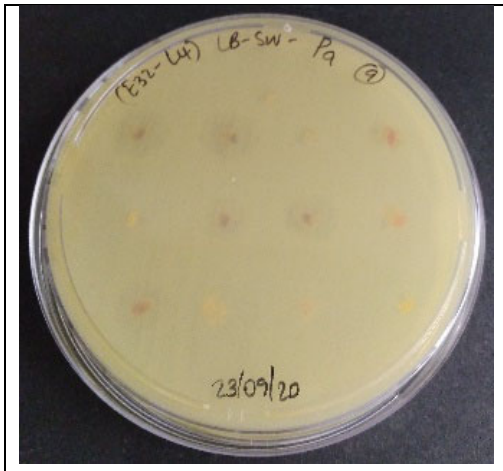
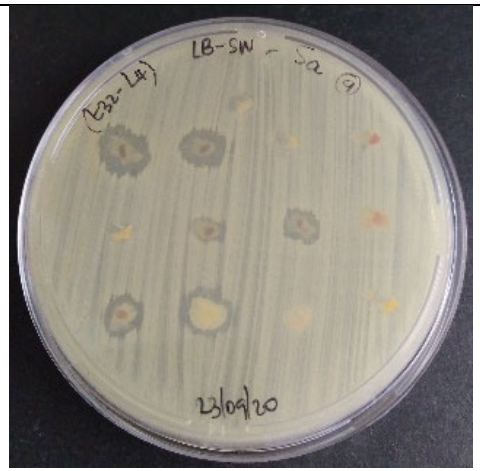
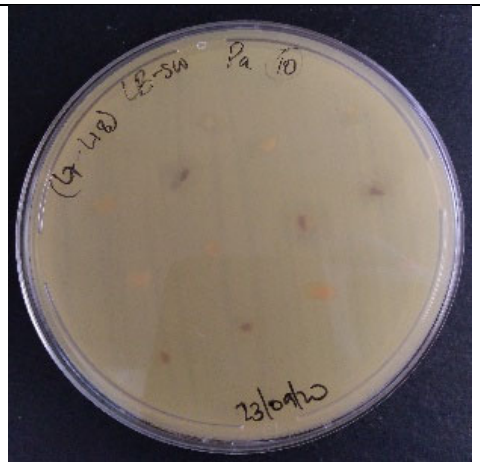
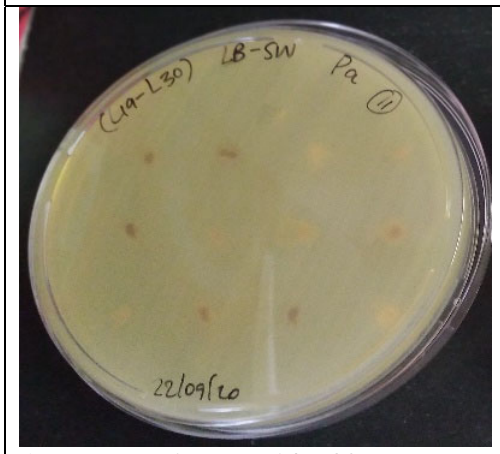
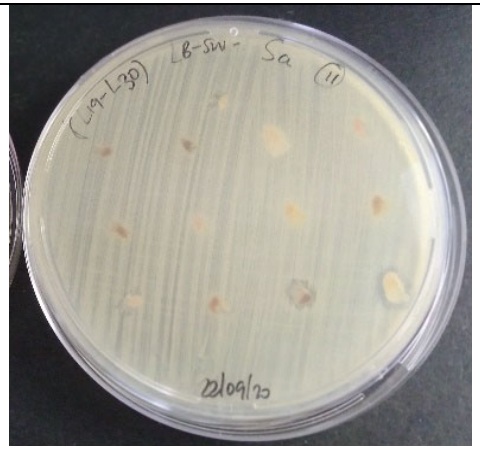
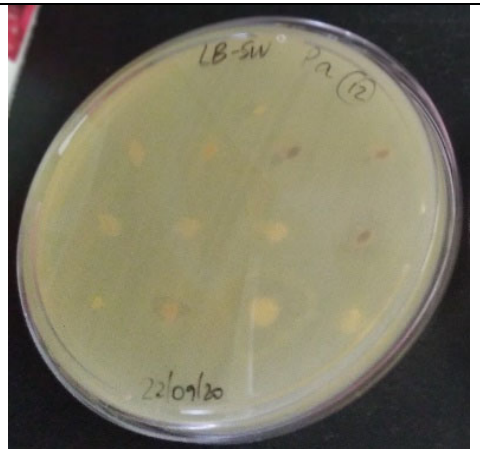
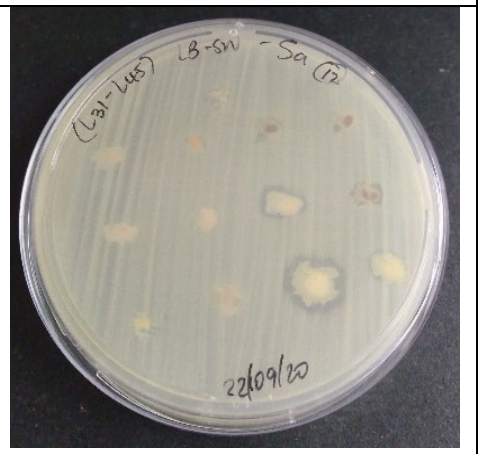
Ac_*S. aureus*_E7-E18

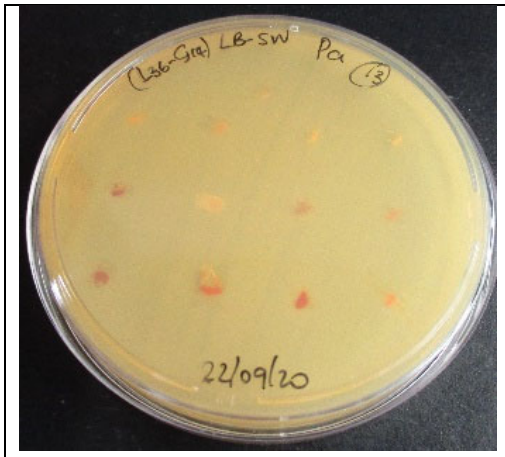
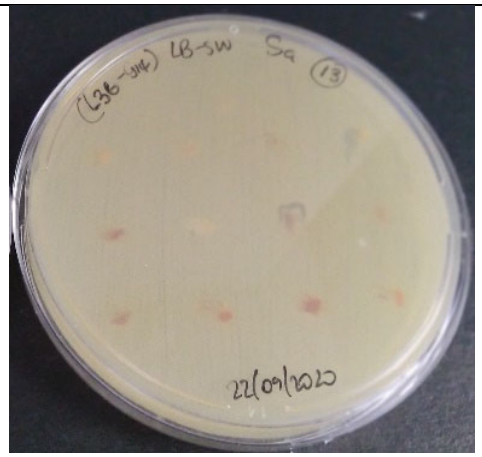
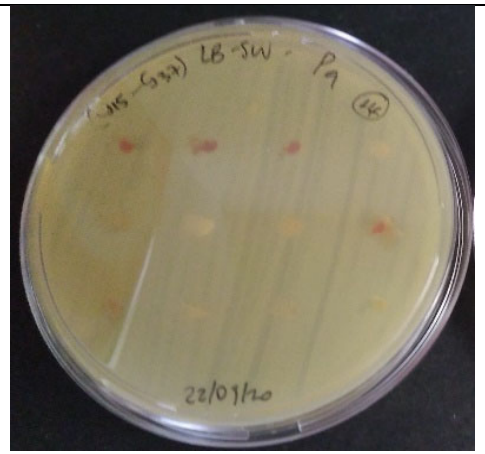
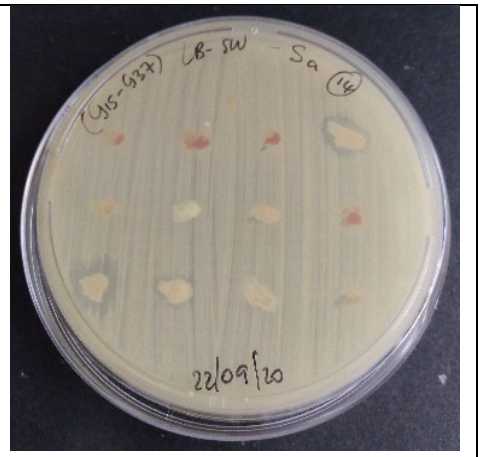
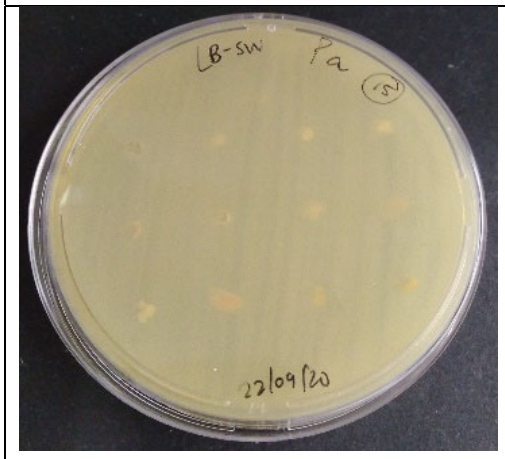
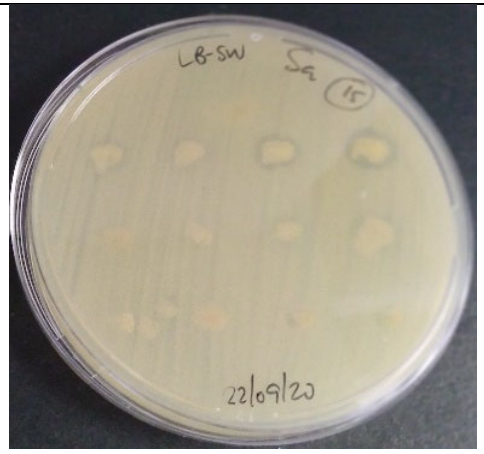
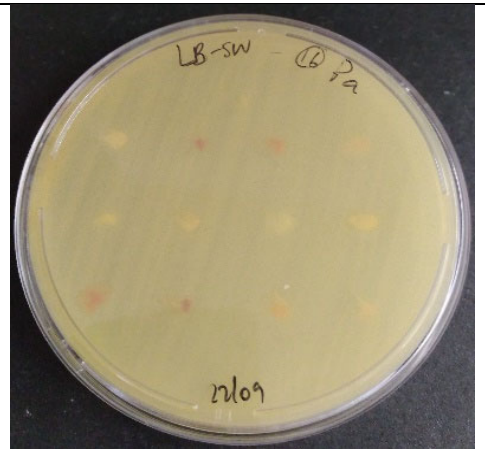
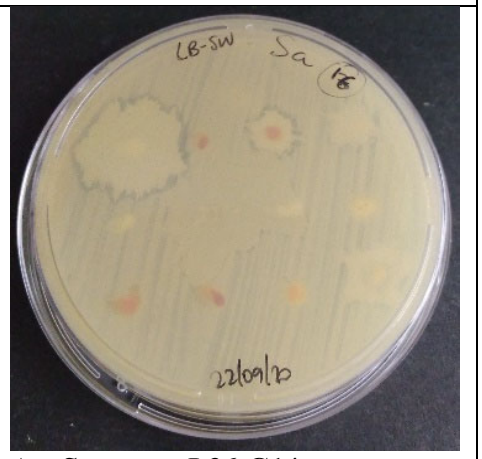


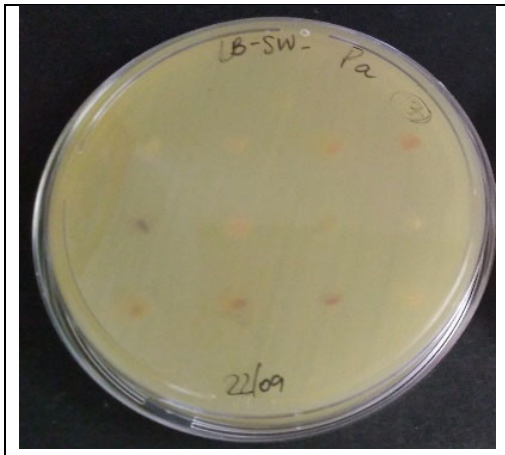
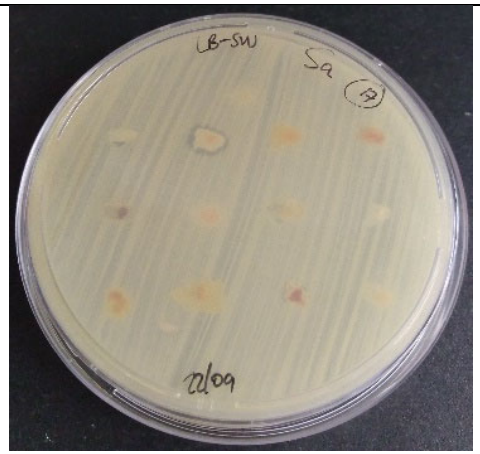
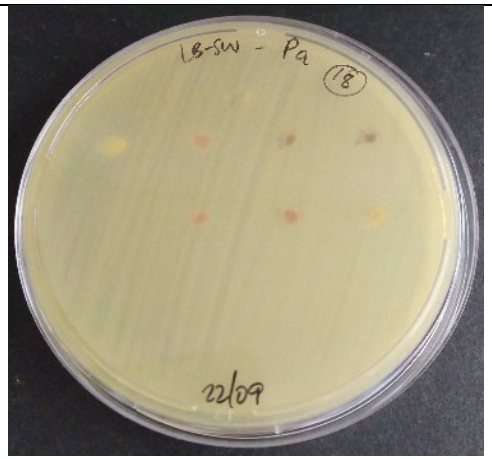
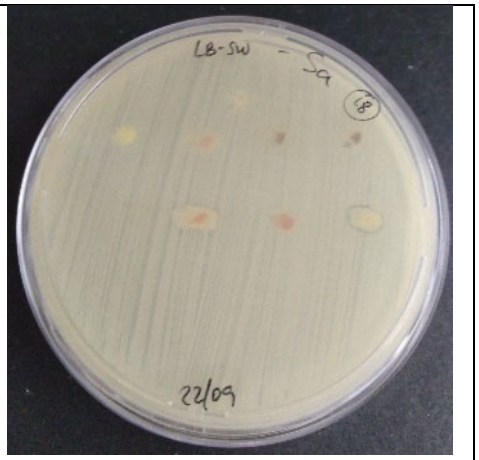
Ac_*P. aeruginosa*_E19-E31



Ac_*S. aureus*_E19-E31

			
Ac_*P. aeruginosa*_E32-L4	**Ac_*S. aureus*_E32-L4**	**Ac_*P. aeruginosa*_L7-L18**	**Ac_*S. aureus*_L7-L18**
			
Ac_*P. aeruginosa*_L14-L30	**Ac_*S. aureus*_L14-L30**	**Ac_*P. aeruginosa*_L31-L45**	**Ac_*S. aureus*_L31-L45**

			
Ac_*P. aeruginosa*_L36-G14	**Ac_*S. aureus*_L36-G14**	**Ac_*P. aeruginosa*_G15-G37**	**Ac_*S. aureus*_G15-G37**
			
Ac_*P. aeruginosa*_L31-L45	**Ac_*S. aureus*_L31-L45**	**Ac_*P. aeruginosa*_L36-G14**	**Ac_*S. aureus*_L36-G14**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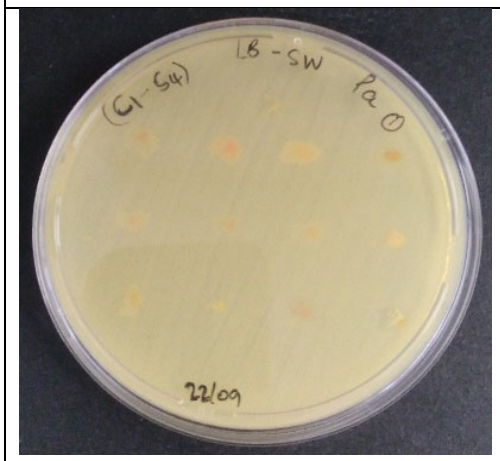	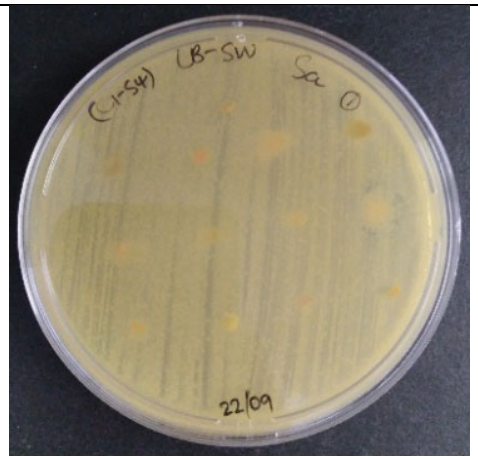	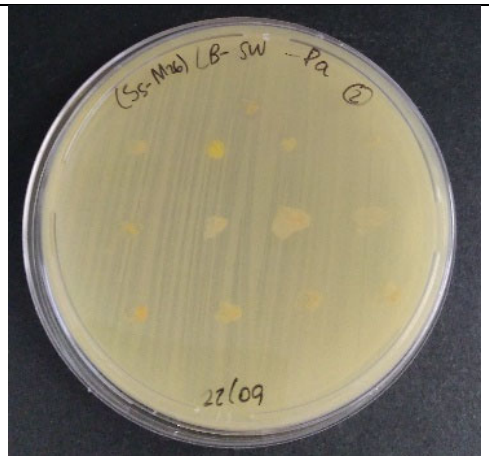	
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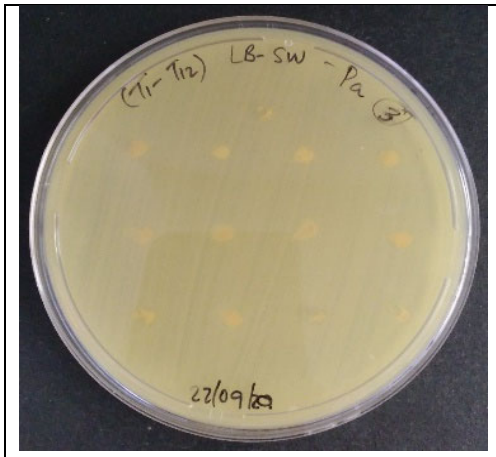

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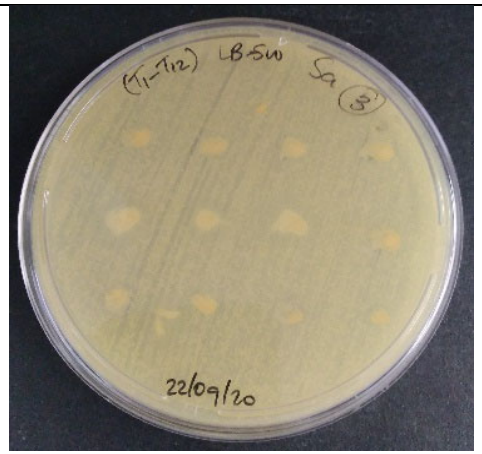
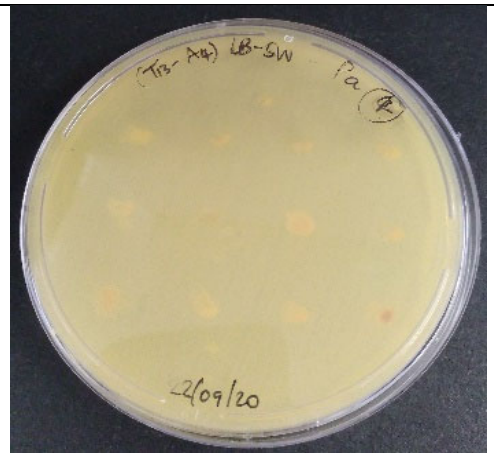


Ac_*S. aureus*_T1-T12



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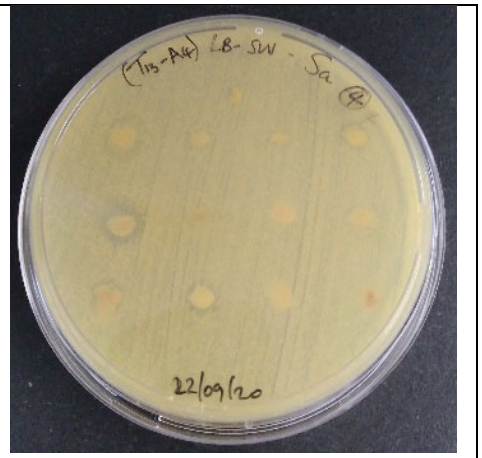
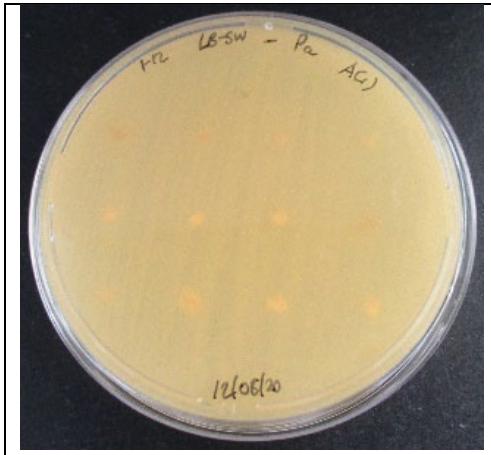
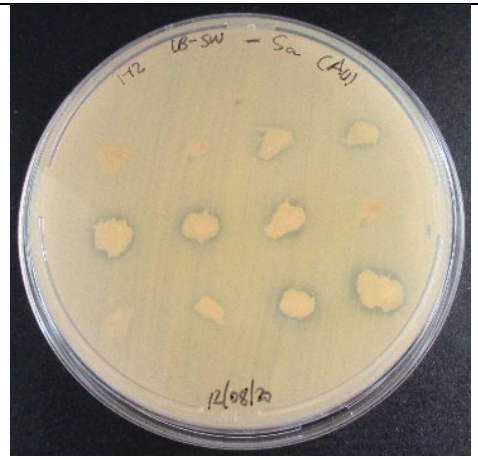


Ac_*S. aureus*_T13-A4

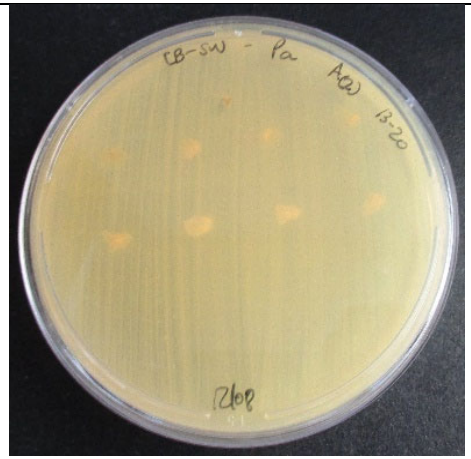
Figure S2.3: Primary screening for antimicrobial activity of *Acropora* bacterial isolates against *Pseudomonas aeruginosa* ATTC 27853 and *Staphylococcus aureus* ATTC 43300 using a colony picking technique. Antimicrobial activity against test organisms *P. aeruginosa* and *S. aureus* was observed by the presence of an opaque or clear zone around the target bacteria.



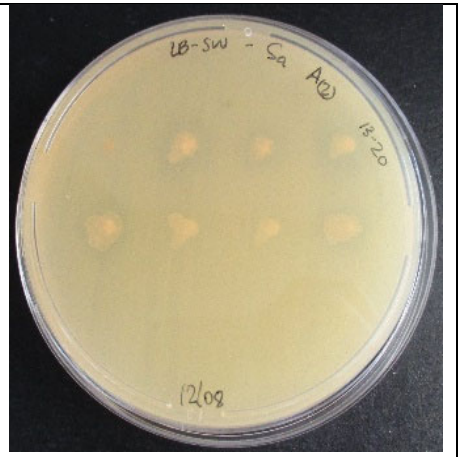
P_*P. aeruginosa*_A1-A12



P_*S. aureus*_A1-A12



P_*P. aeruginosa*_A13-A20



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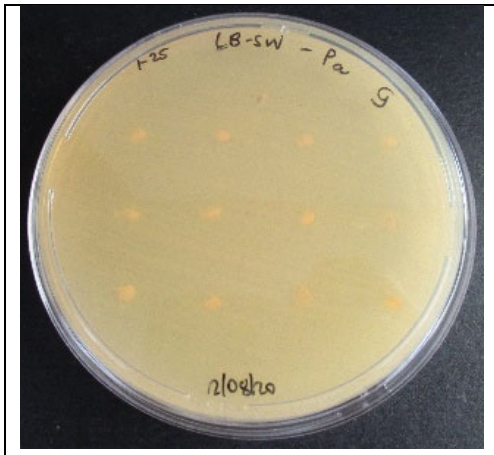
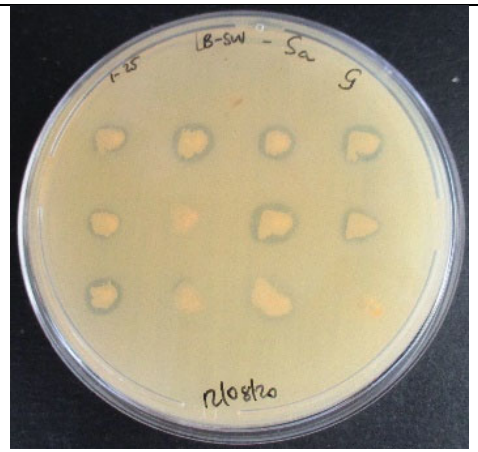
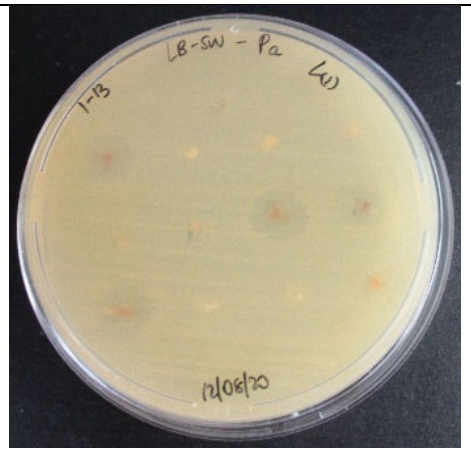


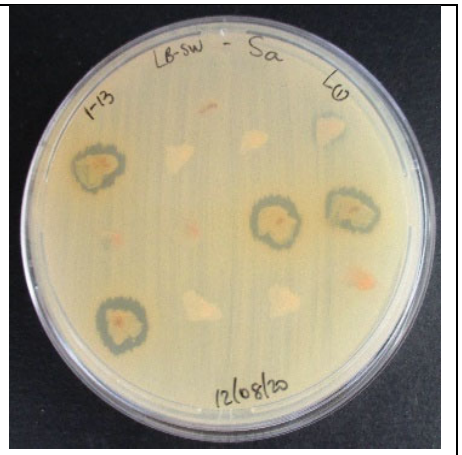
P_*P. aeruginosa*_G1-G25



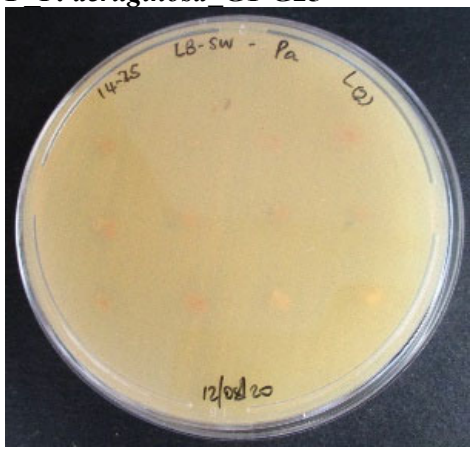
P_*S. aureus*_G1-G25



P_*P. aeruginosa*_L1-L13



P_*S. aureus*_L1-L13



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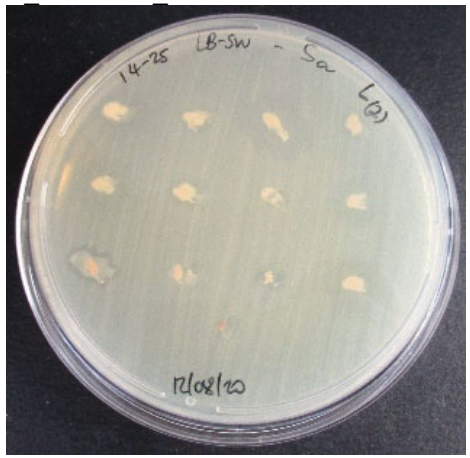
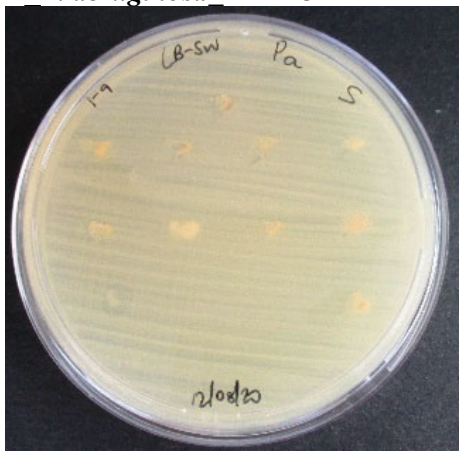
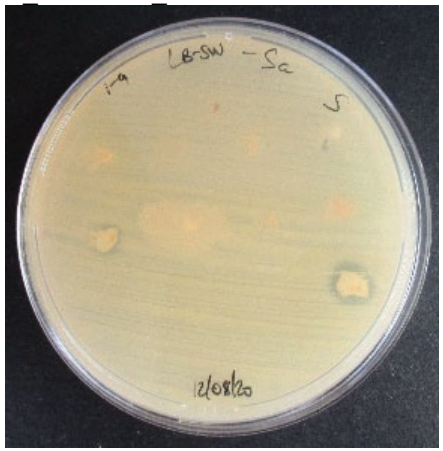


P_*S. aureus*_L14-L25



P_*P. aeruginosa*_S1-S9



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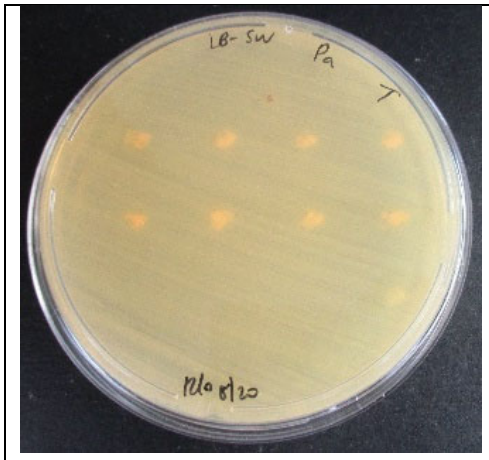

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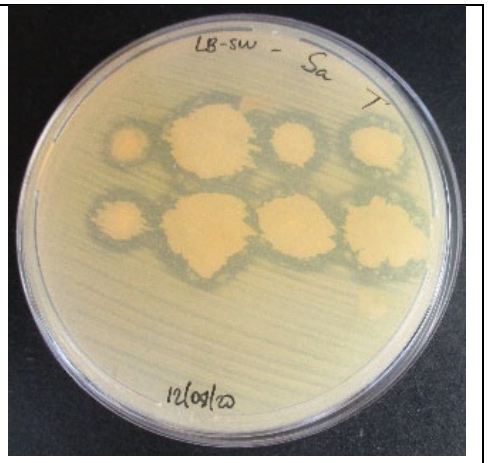
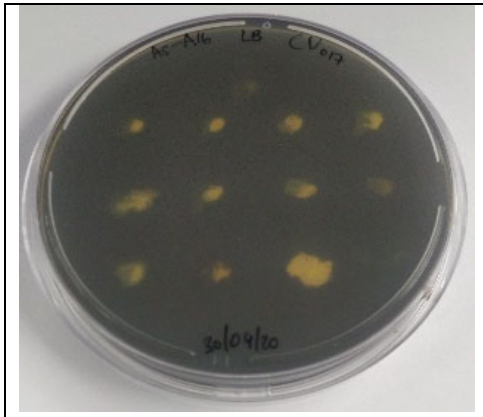
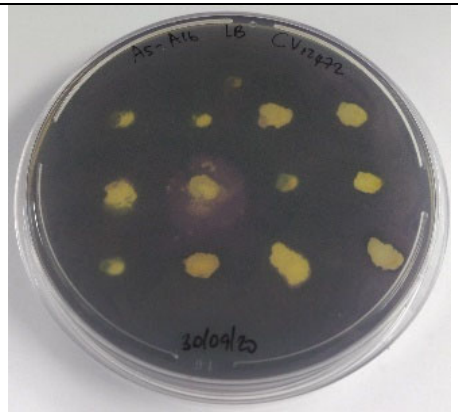
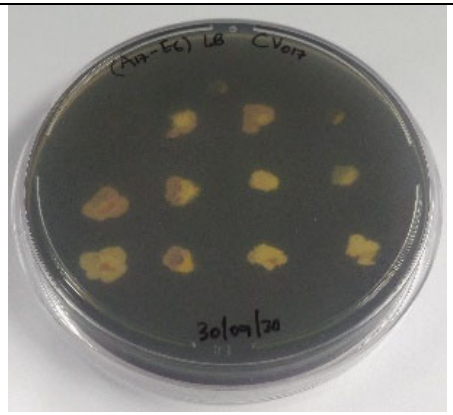
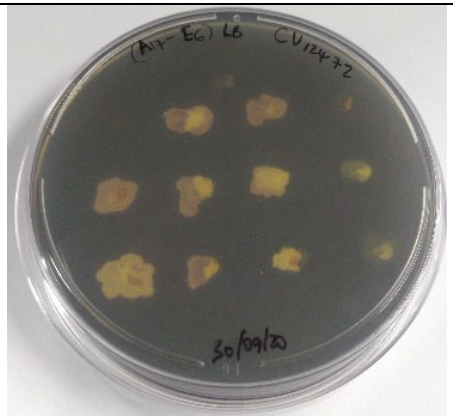
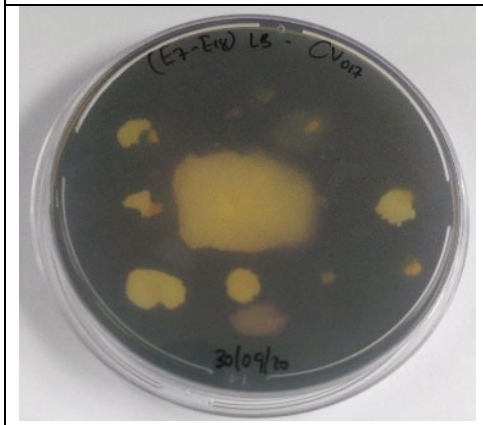
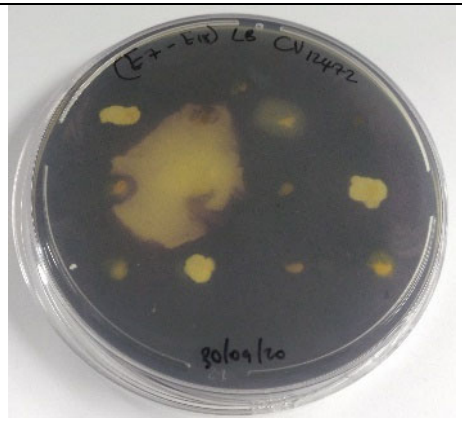
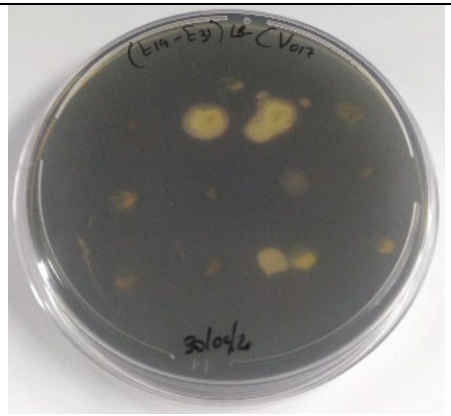
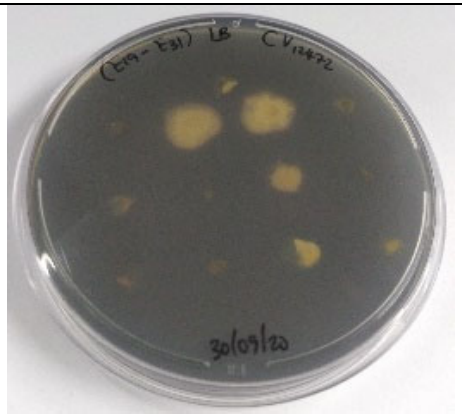


P_*S. aureus*_T1-T9

Figure S2.4: Primary screening for antimicrobial activity of *Pocillopora* bacterial isolates against *Pseudomonas aeruginosa* ATTC 27853 and *Staphylococcus aureus* ATTC 43300 using a colony picking technique. Antimicrobial activity against test organisms *P. aeruginosa* and *S. aureus* was observed by the presence of an opaque or clear zone around the target bacteria.

Quorum sensing inhibition

			
Ac_*C. subtsugae* CV017_A5-A16	**Ac_*C. violaceum* ATCC 12472_A5-A16**	**Ac_*C. subtsugae* CV017_A17-E6**	**Ac_*C. violaceum* CV12472_A17-E16**
			
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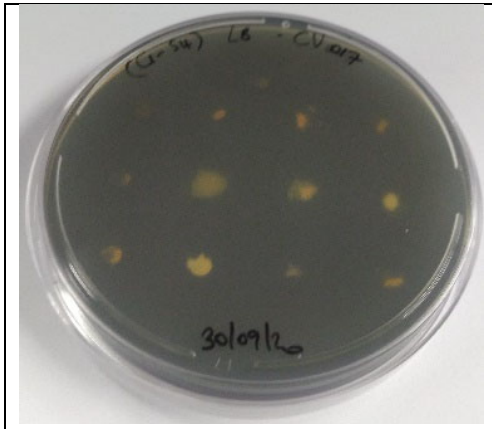
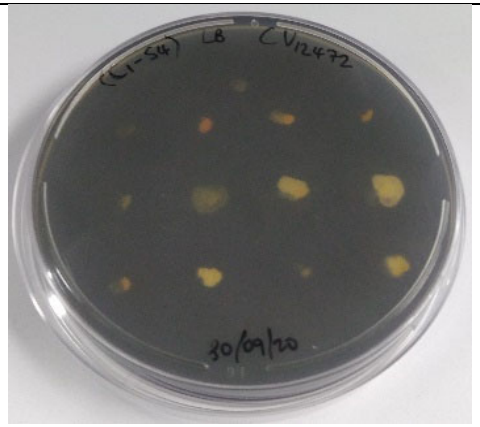
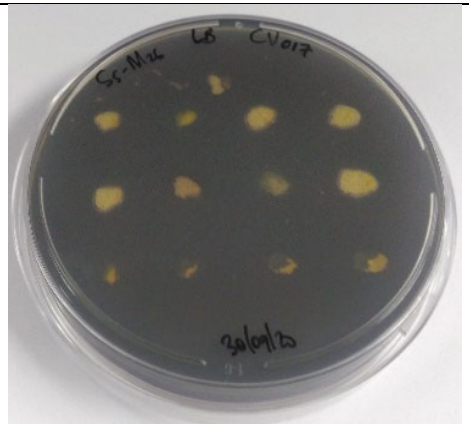
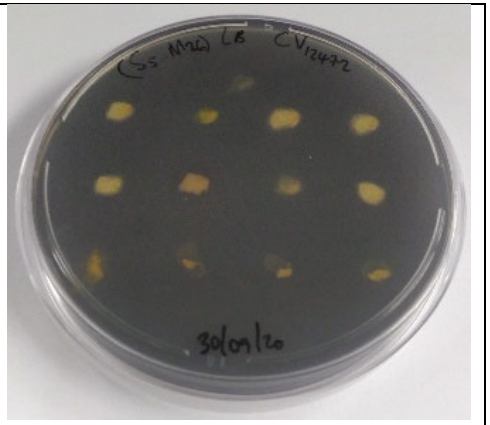
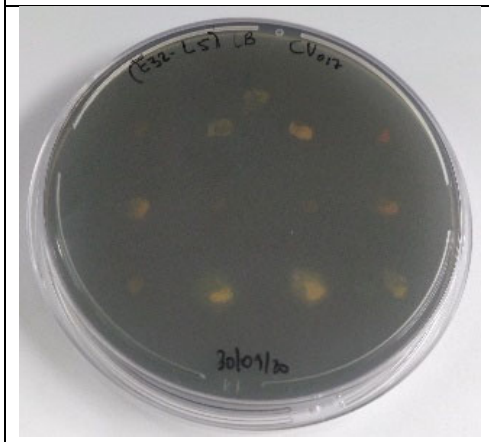
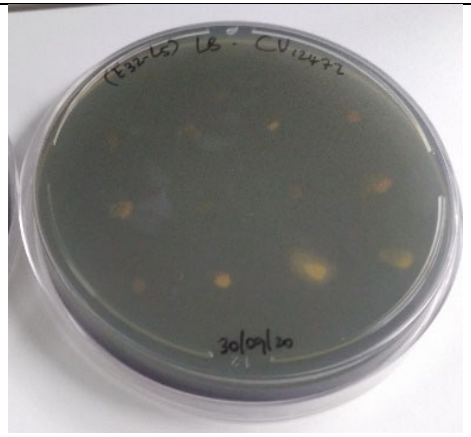
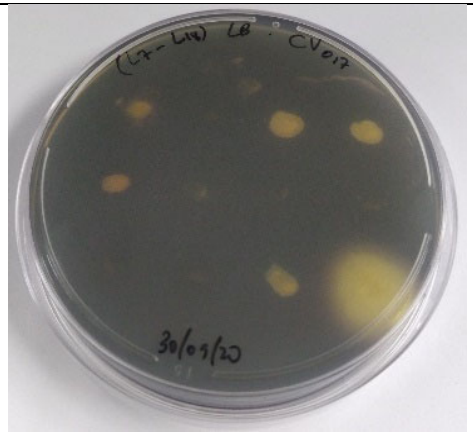
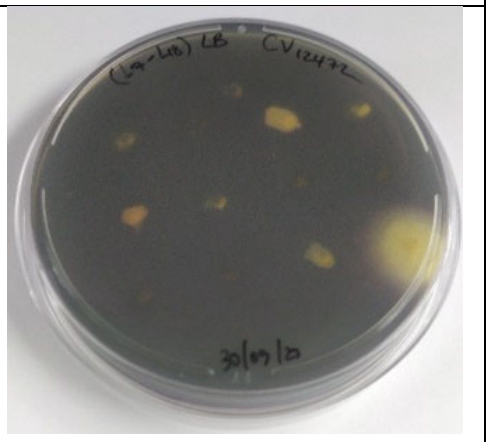
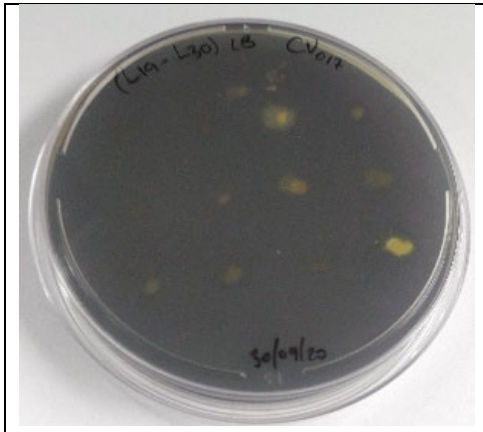
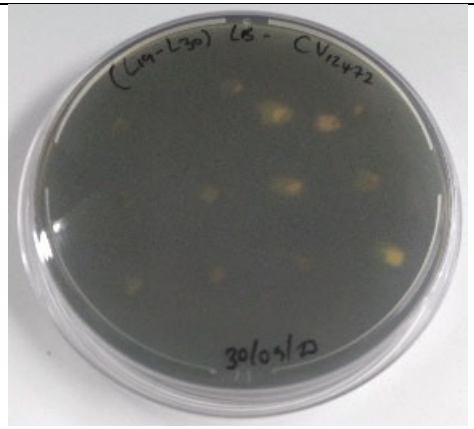
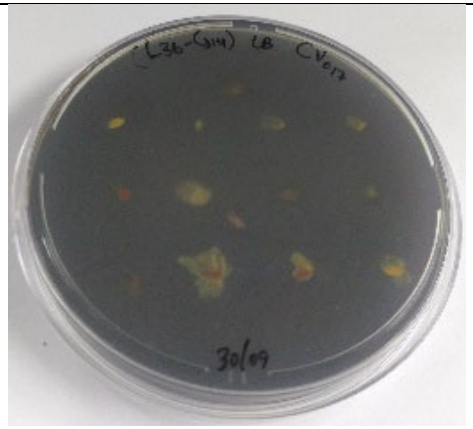
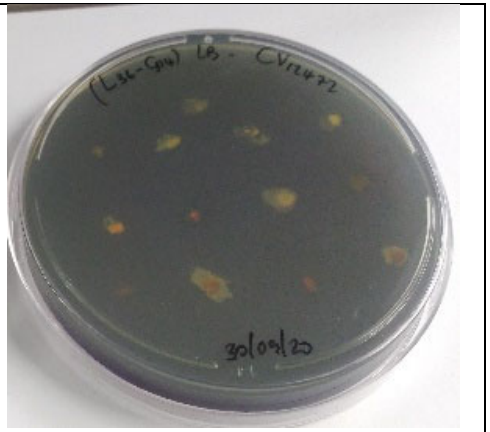
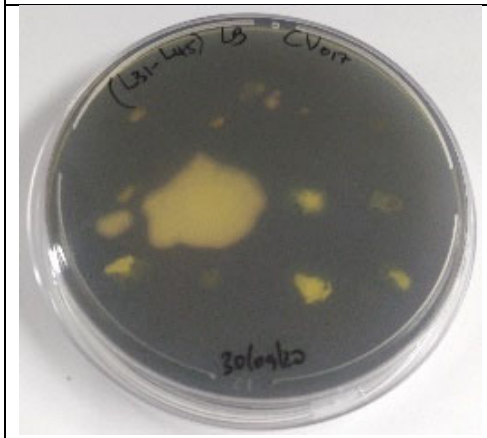
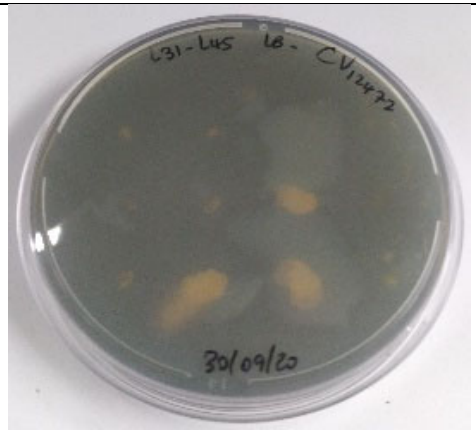
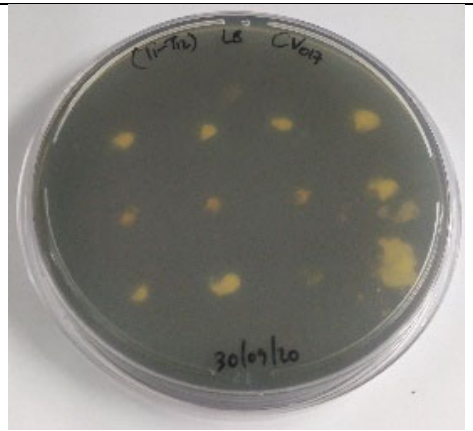
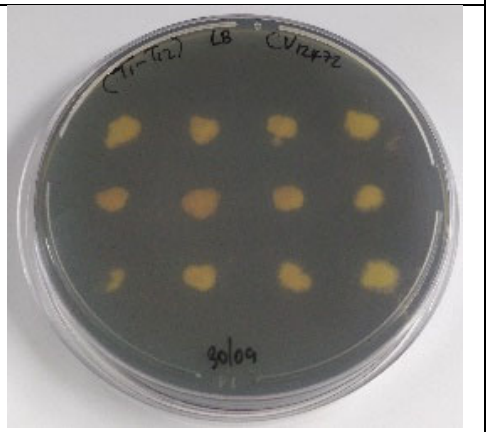
			
Ac_*C. subtsugae* CV017_C1-S4	**Ac_*C. violaceum* ATCC12472_C1-S4**	**Ac_*C. subtsugae* CV017_S5-M26**	**Ac_*C. violaceum* ATCC12472_S5-M26**
			
Ac_*C. subtsugae* CV017_E32-L5	**Ac_*C. violaceum* ATCC12472_E32-L5**	**Ac_*C. subtsugae* CV017_L7-L18**	**Ac_*C. violaceum* ATCC12472_L7-L18**

			
Ac_*C. subtsugae* CV017_L19-L30	**Ac_*C. violaceum* ATCC12472_L19-L30**	**Ac_*C. subtsugae* CV017_L36-G14**	**Ac_*C. violaceum* ATCC12472_L36-G14**
			
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	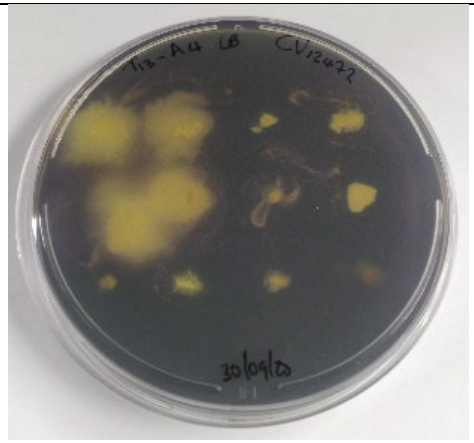	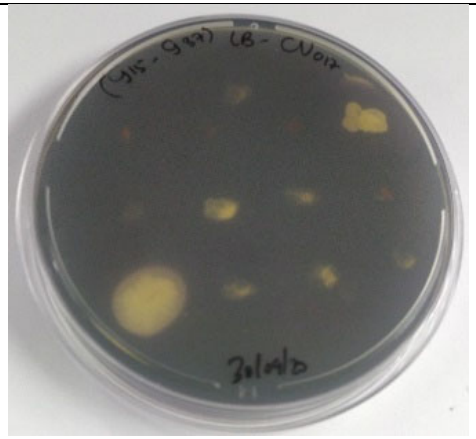	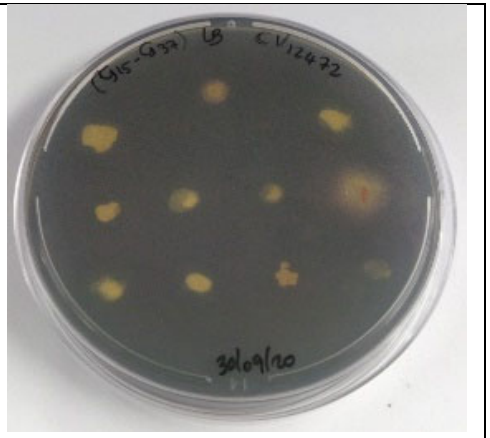
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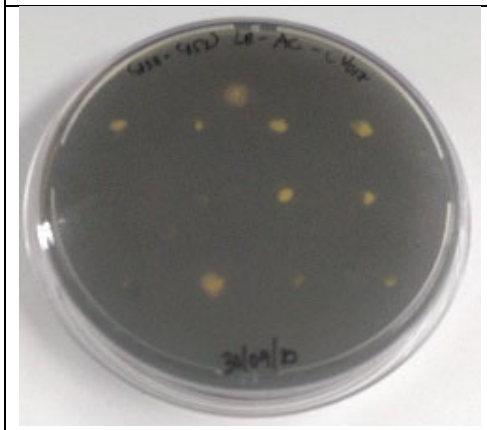	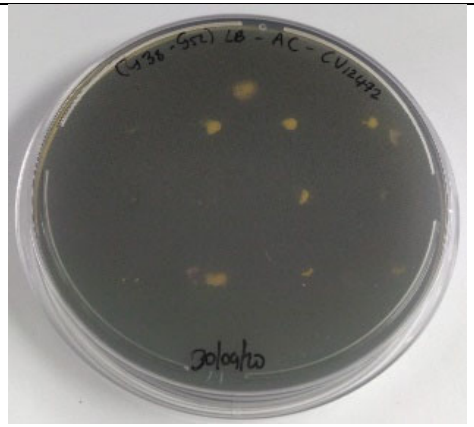	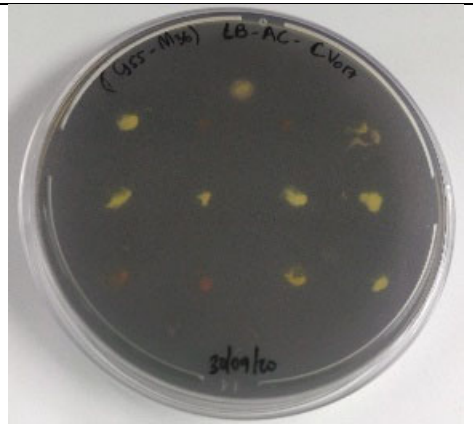	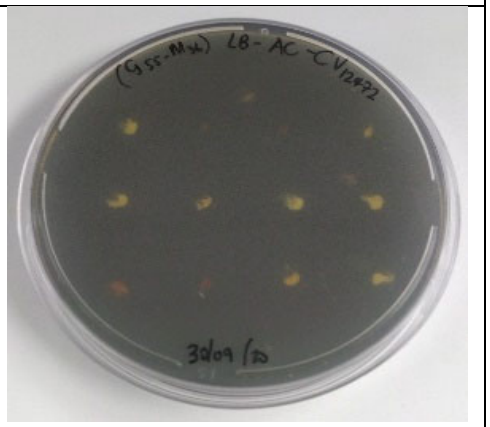
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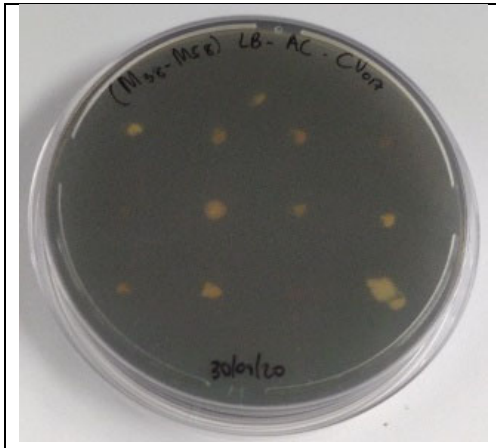
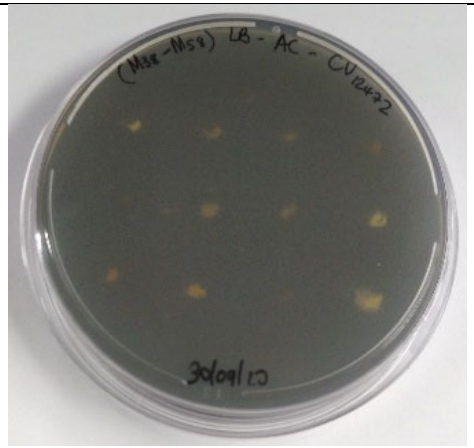
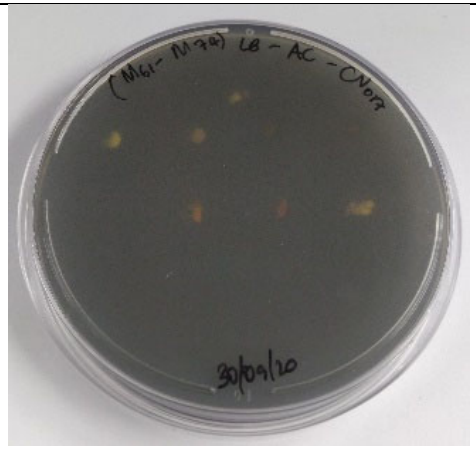
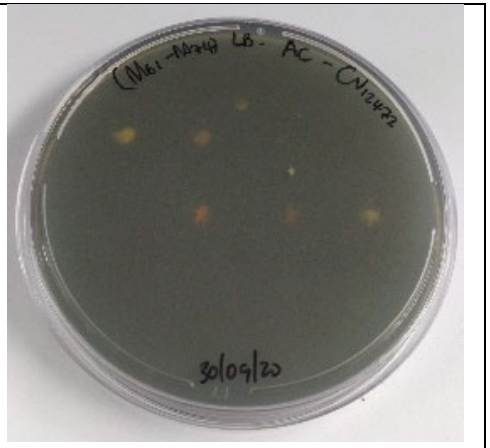
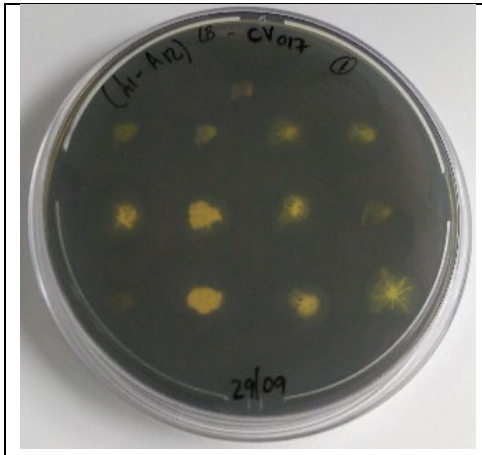
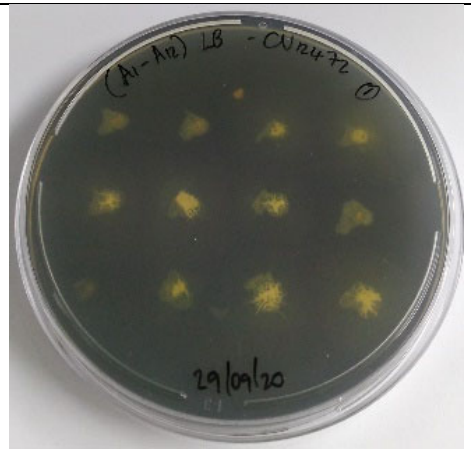
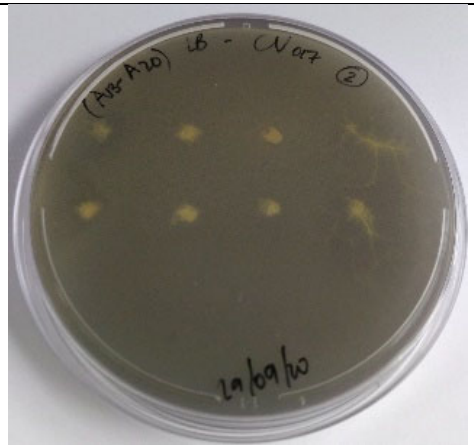
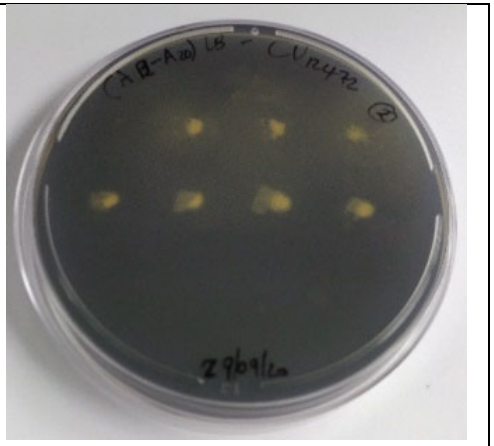
			
Ac_*C. subtsugae* CV017_M38-M58	**Ac_*C. violaceum* ATCC12472_M38-M58**	**Ac_*C. subtsugae* CV017_M61-M74**	**Ac_*C. violaceum* ATCC12472_M61-M74**

Figure S2.5: Gram-negative QS inhibition screening of *Acropora* bacterial isolates against *Chromobacterium subtsugae* CV017 and *Chromobacterium violaceum* ATCC 12472. Quorum sensing inhibition was observed by the presence of an opaque around the target bacteria as a result of loss of the purple pigmentation violacein. Killing was also observed by the appearance of a clear zone around the target bacteria.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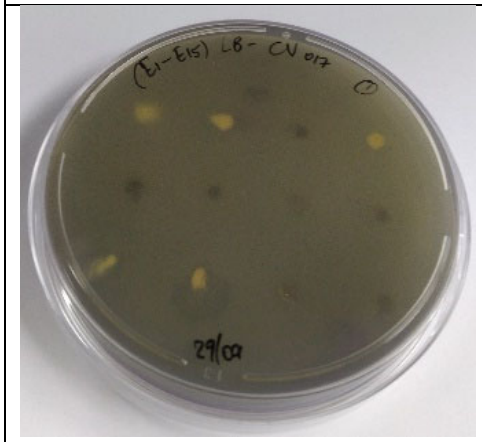
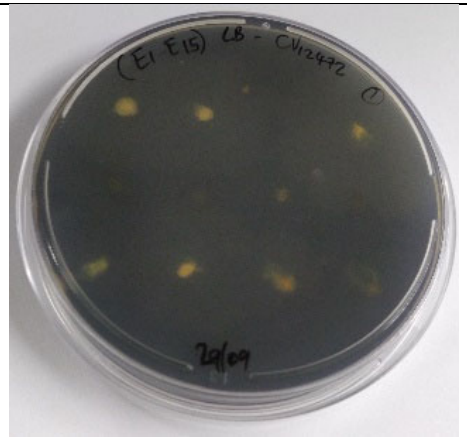
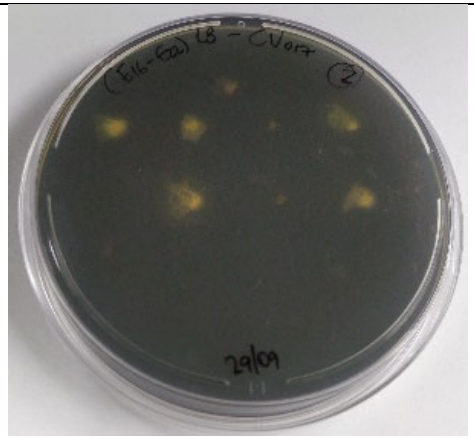
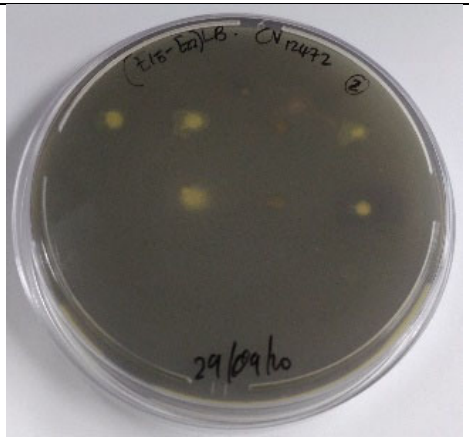
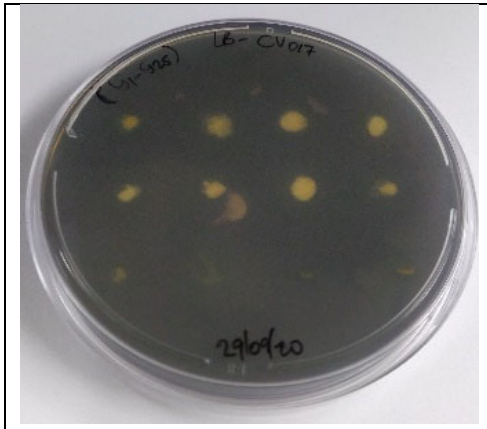
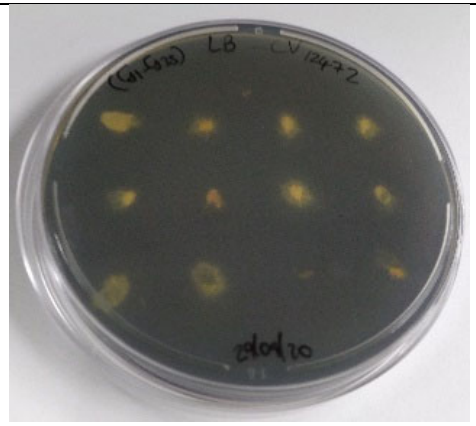
			
P_*C. subtsugae* CV017_E1-E15	**P_*C. violaceum* ATCC12472_E1-E15**	**P_*C. subtsugae* CV017_E16-E22**	**P_*C. violaceum* ATCC12472_E16-E22**



P_*C. subtsugae* CV017_G1-G25



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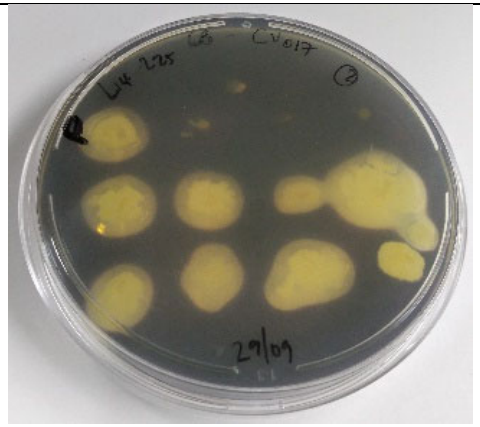

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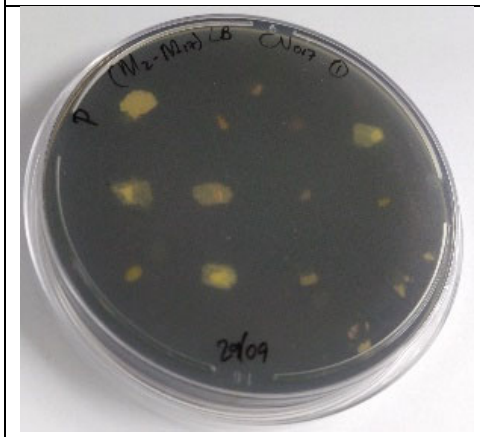

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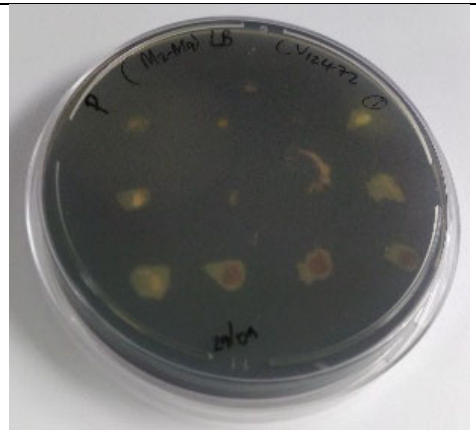

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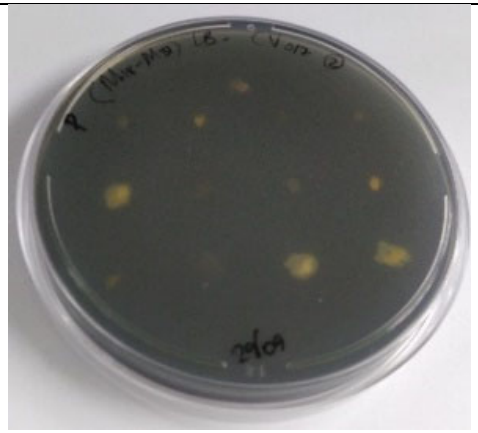
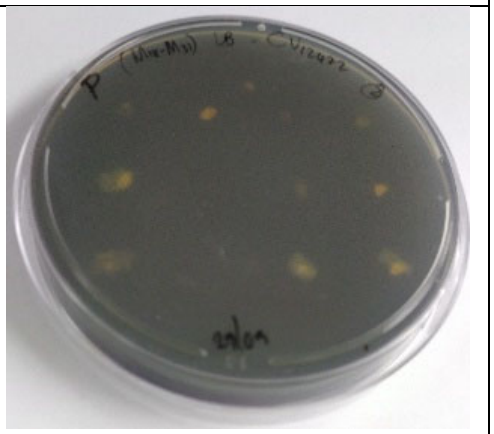
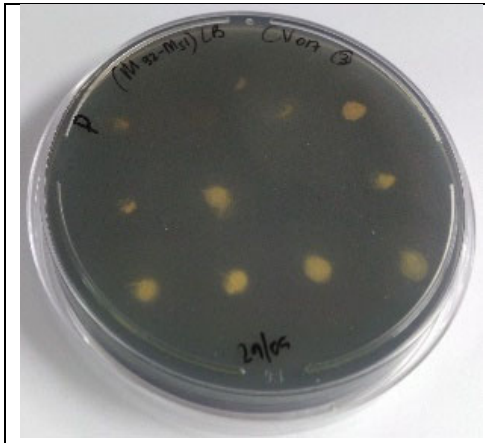


P_*C. subtsugae* CV017_M18-M31



P_*C. violaceum* ATCC12472_M18-M31



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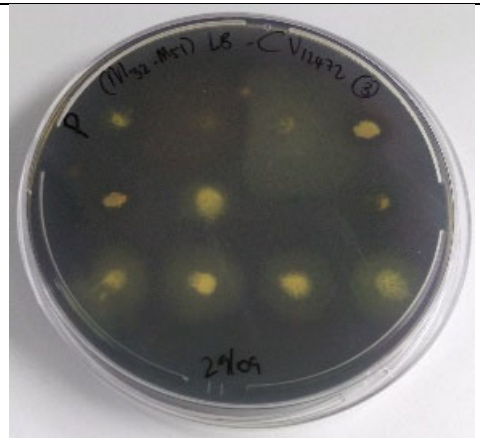
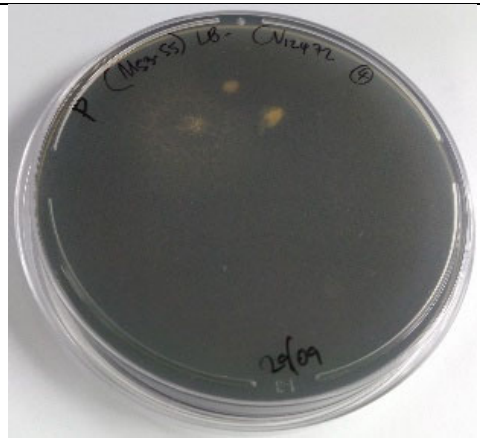


P_*C. violaceum* ATCC12472_M32-M51



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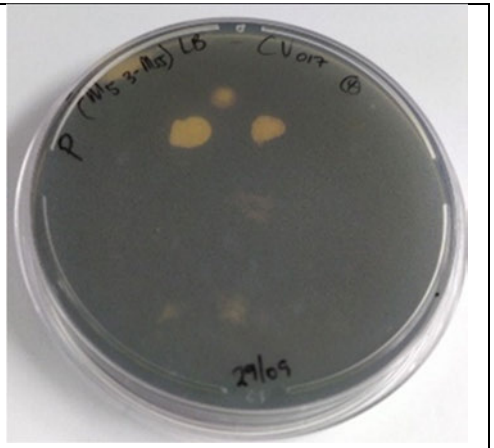
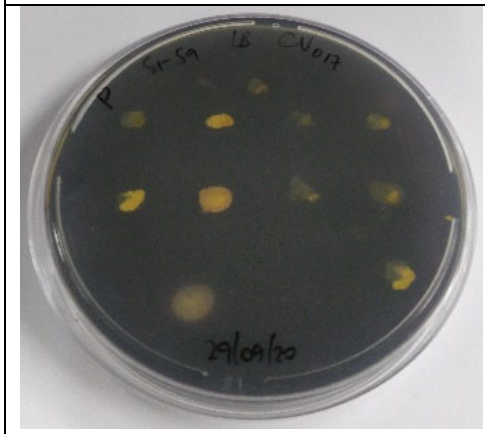
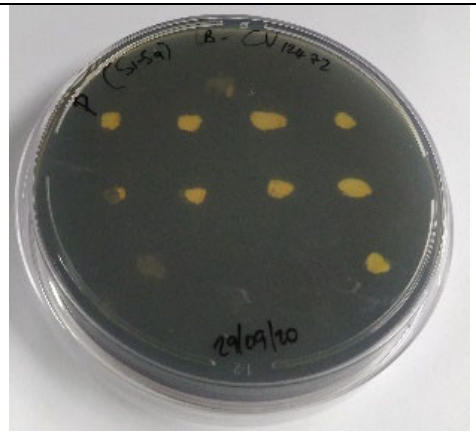


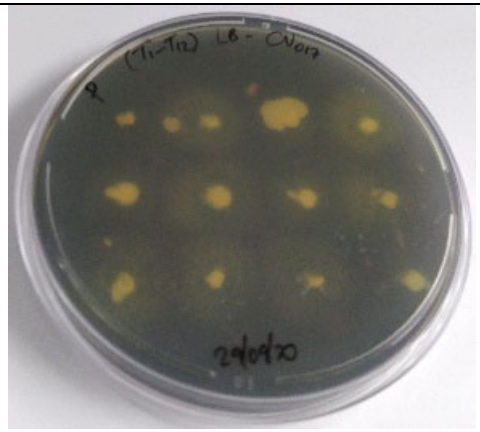
P_*C. violaceum* ATCC12472_M53-M55



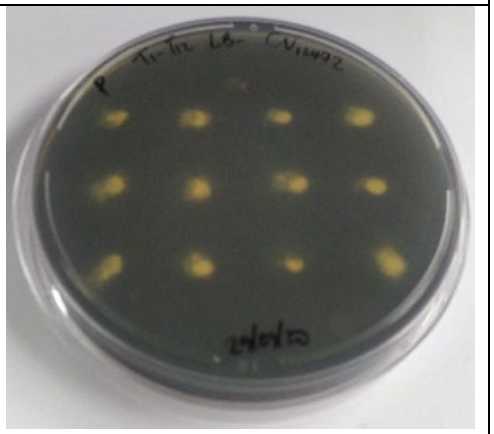
P_*C. subtsugae* CV017_S1-S9



P_*C. violaceum* ATCC12472_S1-S9



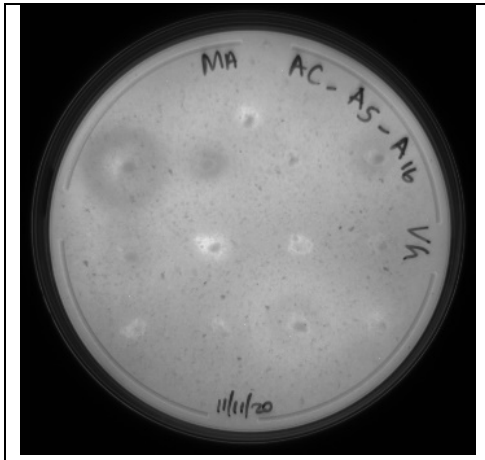
P_*C. subtsugae* CV017_T1-T12



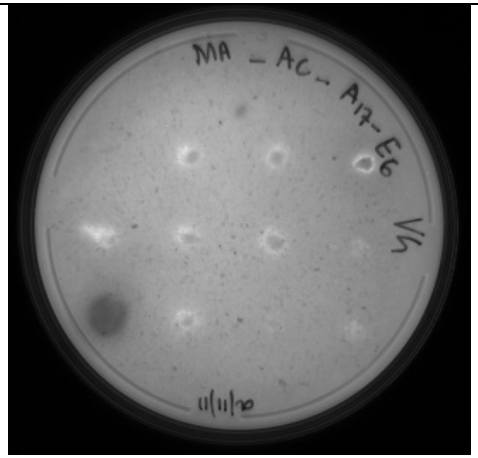
P_*C. violaceum* ATCC12472_T1-T12

Figure S2.6: Gram-negative QS inhibition screening of *Pocillopora* bacterial isolates against *Chromobacterium subtsugae* CV017 and *Chromobacterium violaceum* ATCC 12472. Quorum sensing inhibition was observed by the presence of an opaque around the target bacteria as a result of loss of the purple pigmentation *violacein*. Killing was also observed by the appearance of a clear zone around the target bacteria.

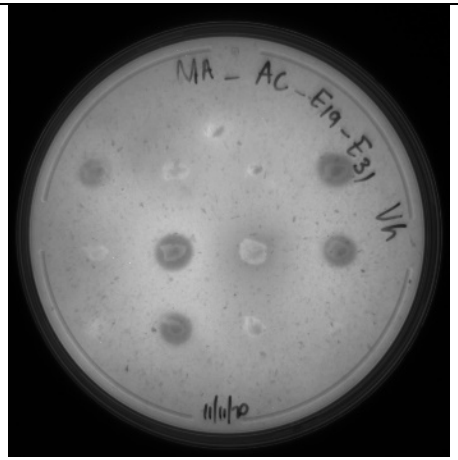
Autoinducer-2 inhibition



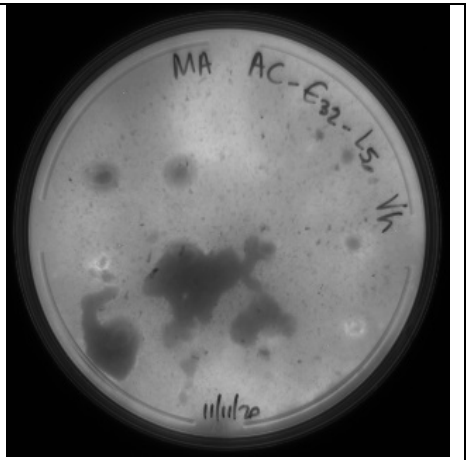
Ac_*V. harveyi*_A5-A16



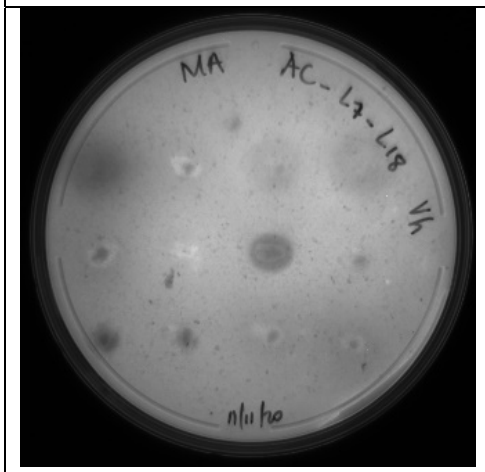
Ac_*V. harveyi*_A17-E6



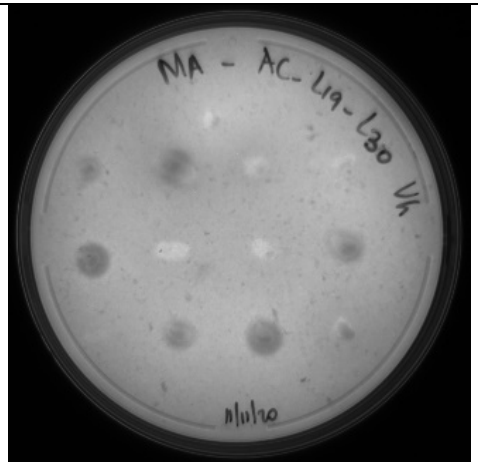
Ac_*V. harveyi*_E19-E31



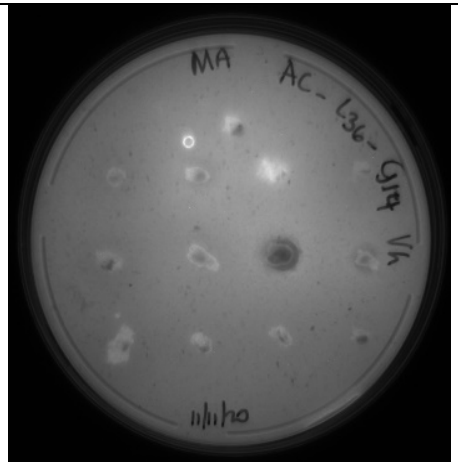
Ac_*V. harveyi*_E32-L5



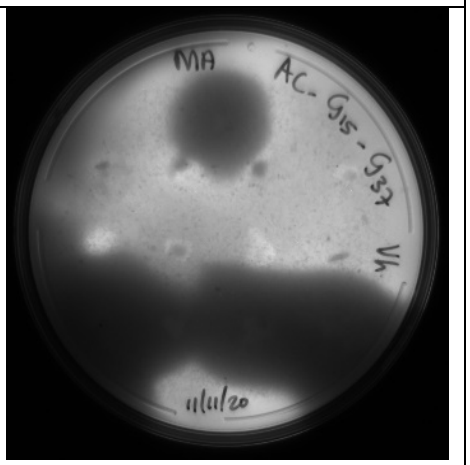
Ac_*V. harveyi*_L7-L18



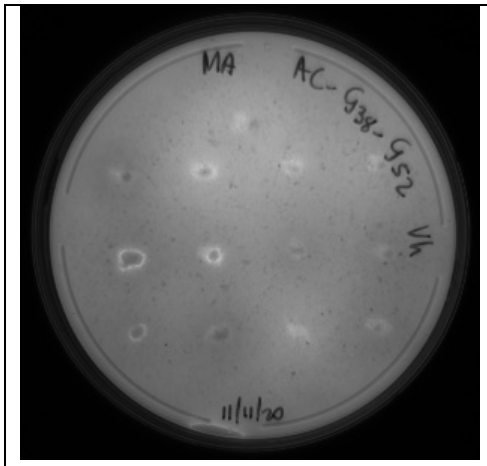
Ac_*V. harveyi*_L19-L30



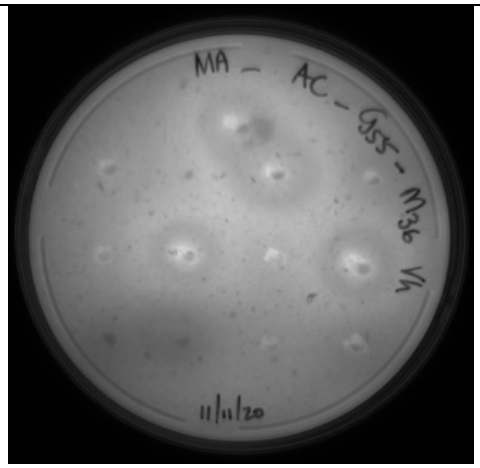
Ac_*V. harveyi*_L36-G14



Ac_*V. harveyi*_G15-G37



Ac_*V. harveyi*_G38-G52



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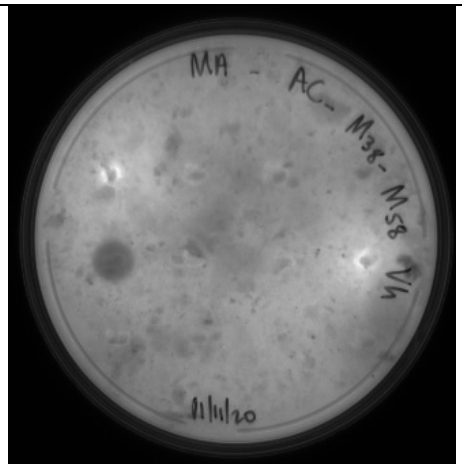

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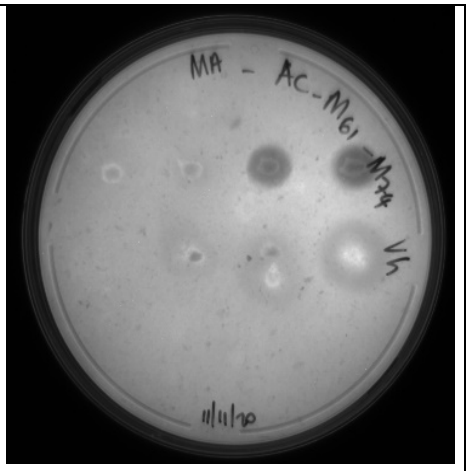

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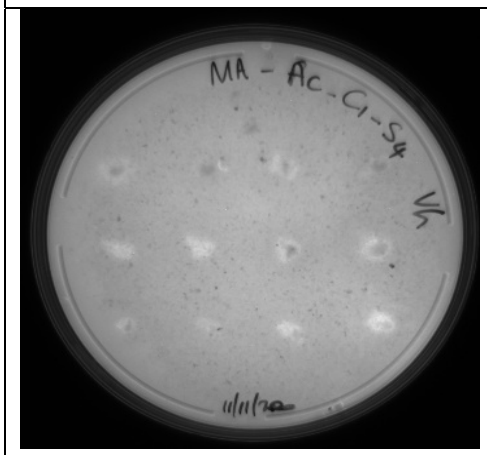
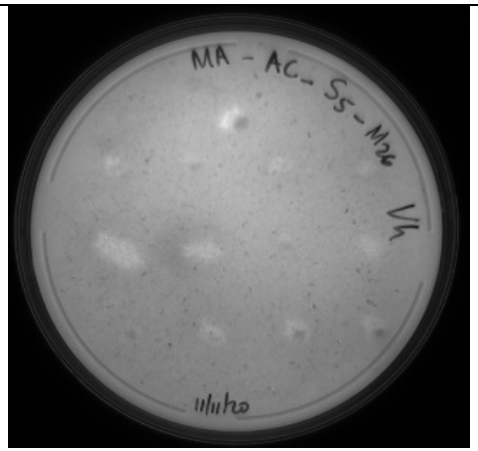
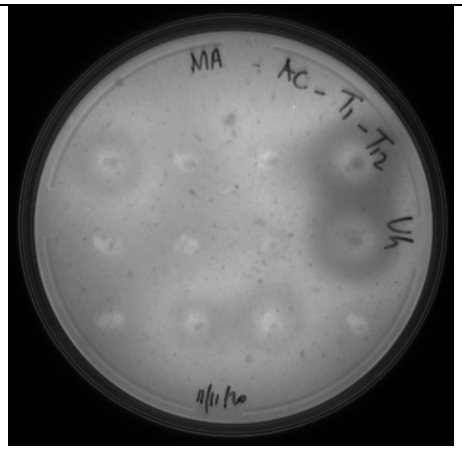


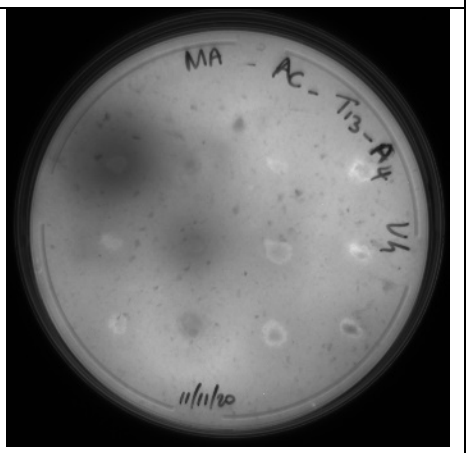
Ac_*V. harveyi*_C1-S4



Ac_*V. harveyi*_S5-M26

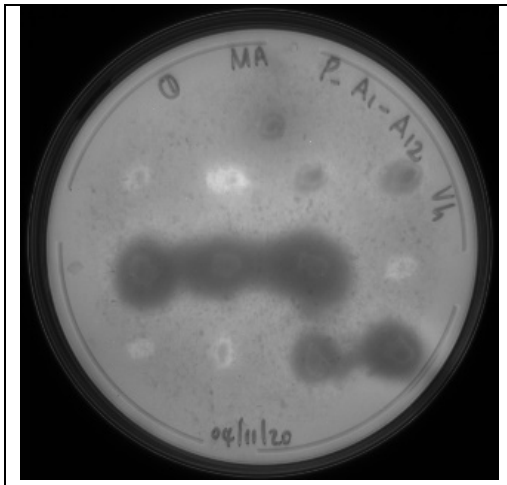
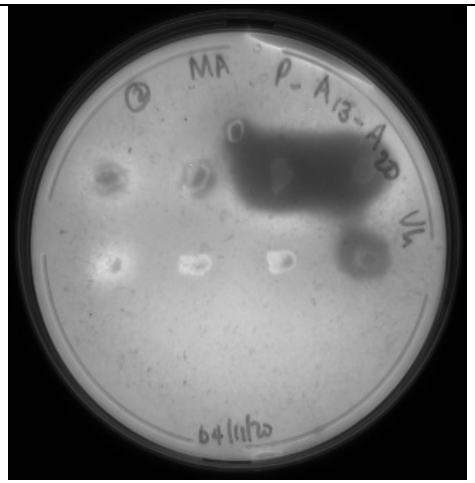
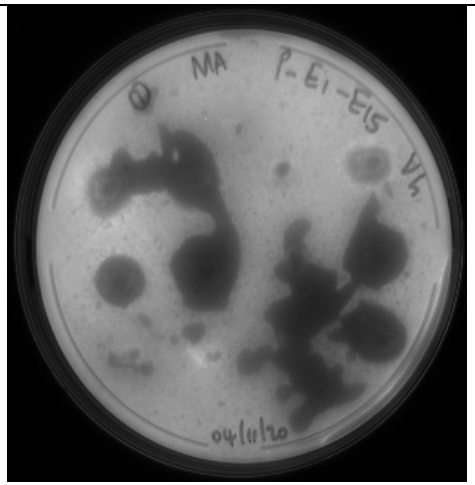
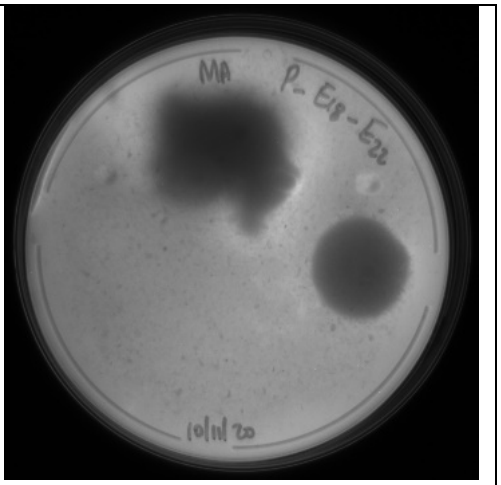
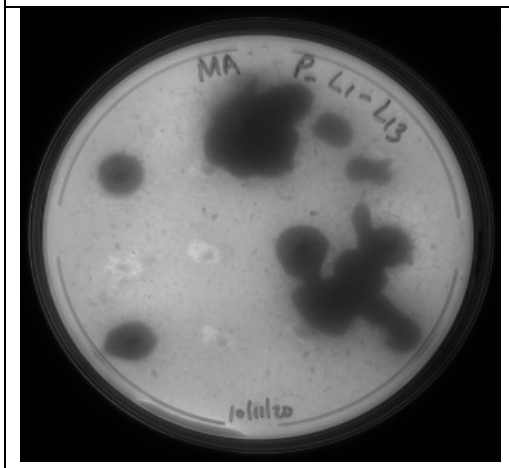
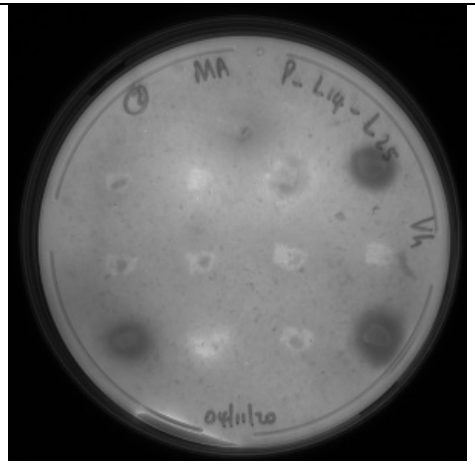
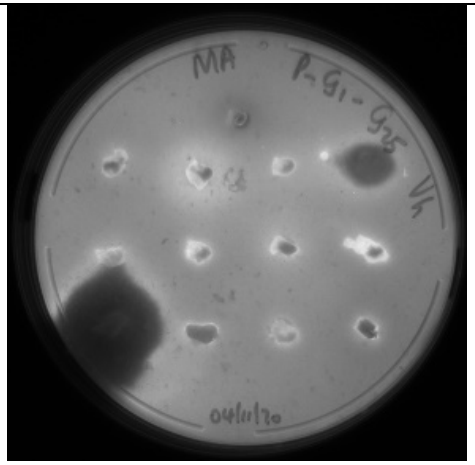
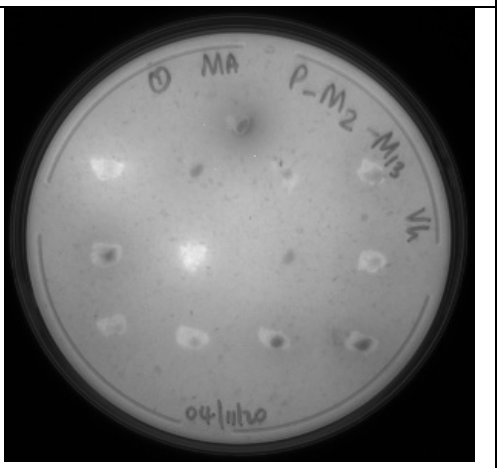


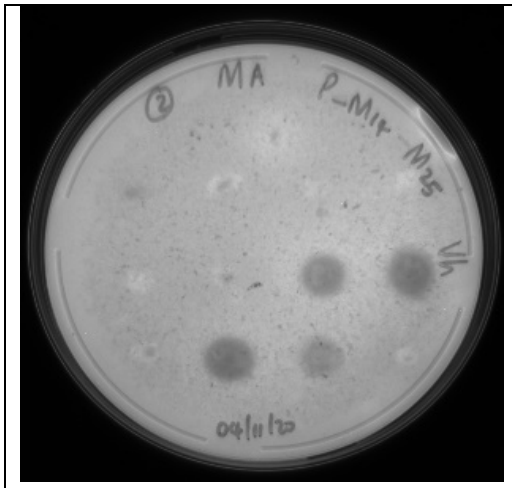
Ac_*V. harveyi*_T1-T12



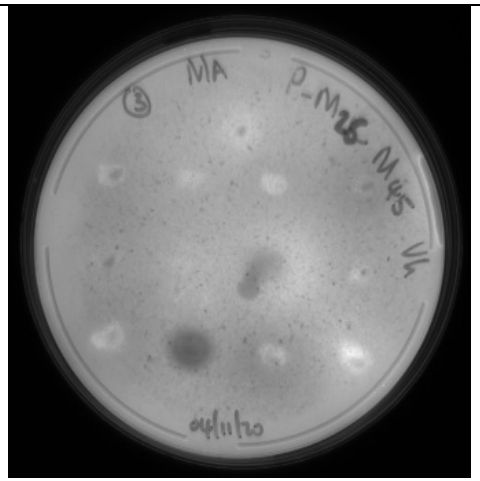
Ac_*V. harveyi*_T13-A4

Figure S2.7: Screening for AI-2 inhibition of *Acropora* bacterial isolates against *Vibrio harveyi* BB120 for detection of bioluminescence inhibition. AI-2 inhibition was observed by the appearance of dark zones lacking bioluminescence around the bacterial isolates by viewing plates using a GeneSys image analyzer (Syngene).

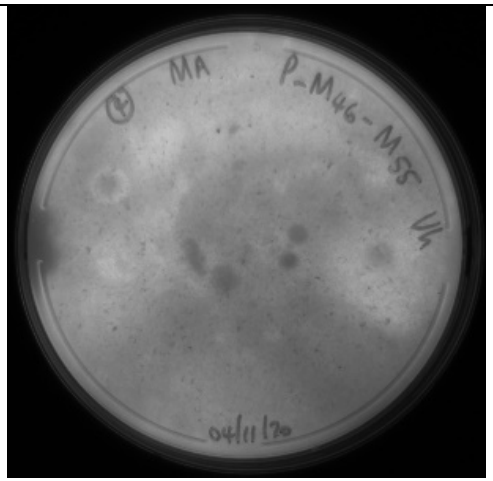
			
P_*V. harveyi*_A1-A12	**P_*V. harveyi*_A13-A20**	**P_*V. harveyi*_E1-E15**	**P_*V. harveyi*_E18-E22**
			
P_*V. harveyi*_L1-L13	***P_V. harveyi*_L14-L25**	**P_*V. harveyi*_G1-G25**	**P_*V. harveyi*_M2-M13**



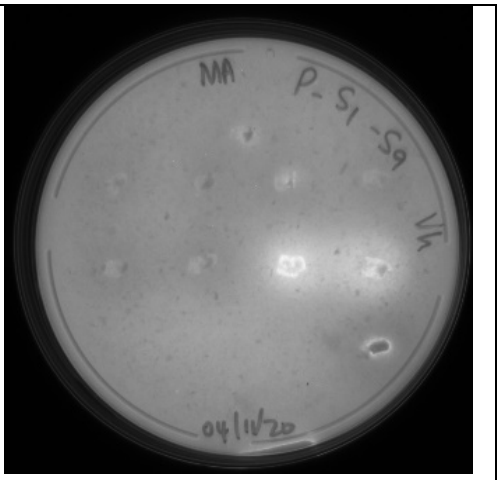
P_*V. harveyi*_M14-M25



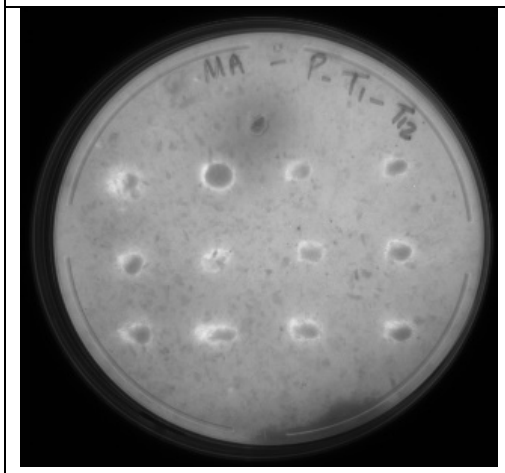
P_*V. harveyi*_M26-M45



P_*V. harveyi*_M46-M55



P_*V. harveyi*_S1-S9



P_*V. harveyi*_T1-T12

Figure S2.8: Screening for AI-2 for *Pocillopora* bacterial isolates against *Vibrio harveyi* BB120 for detection of bioluminescence inhibition. AI-2 inhibition was observed by the appearance of dark zones lacking bioluminescence around the bacterial isolates by viewing plates using a GeneSys image analyzer (Syngene).

CHAPTER 3

ANTIMICROBIAL, ANTI-QUORUM SENSING AND ANTIBIOFILM POTENTIAL OF *Pocillopora* SPECIES-ASSOCIATED BACTERIAL EXTRACTS AGAINST CLINICAL AND MARINE PATHOGENS

Abstract

Coral-associated bacteria are recognized as a potential pool of bioactive compounds for the protection and maintenance of the coral microbiome and discovery of pharmaceutical compounds. The antimicrobial, anti-quorum sensing and anti-biofilm potential of selected *Pocillopora* coral-associated bacteria (CAB) extracts (n=16) was assessed. Shake-flask fermentations and ethyl acetate extractions of the selected bacterial isolates were carried out. Bacterial isolates were identified by 16S rRNA gene amplification and sequencing. Extracts were assessed for antimicrobial activity using antimicrobial susceptibility testing (AST) against selected indicator organisms, *Pseudomonas aeruginosa* ATCC 27853*, Staphylococcus aureus* ATCC 43300*, Shewanella putrefaciens* ATCC 8071*, Vibrio alginolyticus* ATCC 17749*, Vibrio coralliilyticus* ATCC_BAA 450*, Vibrio parahaemolyticus* ATCC 17802*,* and *Vibrio shilonii* ATCC_BAA 91. Quorum sensing (QS) inhibition was assessed using qualitative and quantitative violacein inhibition assays against biosensors *Chromobacterium violaceum* ATCC 12472 and *Chromobacterium subtsugae* CV017, while autoinducer-2 (AI-2) inhibition was detected using *Vibrio harveyi*. Anti-biofilm potential was assessed using violacein inhibition microtiter plate assay against the selected indicators. Antimicrobial activity was demonstrated by 6.25% (1/16) of the extracts against all clinical pathogens, 43.75% (7/16) against marine indicators, with two extracts showing strong activity. Qualitatively, QS inhibition was demonstrated by 93.75% (15/16) of the extracts against *C. violaceum* and 100% (16/16) against *C. subtsugae*. Quantitatively, QS inhibition was demonstrated by 56.25% (9/16) of the extracts against *C. violaceum* and 37.5% (6/16) against *C. subtsugae*. Autoinducer-2 inhibition was exhibited by 68.75% (11/16) of the extracts. For initial adhesion reduction, 75% (12/16) of the extracts demonstrated activity, with five demonstrating activity against two indicators. Mature biofilm reduction (≥50%) was exhibited by 75% (12/16) of the extracts with one extract *Phaeobacter gallaeciensis* (P_M18) showing activity against three indicators and six extracts showing activity against two indicators. For initial adhesion, *S. putrefaciens* and *V. shilonii* were inhibited the most. For mature biofilms, *V. coralliilyticus* was mostly inhibited. *Phaeobacter gallaeciensis* extract P_M18 demonstrated a broad spectrum of inhibitory effects

across all bioassays, which may be due to its previously reported antagonistic effects. This study indicates that coral-associated bacteria may be used as potential probiotics in both marine and clinical settings as they have been observed to produce secondary metabolites with potential bioactivity.

3.1 Introduction

Marine environments are known to be the richest ecosystems with diverse life forms (McDevitt-Irwin *et al*., 2017; Rosado *et al.,* 2019). Marine invertebrates such as corals host a variety of microbes to help maintain coral host homeostasis through chemical defense mechanisms to protect the corals from pathogens and stressful environmental conditions (Boilard *et al*., 2020; Hou *et al.,* 2019). Coral-associated bacteria (CAB) play a vital role in maintaining coral health as they produce a wide range of bioactive natural products with unique features and pharmaceutical importance (Rodrigues *et al*., 2019; Sang *et al*., 2019). Importantly, novel compounds isolated from CAB show a spectrum of bioactivities such as antimicrobial, anti-quorum sensing, antiviral and anti-biofilm activities against various coral pathogens, disrupting their cell-to-cell communication and inhibiting their catabolic enzymes (El-Kurdi *et al*., 2021; Hugget *et al*., 2019; Sang *et al*., 2019; Zhou *et al.,* 2020).

Increasing antimicrobial resistance to existing antibiotics has become a serious health problem leading to a great demand for novel antimicrobial compounds from marine microbial communities (Carroll *et al.,* 2019; Puglis *et al.,* 2019). Antimicrobial compounds have been isolated from CAB, with some compounds displaying strong activity against different pathogens (Chang *et al*., 2017; Pollock *et al*., 2018). Most bioactive compounds from CAB display antimicrobial activity (Modolon *et al.,* 2020; Raina *et al.,* 2016; Sharma *et al*., 2019). Actinobacteria isolated from a soft coral were identified to produce a secondary metabolite thiocoraline which exhibits antimicrobial and antitumor properties (Fu *et al*., 2013). The activity of this metabolite is reported to be broad spectrum as it is antiparasitic and antiviral (Fu *et al*., 2013). Kvennefors *et al.* (2012) reported strong antimicrobial activity of *Vibrio coralliilyticus* and *Pseudoalteromonas* isolated from coral *Acropora millepora* against other CAB. Some of the CAB antimicrobial compounds are effective for controlling opportunistic microbes and clinical pathogens such as *Aspergillus fumigatus*, *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus* (Atencio *et al.,* 2018).

Several studies have shown that CAB produce acyl-homoserine lactones (AHLs) (Ishaque *et al*., 2020; Wu *et al*., 2018). These AHLs have been demonstrated to be drivers of ecological behaviors such as biofilm formation and antibiotic production (Ma *et al*., 2016).

Goldberg *et al.* (2011) screened 100 CAB for their ability to produce AHLs using reporter strains *Agrobacterium tumefaciens* and *E. coli*. It was found that 30% of the isolates produced AHLs. The CAB producing AHLs were mainly affiliated with Proteobacteria, commonly found in the coral holobiont. Majority of the sequenced isolates were most closely related to the *Vibrio* genus, with other *Erythrobacter, Photobacterium, Pseudoalteromonas, Ruegeria* and *Thalassomonas*. Tait *et al*. (2010) used *E. coli* and *Vibrio harveyi* to detect AHLs and AI-2 production by CAB, *Photobacterium rosenbergii* and *Vibrio* species. Twenty-nine isolates were tested and approximately 59% (17/29) produced AHLs and 100% (29/29) produced AI-2. Therefore, these studies suggest that the presence and control of AHL signalling may play an important role in the ecology of corals. A *V. harveyi* strain isolated from a *Favia* species demonstrated evidence of quorum-quenching that inhibited biofilm formation against *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (Golberg *et al.,* 2013). Coral-associated bacterial compounds are also suggested to exhibit quorum sensing (QS) inhibitory potential by disrupting QS signals produced by pathogens that are known to destabilize the coral microbiome structure and health (Su *et al.,* 2017; van de Water *et al*., 2017). Kvennefors *et al*. (2012) reported strong inhibitory activity of CAB *V. coralliilyticus* and *Pseudomonas* against other isolated CAB. Coral-associated bacteria reduce the expression of QS-dependent virulence factors by pathogenic microorganisms (Ma *et al*., 2018). Martinez-Matamoros *et al*., (2016) reported that the compounds tyrosol and tyrosol acetate found from CAB *Oceanobacillus* to demonstrate anti-QS activity. Evidence of anti-QS properties of CAB *Staphylococcus hominis* D11 against *P. aeruginosa* in a dose-dependent manner was demonstrated (Ma *et al*., 2018). This supports the hypothesis that CAB are a source of novel anti-QS and anti-biofilm compounds and that CAB demonstrate more anti-QS activity compared to other marine sources (El-Kurdi *et al*., 2021). Song *et al*. (2019) demonstrated that from the 200 strains isolated from *Pocillopora damicornis,* 15% exhibited inhibition activity against *Chromobacterium violaceum* ATCC 12472 (Rosado *et al*., 2019).

Multiple antifouling bioactive compounds such as 6, 8, 5', 6' – tetrahydroxy-3'-mrthylflavone (223), emodin (224) and citrorosein. has also been identified from CAB with potent antifouling activities (Song *et al.,* 2018). Bacteria isolated from corals exhibit more antifouling properties than free-floating bacteria (Eskander *et al*., 2018). These bacteria play a vital role in the protection of mature corals by the production of QS inhibitory compounds for the inhibition of initial bacterial adhesion, thus preventing the growth of parasites that use coral surfaces for substrate settlement, because QS signals drive biofilm formation (Eskander *et al*., 2018). Rosado *et al*. (2019) demonstrated that the presence of CAB with the potential to

produce anti-biofilm compounds is essential for the coral to fight infections. A *Pseudoalteromonas* strain exhibited anti-biofilm activity against the marine pathogen *V. coralliilyticus*, which caused disease in the coral *P. damicornis*.

Anti-biofilm CAB metabolites are also strong candidates for controlling clinically important pathogens. For example, 100 µg ml/1 of *Bacillus firmus* CAB-E2 liquid-culture ethyl-acetate extracts isolated from *Acropora digitifera* reduced a Methicillin-Resistant *Staphylococcus Aureus* biofilm by 83% and exopolysaccharide formation by 79% (Gowrishankar *et al.,* 2012). Under the same conditions, *Vibrio parahemolyticus* CAB-E4 extracts from *A. digitifera* inhibited MRSA biofilm formation by 79% (Gowrishankar *et al*., 2012). *Bacillus horikoshii* (Thenmozhi *et al.,* 2009), *Streptomyces akiyoshiensis* (Bakkiyaraj and Pandian, 2010), *S. rochei, Propionibacterium* and *M. luteus* are other CAB isolated from *A. digitifera* that produce antifouling compounds (Nithyanand et al., 2010). *Bacillus firmus* extracts isolated from a coral reduced MRSA biofilm by 83%, while *V. parahaemolyticus* isolated from the same coral inhibited *S. aureus* biofilm formation by 79% (Gowrishankar *et al*., 2012). Genera such as *Bacillus* and *Streptomyces* were other CAB previously reported to produce anti-biofilm compounds (Bakkiyaraj and Pandian, 2010; Nithyanand *et al.,* 2010; Thenmozhi *et al.,* 2009).

Coral-associated bacteria have ecological and clinical significance which may be useful in pharmaceutical and coral applications (Modolon *et al.,* 2020). This study examines the antimicrobial, anti-quorum sensing and anti-biofilm inhibitory potential of selected *Pocillopora* bacterial extracts against clinical indicators, marine fouling bacteria and marine pathogens.

3.2 Materials and Methods

3.2.1 Fermentation and ethyl acetate extraction

Sixteen bacterial isolates cultured from the *Pocillopora* species which demonstrated antimicrobial and/or quorum sensing inhibition activity in the colony drop assay (Chapter 2) were selected for fermentation and further screening (Suppl. Fig. S3.1). These isolates were pre-cultured in 5 ml ISP2 broth per litre (4g yeast extract powder, 10g malt exrtact powder, 4g dextrose, 1L distilled water) in duplicate for 2 d and 3 ml of each pre-culture was inoculated into 250 ml of medium Mannitol. Isolates were incubated with 150 rpm shaking at 30 °C for 7 d (Varela *et al*., 2021). Bacterial cells were centrifuged at 9 500 rpm for 10 min to collect cell-free supernatant (CFS). The organic layer was collected using a separatory funnel, where the solvent and the liquid separate into two layers, then majority of the bottom layer is drained into

a labelled flask for extraction. An equal volume (1:1 of ethyl acetate) was added to each CFS followed by a 150 rpm agitation of 1 h at 30 °C. The organic layer was collected and the remaining CFS was subjected to a second (1:1) ethyl acetate extraction and 150 rpm agitation for 4 h to collect the organic layer. Both layers were combined, and the ethyl acetate was evaporated in a rotary evaporator (Ilmvac, ROdist digital 230V 50/60Hz). Each extract was solubilized in 3 ml methanol and was added into a pre-weighed vial. Methanol was allowed to evaporate for 5-7 d and each extract obtained was weighed and dissolved in 10% v/v dimethyl sulfoxide (DMSO) or 100% methanol for oily extracts to a final standardized concentration of 20 mg/ml.

3.2.2 Molecular identification of bacterial isolates

3.2.2.1 Genomic DNA isolation purification

The 16 selected bacterial strains from the *Pocillopora* species were subjected to phylogenetic analysis. Genomic DNA isolation was carried out using the GeneJet Genomic DNA purification (Thermo Scientific) or ZR Fungal/Bacterial DNA Miniprep (Zymo Research) kits according to manufacturers' instructions. Plates with 24-48h cultures were flooded with 1 ml of sterile distilled water and bacterial cells were scraped off using a sterile glass Pasteur pipette into 1.5 ml sterile microcentrifuge tubes. Thereafter the contents in the microfuge tube were centrifuged for 10 min at 12 000 rpm. The DNA was eluted with 75 µl of DNA elution buffer and stored at -20 °C until required.

3.2.2.2 16S rRNA gene amplification and sequencing

The 16S ribosomal RNA genes (1.5 kb) from selected isolates were amplified using the universal primer sets F1 5'- AGAGTTTGATCITGGCTCAG-3-3'; R5 5'- ACGGITACCTTGTTACGACTT -3' (Coram and Rawlings *et al*., 2002) and 27F-DEG 5'- AGAGTTTGATCMTGGCTCAG -3'; 1492R-deg 5′-GGYTACCTTGTTACGACTT-3' (Weisburg *et al*., 1991). PCR reaction mixtures included 2 µl of template DNA, 18 µl ddH_2O, 2.5 µl buffer, 1.2 µl dNTPs, 0.2 µl of each primer set, 1.2 µl $MgCl_2$ and 1 U DNA Taq polymerase (SuperTherm). Amplification was performed in a PCR machine (MJ MINI^{TM} personal Thermal cycler; Bio-Rad) using the following conditions: DNA denaturation at 94 °C for 1 min, followed by 35 cycles of denaturation at 94 °C for 30 s, primer annealing at 52 °C for 1 min and extension at 72 °C for 1 min. A total of 5 µl of PCR mixture and 3 µl gel loading buffer were loaded into agarose gels together with the molecular weight marker, GeneRuler 100 bp plus DNA ladder (Thermo Scientific). Amplified PCR products were subjected to gel

electrophoresis in 1% (w/v) agarose gels at 80 V for 90 min in 1× TAE buffer. PCR products were visualized by UV transillumination (Syngene, UK) after staining in 0.1 mg/ml ethidium bromide for 15 min. PCR amplimers were sequenced at Stellenbosch University, raw sequences were processed using BioEdit (version 7.0) and subjected to identification using the NCBI-Blast nucleotide database.

3.2.3 *Antimicrobial susceptibility testing of the extracts against clinical and marine pathogens by disc diffusion method*

Antimicrobial activity of the bacterial extracts from the *Pocillopora* species (n=16) was assessed using the disc diffusion assay against clinical pathogens (methicillin-resistant *S. aureus* ATCC 43300, multidrug-resistant *P. aeruginosa* ATCC 27853) and marine indicators *Shewanella putrefaciens* ATCC 8071, *V. alginolyticus* ATCC 17749, *V. coralliilyticus* ATCC-BAA 450, *V. parahaemolyticus* ATCC 17802 and *V. shilonii* ATCC-BAA 91. *Staphylococcus aureus* ATCC 43300, *P. aeruginosa* ATCC 27853*, S. putrefaciens* ATCC 8071, *V. alginolyticus* ATCC 17749 and *V. parahaemolyticus* ATCC 17802 were grown on Mueller-Hinton (MH) agar plates and incubated at 30 °C for 24 h, while *V. coralliilyticus* ATCC-BAA 450, and *V. shilonii* ATCC-BAA 91 were grown on Marine Agar (MA) (Difco^{TM}) plates and incubated at 30 °C for 24 h. Following incubation, turbid cell suspensions equivalent to a 0.5 McFarland standard were prepared (Chenia, 2013) and cultures were uniformly swabbed over the entire surface of MH and MA plates. Extracts (200, 400, 600 and 800 µg) were loaded into 6 mm antimicrobial susceptibility test blank discs-oxoid. Ampicillin (AP10 µg), ceftriaxone (CRO30 µg), ciprofloxacin (CIP5 µg), erythromycin (E15 µg), gentamicin (GN10 µg), streptomycin (S10 µg) and tetracycline (T30 µg) discs were used as antibiotic controls (Chenia, 2013) and 10% v/vDMSO and 100% methanol served as the solvent control. *Pseudomonas aeruginosa* and *S. aureus* plates were incubated at 37 °C (optimal temperature) for 24 h, while *S. putrefaciens*, *V. alginolyticus*, *V. coralliilyticus*, *V. parahaemolyticus* and *V. shilonii* were incubated at 30 °C for 24 h. Following incubation, the diameter of zones of inhibition was measured and considered to be indicative of antimicrobial activity (Nithya *et al*., 2011). The following criteria of zone diameter were used to indicate activity of tested extracts: Strong activity ≥16 mm, Intermediate activity = 11 – 15 mm, and weak activity ≤11 mm (Chenia, 2013).

3.2.4 Anti-quorum sensing ability screening

3.2.4.1 Qualitative anti-quorum sensing ability

The quorum quenching (QQ) potential of the 16 selected bacterial extracts was assessed using the agar-overlay assay. Wild-type pigmented biosensor strains *C. violaceum* ATCC 12472 and *C. subtsugae* CV017 were grown overnight in Luria Bertani (LB) broth at 150 rpm at 30 °C using a rotary shaker (Chenia, 2013). Molten soft LB agar (0.5% w/v) was prepared and 5 ml was inoculated with 150 µl of the respective biosensor strains equivalent to 0.5 McFarland standard. The soft agar-culture solution was immediately poured over the LB agar plates and left to solidify. Thereafter, 6 mm blank discs were loaded with extracts (200, 400, 600, and 800 µg) and incubated in an upright position overnight at 30 °C. The inhibition of the violacein pigment production appearing as opaque zones indicated quorum quenching and clear zones around the discs were indicative of killing (Chenia, 2013). (*Z*-)-4-bromo-5-bromoethylene)-2-(5*H*)-furanone (Sigma) (20 µg/ml) was used as a positive control and 10% (v/v) DMSO and 100% methanol were used as a solvent controls (Chenia, 2013).

3.2.4.2 Quantitative quorum sensing inhibition using violacein assay

The QS inhibitory potential of the CAB extracts was quantified using the violacein inhibition assay (Chenia, 2013). The biosensor strains *C. violaceum* ATCC 12472 and *C. subtsugae* CV017 were grown overnight and 150 µl was inoculated in 3 ml of LB broth in 36 ml test tubes and varying concentrations of the extracts (200, 400, 600, 800 and 1000 µg/ml) were added and incubated at 30 °C for 24 h with shaking. Broth tubes containing *C. violaceum* ATCC 12472 and *C. subtugae* CV017 with no extracts were used as growth controls. Following incubation, 200 µl of the overnight culture was added to microtiter plate wells, and growth absorbance readings were taken at $OD_{600\ nm}$ using a microtitre plate reader (Glomax Multi+ Detection System, Promega) to determine the percentage growth inhibition (GI). If the %GI at $OD_{600\ nm}$ was ≥ 40% of the positive, untreated control, the extract was considered to have growth inhibitory activity and not considered to be good quorum sensing inhibition (QSI) indicator.

One ml of the culture supernatant was centrifuged at 12 000 rpm for 10 min to remove the cells and the pellet was evenly resuspended in 1 ml of DMSO and centrifuged at 12 000 rpm for 10 min and the violacein supernatant was collected. Violacein was quantified using a microtitre plate reader at $OD_{560\ nm}$ using Glomax Multi+ Detection System (Promega). Cinnamaldehyde and vanillin (20 µg/ml) were used as a QS inhibition positive control and LB broth as a purity control (Chenia, 2013). The percentage of violacein inhibition (VI) was

calculated as follows (Packiavathy *et al*., 2012): %VI = (control $OD_{560\ nm}$ – test $OD_{560\ nm}$ /control $OD_{560\ nm}$) × 100 (Chenia, 2013). Violacein inhibition was assessed in triplicate on two separate occasions.

3.2.5 Qualitative autoinducer-2 inhibition

An autoinducer-2 inhibition assay was also carried against the indicator *Vibrio harveyi* BB120, a wild-type bioluminescent strain using an agar overlay assay (Teasdale *et al.,* 2011). *Vibrio harveyi* was cultured on a MA plate (Difco™) and incubated at 30 °C overnight. The culture was then inoculated into Marine broth and incubated with 150 rpm agitation at 30 °C for 24 h. Following incubation, 150 µl of the *V. harveyi* broth equivalent to 0.5 McFarland standard, was inoculated in Marine soft agar (0.5% w/v) and poured over the LB-seawater agar plates and left to solidify. Once the overlay solidified, 6 mm antimicrobial susceptibility sterile blank discs were loaded with extracts (200, 400, 600 and 800 µg) and incubated in an upright position overnight at 30 °C. Zones of growth inhibition were observed visually, while bioluminescence inhibition was observed by using GeneSys image analyzer (Syngene). Autoinducer-2 inhibition was identified by the appearance of dark zones and lack of bioluminescence around the bacterial colonies. Cinnamaldehyde (10 µg/ml) was used as positive AI-2 inhibitor control (Brackman *et al*., 2008).

3.2.6 Detection of anti-biofilm activity of coral-associated bacteria extracts

Prior to the anti-biofilm assay, the 16 selected *Pocillopora* bacterial extracts were tested for antibacterial activity with sub-inhibitory and inhibitory concentrations (200, 400, 600 and 800 µg), using the disc diffusion assay (Basson *et al*., 2008). Extracts were tested against clinical pathogens *P. aeruginosa* ATTC 27853 and *S. aureus* ATCC 43300 and marine indicators *S. putrefaciens* ATCC 27853, *V. coralliilyticus* ATCC_BAA 450, *V. parahaemolyticus* ATCC 17802 and *V. shilonii* ATCC_BAA 91 to assess their effect on initial adhesion and detachment of mature biofilms. Overnight cultures were used to prepare cell suspensions, which were standardized equivalent to a 0.5 McFarland standard (Basson *et al*., 2008). For initial adhesion studies, extracts were added to 90 µl tryptic soy broth (TSB) and 10 µl of standardized cell suspension (to a final volume of 200 µl) and incubated for 24 h at 37 ˚C with agitation. For pre-formed biofilm detachment assays, 24 h biofilms were established following addition of 90 µl TSB and 10 µl of 0.5 McFarland standardized cell suspension to microtiter plate wells, and incubation and shaking at 37 ºC for 24 h. Microtiter plates were washed three times with sterile deionised water and allowed to air-dry. Following air-drying, 90 µl TSB as well as extracts at

the relevant, respective concentrations were added to wells (to a final volume of 200 µl) and microtiter plates were incubated for 24 h with agitation at 37 ˚C. The negative control contained only broth, while positive controls contained respective cell suspensions with no extracts added. After incubation, growth $OD_{600\ nm}$ values were determined using the Glomax multi + detection system (Promega) and wells with ≥40% reduction in growth were considered growth inhibitory. Thereafter, planktonic cells were removed by discarding the liquid media. Plates were processed for biofilm inhibition as described by Basson *et al*. (2008). Microtiter plates were washed three times with sterile dH_2O. Cells were fixed with 200 µl of methanol for 15 min, then air-dried. Wells were stained with 150 µl of 2% (v/) Hucker's crystal violet for 5 min. Wells were rinsed gently under running tap water then plates were allowed to dry. Glacial acetic acid (150 µl; 33% (v/v)) was used to re-solubilise cells (Basson *et al*., 2008). The OD was read at 600 nm using the Glomax multi + detection system (Promega). Tests were conducted in triplicate on two separate occasions. A measure of efficacy called percentage reduction was calculated from the blank, control, and treated absorbance values (Pitts *et al*., 2003): %reduction =[(C−B) − (T−B) C−B]/C-B ×100, where B denotes the average absorbance per well for blank wells (no biofilm, no treatment), C denotes the average absorbance per well for control wells (biofilm, no treatment), and T denotes the average absorbance per well for treated wells (biofilm and treatment).

3.2.7 Statistical analysis

Data was obtained after assays had been performed in either duplicate or triplicate to ensure validity of obtained results. One-way ANOVA analysis was employed to determine the significance of the CAB extracts bioactivity. For all analyses, a p-value of ≤ 0.05 was accepted as significant, such that the null hypothesis may be rejected. The differences in violacein inhibition with and without the addition of varying concentrations of extracts was determined using pair-wise testing based on Student's t-tests using IBM SPSS (Statistical Package for the Social Sciences) (IBM SPSS Statistics for Windows, 2017. Version 27.0. Armonk, NY: IBM Corp.), with $p \leq 0.05$ being considered significant. The difference in violacein inhibition mean values between extracts was determined using One-way repeated measures ANOVA with $p \leq 0.05$ being considered significant (Chenia, 2013).

3.3 Results

3.3.1 Preparation of ethyl acetate bacterial extracts

Sixteen *Pocillopora* bacterial isolates (Suppl. Fig. S3.1) were selected from primary screening for fermentation and secondary metabolite extracts obtained following ethyl acetate extractions and were dissolved in 10% (v/v) DMSO or methanol for further screening.

3.3.2 Molecular identification of selected bacterial isolates

Genomic DNA was isolated from the 16 selected bacterial isolates and amplified to get 16S rRNA gene fragments of ~ 1500 bp. These were compared with 16S rRNA gene sequences in the GenBank database. Gram-positive *Bacillus* spp. (n=6) and Gram-negative *Phaeobacter* spp. (n=6) predominated (Table 3.1).

3.3.3 Secondary screening of coral-associated bacterial isolates using antimicrobial susceptibility test (AST)

All extracts demonstrated no to weak activity (≤10 mm) against multidrug-resistant *P. aeruginosa* ATCC 27853 at 200–800 µg (Table 3.2; Suppl. Fig. S3.2). Only the *Phaeobacter gallaeciensis* P_M18 extract demonstrated intermediate activity against methicillin-resistant *S. aureus* ATCC 43300 at 200–800 µg (Table 3.2; Suppl. Fig. S3.2).

Against *Shewanella putrefaciens* ATCC 8071, two extracts demonstrated antimicrobial activity. *P. gallaeciensis* P_M18 extracts demonstrated intermediate activity (11 mm) at 800 µg, while *Halomonas venusta* P_E13 extract demonstrated intermediate activity at 400 - 600 µg and strong activity (16 mm) at 800 µg (Table 3.3; Suppl. Fig. S3.2). All 16 extracts tested demonstrated weak-to-no activity against *V. alginolyticus* ATCC 17749 and *V. parahaemolyticus* ATCC 17802 (Table 3.3; Suppl. Fig. S3.2).

Two extracts demonstrated activity against *V. coralliilyticus* ATCC-BAA 450. *Staphylococcus equorum* P_L12 extract demonstrated intermediate activity at 800 µg, while the *P. gallaeciensis* P_M18 extract demonstrated strong activity (18 - 24 mm) across from 200 - 800 µg (Table 3.4; Suppl. Fig. S3.2). Five extracts demonstrated activity against *V. shilonii* ATCC-BAA 91. The *P. gallaeciensis* P_E2 and *Streptomyces griseus* P_S9 extracts demonstrated intermediate activity at 800 µg, while the *P. gallaeciensis* P_E12 extract demonstrated intermediate activity at 600 - 800 µg. The *Bacillus altitudinis* P_A9 extract demonstrated intermediate activity at 400 - 800 µg, while *P. gallaeciensis* P_M18 extract demonstrated intermediate activity at 200 - 400 µg but strong activity from 600 - 800 µg (Table 3.4; Suppl. Fig. S3.2).

Table 3.1: 16S rRNA identities of 16 selected *Pocillopora* -associated bacterial isolates with corresponding colony characteristics.

Isolate Code	16S rRNA confirmation	16S rRNA confirmation	Sequence %	Colony description	Gram stain result
P_A9	*Bacillus altitudinis*	MN511801.1	98.27%	Cream white	Gram-positive rods
P_E2	*Phaeobacter gallaeciensis*	CP010784.1	99.85%	Brown	Gram-negative rods
P_E5	*Bacillus pumilus*	KC771039.1	99.58%	Cream white glossy	Gram-positive rods
P_E12	*Phaeobacter gallaeciensis*	KY357427.1	99.85%	Brown	Gram-negative rods
P_E13	*Halomonas venusta*	KC261283.1	99.58%	Pink	Gram-negative rods
P_E20	*Halomonas venusta*	KC261283.1	99.58%	Cream white	Gram-negative rods
P_G1	*Bacillus altitudinis*	MT598007.1	99.93%	Cream white glossy	Gram-positive rods
P_L4	*Phaeobacter gallaeciensis*	KY357427.1	99.85%	Brown	Gram-negative rods
P_L5	*Phaeobacter gallaeciensis*	KY357427.1	99.85%	Brown	Gram-negative rods
P_L12	*Staphylococcus equorum*	MK015791.1	99.79%	Cream white	Gram-positive cocci
P_M18	*Phaeobacter gallaeciensis*	KY357427.1	100%	Brown	Gram-negative rods
P_M24	*Phaeobacter gallaeciensis*	CP010784.1	99.85%	Brown	Gram-negative rods
P_S9	*Streptomyces griseus*	MK134633.1	99.91%	White actinomycete (blue pigment)	Gram-positive rods
P_T3	*Bacillus paralicheniformis*	MN689683.1	99.93%	Cream white	Gram-positive rods
P_T5	*Bacillus* species	KP296556.1	82.54%	Cream white	Gram-positive rods
P_T7	*Bacillus* species	MK720155.1	75.48%	Cream white	Gram-positive rods

Table 3.2: Antimicrobial activity of *Pocillopora* CAB extracts against multi-drug resistant *Pseudomonas aeruginosa* ATCC 27853 and methicillin-resistant *Staphylococcus aureus* ATCC 43300 using the disc diffusion assay.

Extracts	Zone diameter (mm)							
	P. aeruginosa ATCC 27853				*S. aureus* ATCC 43300			
	200 µg	400 µg	600 µg	800 µg	200 µg	400 µg	600 µg	800 µg
P_A9	0	0	8	8	0	0	8	8
P_E2	0	0	0	0	0	0	0	0
P_E5	0	0	0	0	0	0	0	0
P_E12	0	0	0	0	0	0	0	0
P_E13	0	0	8	9	0	0	8	8
P_E20	0	0	8	8	0	0	8	8
P_G1	0	0	8	8	0	0	8	8
P_L4	0	0	0	9	0	0	0	0
P_L5	8	8	8	9	0	0	8	9
P_L12	0	0	0	8	0	0	0	0
P_M18	8	8	9	8	11	12	12	13
P_M24	0	0	0	0	0	0	0	0
P_S9	0	0	0	0	0	0	0	0
P_T3	0	0	8	9	0	0	0	0
P_T5	8	10	10	10	0	0	0	0
P_T7	0	0	0	0	0	0	0	0
Antibiotic Controls								
Ampicillin (AMP10)	0				0			
Ceftriaxone (CTX30)	17				11			
Ciprofloxacin (CIP5)	28				24			
Erythromycin (E15)	15				0			
Gentamicin (GN10)	18				0			
Streptomycin (S10)	12				13			
Tetracycline (TE30)	9				22			
Solvent								
10% (v/v) DMSO	0				0			

*Color codes

*Red – Weak activity ≤ 10 mm

*Yellow – Intermediate activity 11- 15 mm

*Green – Strong activity ≥ 16 mm

Table 3.3: Antimicrobial activity of *Pocillopora* CAB extracts against marine bacteria *Shewanella putrefaciens* ATCC 8071, *Vibrio alginolyticus* ATCC 17749 and *Vibrio parahaemolyticus* ATCC 17802 using the disc diffusion assay.

Extracts	Zone diameter (mm)											
	S. putrefaciens ATCC 8071				*V. alginolyticus* ATCC 17749				*V. parahaemolyticus* ATCC 17802			
	200 µg	400 µg	600 µg	800 µg	200 µg	400 µg	600 µg	800 µg	200 µg	400 µg	600 µg	800 µg
P_A9	0	0	0	0	0	0	0	0	0	0	0	0
P_E2	0	0	0	0	0	0	0	0	0	0	0	0
P_E5	0	0	0	0	0	0	0	0	0	0	0	0
P_E12	0	0	0	0	0	0	0	0	0	0	0	0
P_E13	9	11	15	16	0	0	0	0	0	0	0	0
P_E20	0	0	0	0	0	0	0	0	0	0	0	0
P_G1	0	0	8	9	0	0	0	0	0	0	0	0
P_L4	0	0	0	0	0	0	0	0	0	0	0	0
P_L5	0	0	0	0	0	0	0	0	0	0	0	0
P_L12	0	0	0	0	0	0	0	0	0	0	0	0
P_M18	0	10	10	11	0	0	0	7	0	0	0	0
P_M24	0	0	0	0	0	0	0	0	0	0	0	0
P_S9	0	8	8	9	0	0	0	0	0	0	0	0
P_T3	0	0	0	0	0	0	0	0	0	0	0	0
P_T5	0	0	0	0	0	0	0	0	0	0	0	0
P_T7	0	0	0	0	0	0	0	7	0	0	0	10
Antibiotic Controls												
Ampicillin (AMP10)	9				9				0			
Ceftriaxone (CTX30)	14				15				28			
Ciprofloxacin (CIP5)	31				30				25			
Erythromycin (E15)	10				21				22			
Gentamicin (GN10)	10				10				19			
Streptomycin (S10)	16				19				20			
Tetracycline (TE30)	29				20				21			
Solvent												
10% (v/v) DMSO	0				0				0			

*Color codes

*Red – Weak activity ≤ 10 mm

*Yellow – Intermediate 11- 15 mm

*Green – Strong ≥ 16 mm

Table 3.4: Antimicrobial activity of *Pocillopora* CAB extracts against marine pathogens *Vibrio coralliilyticus* ATCC_BAA 450 and *Vibrio shilonii* ATCC BAA 91 using the disc diffusion assay.

Extracts	Zone diameter (mm)							
	V. coralliilyticus ATCC_BAA_450				*V. shilonii* ATCC_BAA 91			
	200 µg	400 µg	600 µg	800 µg	200 µg	400 µg	600 µg	800 µg
P_A9	0	0	8	9	0	11	11	12
P_E2	0	0	8	9	0	0	0	12
P_E5	0	0	0	0	0	0	0	0
P_E12	0	9	10	10	0	0	13	15
P_E13	0	0	0	8	0	0	0	0
P_E20	0	0	8	8	0	0	0	0
P_G1	0	0	0	8	0	0	0	0
P_L4	0	0	0	0	0	0	0	0
P_L5	0	0	0	0	0	0	10	10
P_L12	0	9	10	11	0	9	9	9
P_M18	18	20	21	24	11	13	16	17
P_M24	0	0	0	0	0	0	0	0
P_S9	0	0	0	0	0	10	10	11
P_T3	0	0	0	0	0	0	0	0
P_T5	0	0	8	8	0	0	0	0
P_T7	0	0	8	8	0	0	0	0
Antibiotic controls								
Ampicillin (AMP10)	0				25			
Ceftriaxone (CTX30)	41				30			
Ciprofloxacin (CIP5)	34				33			
Erythromycin (E15)	24				31			
Gentamicin (GN10)	30				30			
Streptomycin (S10)	20				22			
Tetracycline (TE30)	30				28			
Solvent								
10% (v/v) DMSO	0				0			

*Color codes

*Red – Weak activity ≤ 10 mm

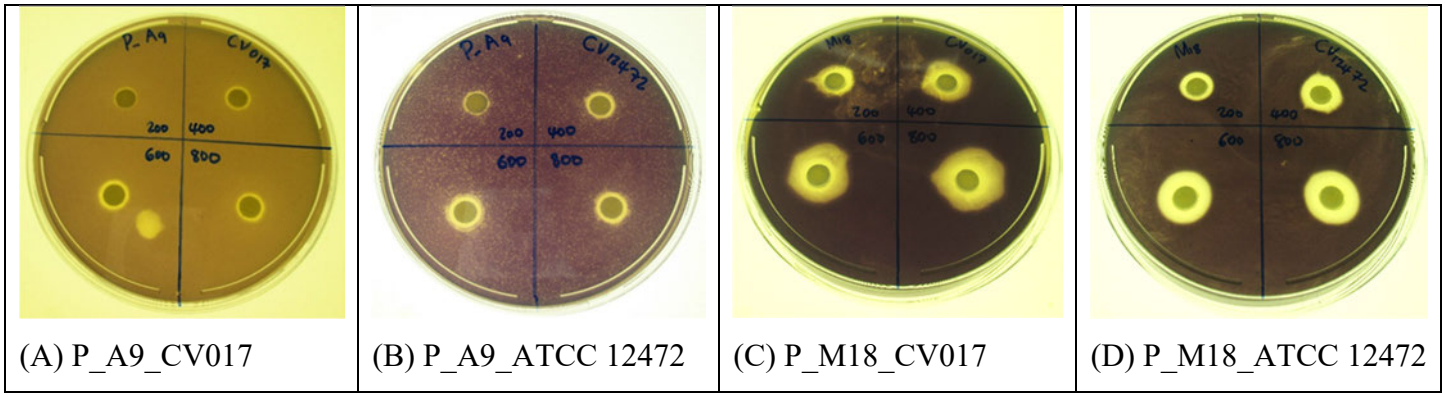
*Yellow – Intermediate 11- 15 mm

*Green – Strong ≥ 16 mm

3.3.4 Gram-negative anti-quorum sensing screening

3.3.4.1 Qualitative agar-overlay assay

All 16 *Pocillopora* CAB extracts demonstrated QS inhibitory activity against both *C. subtsugae* CV017 and *C. violaceum* ATCC 12472 (Fig. 3.1; Tables 3.5 – 3.6; Suppl. Fig. S3.3). Quorum sensing inhibition against *C. subtsugae* CV017 was demonstrated by 100% (16/16) of extracts across 200 µg – 800 µg, however, killing activity was also observed with the *P. gallaeciensis* P_M18 extract at 200 µg – 800 µg (Fig. 3.1; Table 3.5; Suppl. Fig. S3.3).



(A) P_A9_CV017 (B) P_A9_ATCC 12472 (C) P_M18_CV017 (D) P_M18_ATCC 12472

Figure 3.1: Anti-quorum sensing inhibition of selected *Pocillopora* extracts against *Chromobacterium substugae* CV017 and *Chromobacterium violaceum* ATCC 12472. (A-B) P_A9; (C-D) P_M18. The inhibition of the violacein pigment production appearing as opaque zones was indicative of QS inhibition and clear zones around the discs were indicative of killing.

Inhibition activity against long chain AHL-producing *C. violaceum* ATCC 12472 was demonstrated by 93.75% (15/16) of the extracts, with only the *P. gallaeciensis* P_M24 extract showing no QSI across all concentrations tested. The *Halomonas venusta* P_E13 and P_E20 and *Bacillus pumilus* P_G1 extracts demonstrated QSI at 400 µg – 800 µg, however, no killing activity was observed with any of the extracts. The best activity was observed with the *P. gallaeciensis* P_M18 extract (Fig. 3.1; Table 3.6; Suppl. Fig. S3.3).

Table 3.5: Quorum sensing inhibitory potential (opaque zones) demonstrated by *Pocillopora* CAB extracts using the *Chromobacterium substugae* CV017 agar-overlay assay.

Extracts	*Chromobacterium subtsugae* CV017											
	Zone diameter (mm)				Clear zone (mm)				QSI zone (mm)			
	200 µg	400 µg	600 µg	800 µg	200 µg	400 µg	600 µg	800 µg	200 µg	400 µg	600 µg	800 µg
P_A9	9	9	10	10	0	0	0	0	9	9	10	10
P_E2	9	9	9	9	0	0	0	0	9	9	9	9
P_E5	9	9	9	10	0	0	0	0	9	9	9	10
P_E12	8	8	8	8	0	0	0	0	8	8	8	8
P_E13	9	9	10	11	0	0	0	0	9	9	10	11
P_E20	9	10	10	11	0	0	0	0	9	10	10	11
P_G1	8	9	10	11	0	0	0	0	8	9	10	11
P_L4	8	9	9	9	0	0	0	0	8	9	9	9
P_L5	8	9	10	11	0	0	0	0	8	9	10	11
P_L12	9	9	9	9	0	0	0	0	9	9	9	9
P_M18	11	14	16	19	8	8	8	8	3	6	8	11
P_M24	8	8	8	8	0	0	0	0	8	8	8	8
P_S9	0	0	8	8	0	0	0	0	0	0	8	8
P_T3	7	8	8	8	0	0	0	0	7	8	8	8
P_T5	10	10	10	10	0	0	0	0	10	10	10	10
P_T7	9	9	9	9	0	0	0	0	9	9	9	9
Furanone (10 µg/ml)	9				0				9			

*Color codes

*Green – selected extracts showing QSI activity

*Yellow – extracts showing killing activity

Orange – extracts showing killing and QSI activity

*Purple – extracts demonstrating QSI activity

*If an opaque halo was present, the QSI zone was calculated as follows: Zone diameter – Clear zone.

Table 3.6: Quorum sensing inhibitory potential (opaque zones) demonstrated by *Pocillopora* CAB extracts using the *Chromobacterium violaceum* ATCC 12472 agar-overlay assay.

Extracts	*Chromobacterium violaceum* ATCC 12472											
	Zone diameter (mm)				Clear zone (mm)				QSI zone (mm)			
	200 µg	400 µg	600 µg	800 µg	200 µg	400 µg	600 µg	800 µg	200 µg	400 µg	600 µg	800 µg
P_A9	8	10	11	11	0	0	0	0	8	10	11	11
P_E2	9	9	9	9	0	0	0	0	9	9	9	9
P_E5	8	8	8	8	0	0	0	0	8	8	8	8
P_E12	8	8	8	8	0	0	0	0	8	8	8	8
P_E13	0	8	9	10	0	0	0	0	0	8	9	10
P_E20	0	8	9	10	0	0	0	0	0	8	9	10
P_G1	0	8	10	11	0	0	0	0	0	8	10	11
P_L4	8	8	9	9	0	0	0	0	8	8	9	9
P_L5	9	9	9	10	0	0	0	0	9	9	9	10
P_L12	9	9	9	9	0	0	0	0	9	9	9	9
P_M18	10	13	14	15	0	0	0	0	10	13	14	15
P_M24	0	0	0	0	0	0	0	0	0	0	0	0
P_S9	8	8	8	8	0	0	0	0	8	8	8	8
P_T3	8	8	8	8	0	0	0	0	8	8	8	8
P_T5	8	8	8	9	0	0	0	0	8	8	8	9
P_T7	8	8	8	8	0	0	0	0	8	8	8	8
Furanone ((10 µg/ml)	11				0				11			

* **Color codes**

*Green – selected extracts showing activity

*Yellow – No killing activity

*Purple – extracts demonstrating QSI activity

*If an opaque halo was present, the QSI zone was calculated as follows: Zone diameter – Clear zone.

3.3.4.2 Quantitative violacein inhibition assay

This assay was carried out to quantify violacein inhibition and ascertain whether the inhibition was a result of cell death or QSI (Figs. 3.2 - 3.3; Suppl. Figs. S3.4). Extracts that demonstrated GI ≥40% were considered bactericidal rather than demonstrating QS inhibition. Optimal QS inhibition of extracts was indicated by a VI ≥50% with <40% GI.

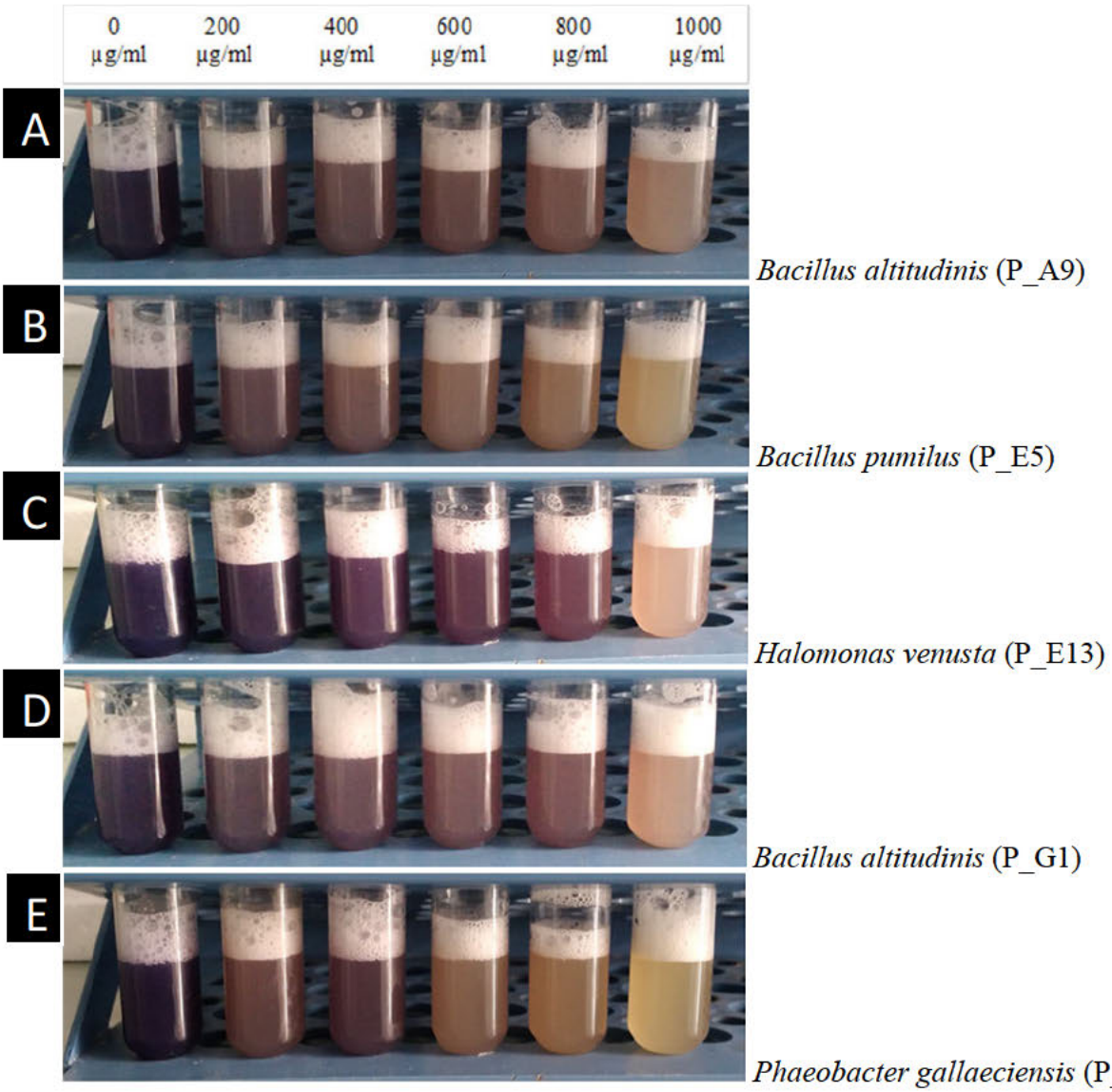


Figure 3.2: Violacein inhibitory effects of *Pocillopora* CAB extracts on the production of violacein by *Chromobacterium subtsugae* CV017 at 200-1000 µg/ml. Strong purple violacein pigment indicated less or no QS inhibition, while loss of it indicated QS inhibition or killing. (A) P_A9, (B) P_E5, (C) P_E13, (D) P_G1, (E) P_M18.

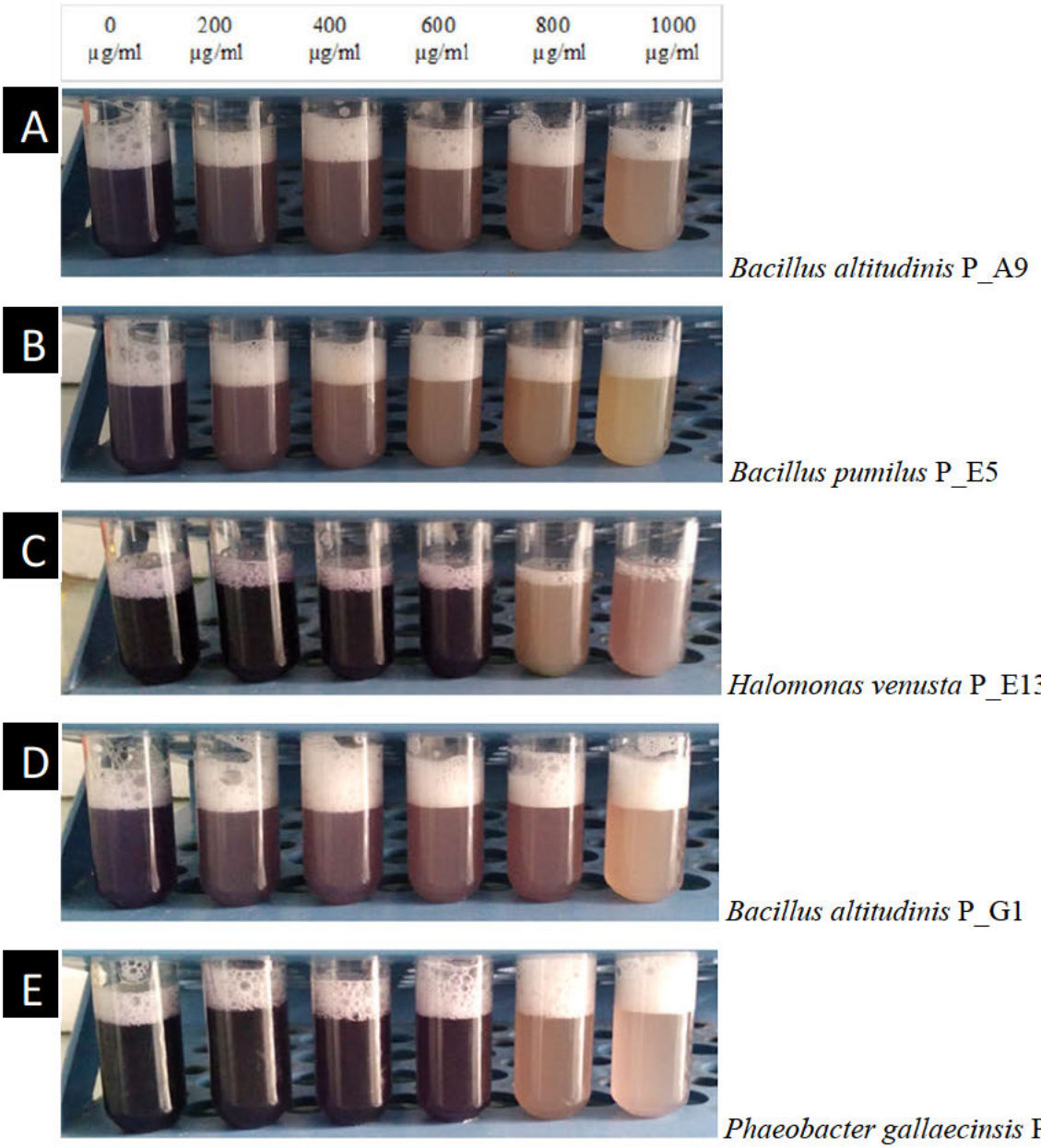


Figure 3.3: Violacein inhibitory effects of *Pocillopora* CAB extracts on the production of violacein by *Chromobacterium violaceum* ATCC 12472 at various concentrations (200 – 1000 µg/ml). Strong purple violacein pigment indicated less or no inhibition, while loss of it indicated inhibition of QS or killing. (A) P_A9, (B) P_E5, (C) P_E13, (D) P_G1, (E) P_M18.

Six extracts (37.5%) demonstrated the desired range of activity, i.e., ≥50% VI and <40% GI against *C. subtsugae* CV017 (Figs. 3.4 - 3.5; Suppl. Fig. S3.4). A single *P. gallaeciensis* extract (P_M18) demonstrated activity at 600 µg/ml – 800 µg/ml but demonstrated bactericidal activity at 1000 µg/ml (Fig. 3.5). Two extracts, P_E5 and P_S9 demonstrated activity at 800 - 1000 µg/ml, with P_E5 demonstrating greater activity at 1000 µg /ml (Fig. 3.5). Three extracts,

P_A9, P_E13 and P_G1 demonstrated activity at 1000 µg/ml, with P_G1 demonstrating the best activity, followed by P_E13 then P_A9 (Figs. 3.4 - 3.5; Suppl. Figs. S3.4).

Nine extracts (56.25%) demonstrated ≥50% VI and <40% GI against *C. violaceum* ATCC 12472. A single extract P_E20 demonstrated activity at 600 - 1000 µg/ml (Figs. 3.4 - 3.5; Suppl. Fig. S3.4). Three extracts, P_E5, P_E13 and P_M18 demonstrated activity at 800 - 1000 µg/ml, while extract P_T5 demonstrated activity at 800 µg/ml, but was bactericidal at 1000 µg/ml. Four extracts, P_A9, P_G1, P_L4 and P_T3 demonstrated activity at 1000 µg/ml (Figs. 3.4 - 3.5; Suppl. Fig. S3.4).

Against *C. subtsugae* CV017, 50% of the *Bacillus* species extracts demonstrated short-chain inhibition, whilst against *C. violaceum* ATCC 12472, 83.33% (5/6) of *Bacillus* species extracts demonstrated long-chain inhibition. Although 100% (2/2) of the *Halomonas* species extracts demonstrated long-chain inhibition, only P_E13 demonstrated short-chain inhibition (Figs. 3.4 - 3.5; Suppl. Figs. S3.4). While 33.33% (2/6) of *Phaeobacter* species extracts demonstrated QS inhibition potential against long-chain AHL producers, only one demonstrated short-chain inhibition. *Phaeobacter* spp. extract QS inhibitory activity was not as good as that observed with *Bacillus* species extracts. *Phaeobacter* species extracts demonstrated better antimicrobial activity, while *Bacillus* species extracts demonstrated better QS inhibition.

Overall, five extracts P_A9, P_E5, P_E13, P_G1 and P_M18 significantly demonstrated QS inhibition activity against both biosensors ($p \leq 0.05$), thus demonstrating broad-spectrum activity. These extracts were most effective at higher concentrations, i.e., 600 - 1000 µg /ml for both biosensors (Figs. 3.4 - 3.5; Suppl. Figs. S3.4). The P_M18 extract demonstrated better overall activity at 800 µg/ml compared to P_E5, P_E13, P_G1 and P_A9 (Figs. 3.4 - 3.5; Suppl. Figs. S3.4). Although the *P. gallaeciensis* extract (P_M18) demonstrated the best overall activity, broad spectrum QS inhibition activity was observed with 50% (3/6) of the *Bacillus* species extracts.

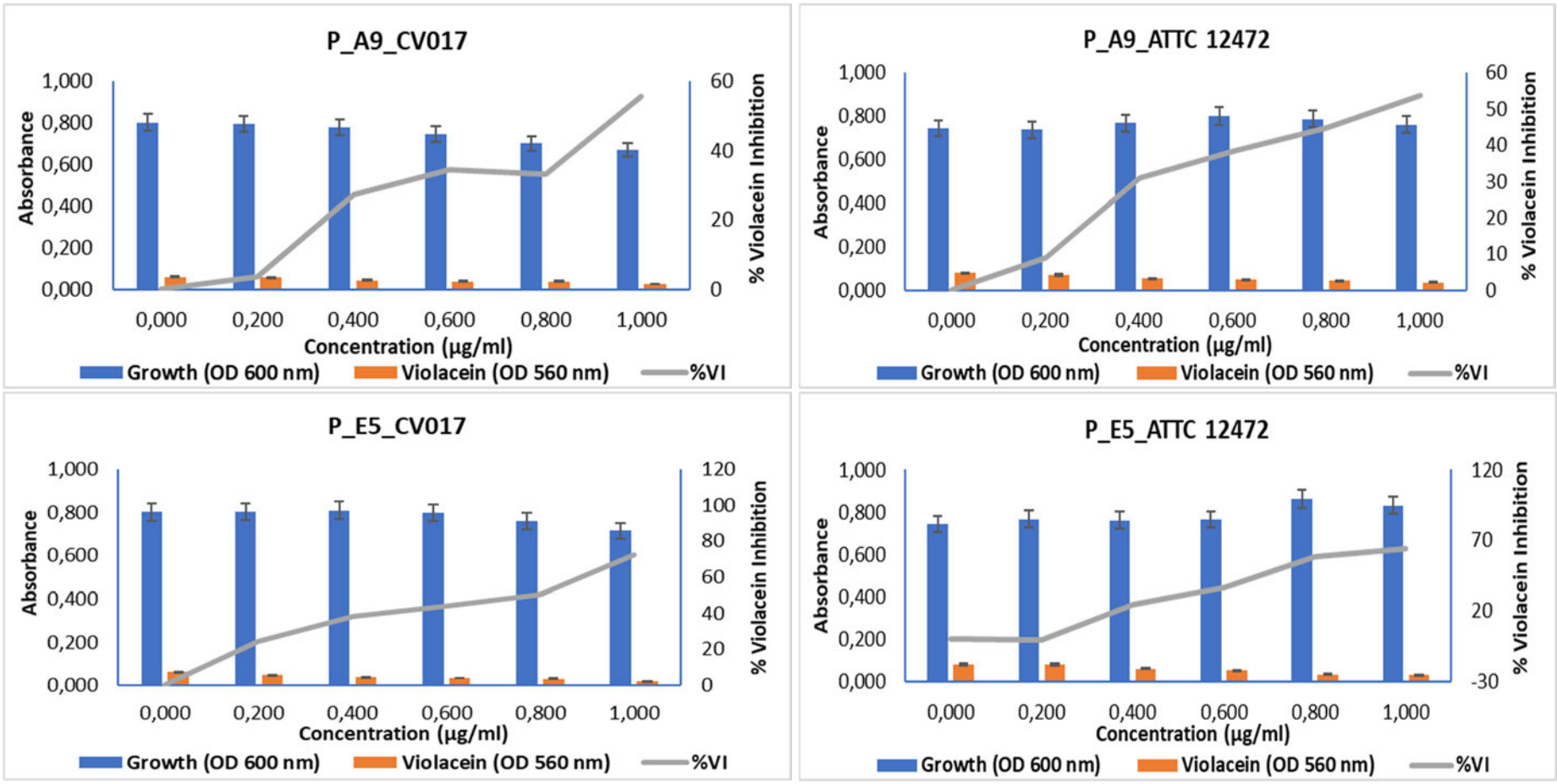


Figure 3.4: Quantitative assessment of violacein inhibitory effects of P_A9 and P_E5 extracts against *Chromobacterium subtsugae* CV017 and *Chromobacterium violaceum* ATCC 12472. Bacterial growth measured at $OD_{600\ nm}$ and violacein production at $OD_{560\ nm}$ are indicated as bar graphs, while the percentage violacein inhibition (%VI) is indicated by the line graph. Data represent the mean of two independent experiments performed in triplicate.

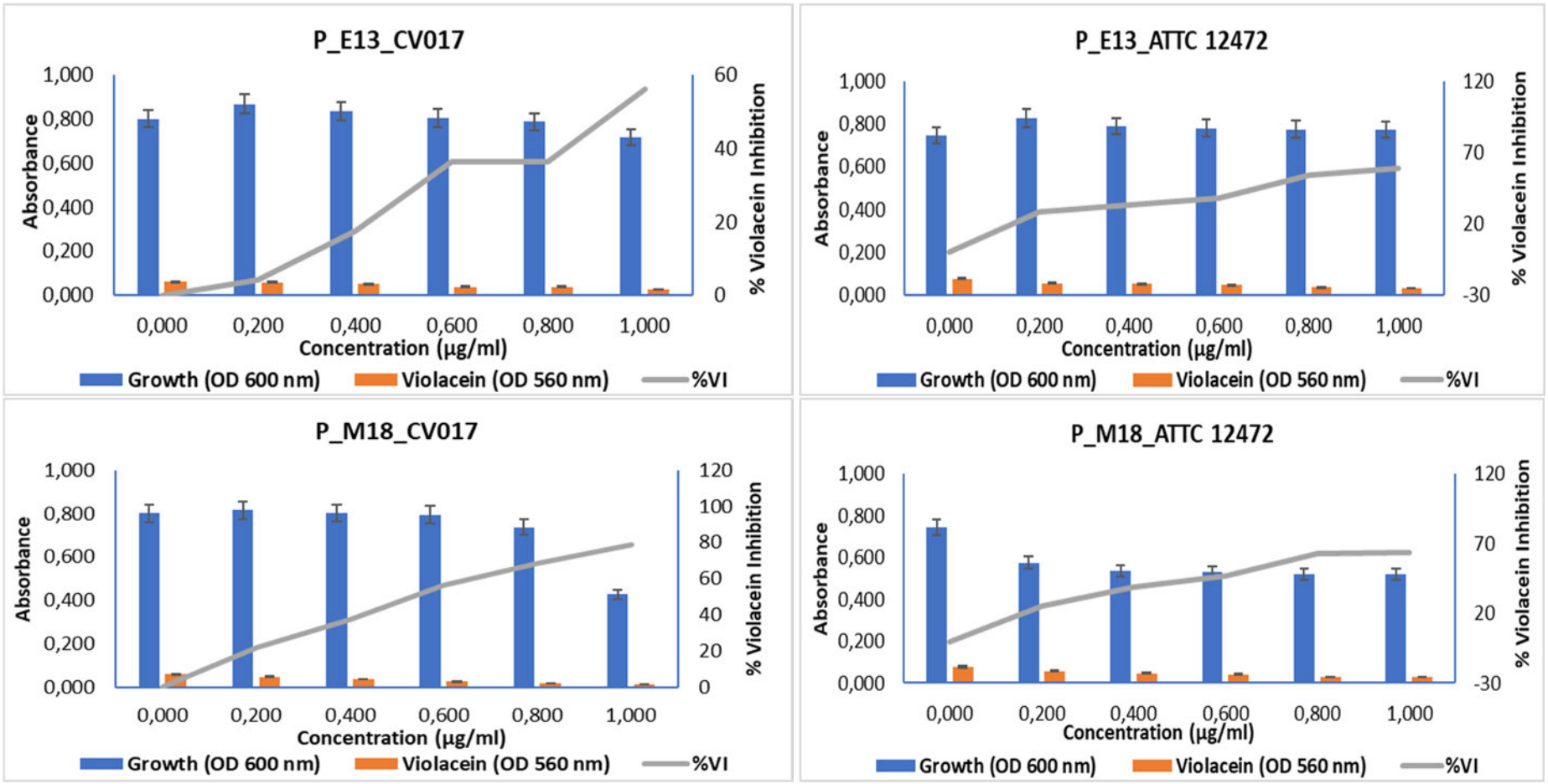
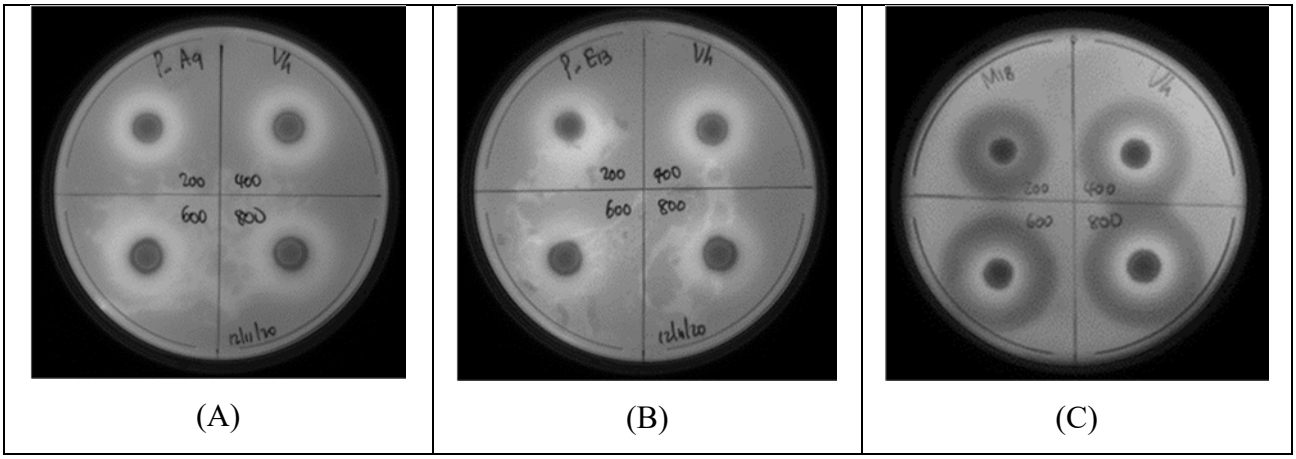


Figure 3.5: Quantitative assessment of violacein inhibitory effects of P_A9, P_E5, P_E13 and P_G1 extracts against *Chromobacterium subtsugae* CV017 and *Chromobacterium violaceum* ATCC 12472. Bacterial growth measured at $OD_{600\ nm}$ and violacein production at $OD_{560\ nm}$ are indicated as bar graphs, while the percentage violacein inhibition (%VI) is indicated by the line graph. Data represent the mean of two independent experiments performed in triplicate.

3.3.5 Qualitative autoinducer-2 inhibition

Autoinducer-2 inhibition of *Pocillopora* species CAB extracts was assessed using a bioluminescent, marine *V. harveyi* BB120. Growth inhibition was denoted by the presence of dark zones, lacking bioluminescence and growth around the discs (Fig. 3.6; Table 3.7; Suppl. Fig. S3.5).

AI-2 inhibition activity against *V. harveyi* was demonstrated by 68.75% (11/16) of extracts, while 31.25% (5/16) of extracts showed both AI-2 inhibition and killing across all concentrations tested, i.e., 200 – 800 µg (Table 3.7). Killing of *V. harveyi* was observed with five extracts, *P. gallaeciensis* P_E12, *P. gallaeciensis* P_L4, *P. gallaeciensis* P_L5, *Staphylococcus equorum* P_L12 and *P. gallaeciensis* P_M18, where a transparent zone was observed around the discs (Table 3.7; Fig. 3.6 A-C; Suppl. Fig. S3.5). All the extracts with AI-2 inhibition potential exhibited weak intermediate AI-2 inhibition activity across varying concentrations, i.e., 200 – 800 µg, with the *P.r gallaeciensis* P_M18 extract being the exception and demonstrating strong inhibition across all concentrations, even with its bactericidal activity (Table 3.7; Fig. 3.6 C; Suppl. Fig. S3.5).



(A) (B) (C)

Figure 3.6: Autoinducer-2 inhibition of *Pocillopora* CAB extracts against marine bacterium *Vibrio harveyi* BB120. AI-2 inhibition was indicated by the appearance of dark zones and lack of bioluminescence around the discs. (A) P_A9; (B) P_E13; (C) P_M18.

Table 3.7: Qualitative autoinducer-2 inhibitory potential (opaque zones) demonstrated by *Pocillopora* CAB extracts against marine pathogen *Vibrio harveyi* BB120 using agar-overlay assay.

Extract	*Vibrio harveyi* BB120											
	Zone diameter (mm)				Clear zone (mm)				QSI zone (mm)			
	200 µg	400 µg	600 µg	800 µg	200 µg	400 µg	600 µg	800 µg	200 µg	400 µg	600 µg	800 µg
P A9	8	9	9	10	0	0	0	0	8	9	9	10
P E2	0	0	0	0	0	0	0	0	0	0	0	0
P E5	0	0	0	0	0	0	0	0	0	0	0	0
P E12	9	9	10	10	7	7	8	8	2	2	2	2
P E13	8	8	9	10	0	0	0	0	8	8	9	10
P E20	8	8	9	9	0	0	0	0	8	8	9	9
P G1	8	8	9	9	0	0	0	0	8	8	9	9
P L4	7	8	9	10	6	7	7	8	1	1	2	2
P L5	9	9	9	10	7	7	7	8	2	2	2	2
P L12	8	9	10	10	6	6	7	7	2	3	3	3
P M18	18	27	30	33	8	10	12	15	10	17	18	18
P M24	0	0	0	0	0	0	0	0	0	0	0	0
P S9	0	8	8	8	0	0	0	0	0	8	8	8
P T3	0	0	0	0	0	0	0	0	0	0	0	0
P T5	0	0	9	12	0	0	0	0	0	0	9	12
P T7	0	0	0	0	0	0	0	0	0	0	0	0
Control												
Cinnamaldehyde (10 µg/ml)	38				24				14			

*Color codes

*Green – selected extracts showing AI-2 activity

*Yellow – extracts showing killing activity

Orange – extracts showing both AI-2 inhibition and killing activity

*Blue – extracts demonstrating AI-2 inhibition

*AI-2 inhibition zone = Total zone diameter – Clear zone.

3.3.6 Detection of anti-biofilm activity of coral-associated bacteria extracts

Selected *Pocillopora* bacterial extracts (n = 16) were assessed for their potential to disrupt initial and mature biofilm formation using the crystal violet microtitre plate assay. These were screened against clinical indicators *S. aureus* ATCC 43300 and *P. aeruginosa* ATCC 27853 and marine pathogens *S. putrefaciens* ATCC 8071, *V. coralliilyticus* ATCC_BAA 450, *V. parahaemolyticus* ATCC 17802 and *V. shilonii* ATCC_BAA 91. Promising biofilm reduction (BFR) was defined as extracts with ≥50% BFR with <40% growth inhibition, while antimicrobial activity was defined as extracts with ≥40% GI (Figs. 3.7 - 3.9; Suppl. Tables S3.1 - S3.6). Differences in mean values following exposure to 200 µg – 800 µg of extracts were statistically significant ($p \leq 0.05$) based on indifferences in values**.**

Initial adhesion inhibition by extracts against clinical pathogens *P. aeruginosa* and *S. aureus* was not effective at initial adhesion, but only one extract P_T7 demonstrated bactericidal activity at 600 - 800 µg/ml against *P. aeruginosa* and at 400 – 800 µg/ml against *S. aureus* (Suppl. Tables S3.1 – 3.2). For mature biofilms, there was no ≥ 50% BFR against *P. aeruginosa*, however, three extracts demonstrated bactericidal activity, with one extract P_T7 showing bactericidal activity at 600 – 800 µg/ml and two extracts (P_E12, P_M18) showing growth inhibitory activity at 800 µg/ml. Against *S. aureus*, three extracts demonstrated ≥50% BFR, with <40% GI, with two extracts, *Bacillus* spp. P_E5 and *Phaeobacter* sp. strain P_L4 showing biofilm inhibition at 800 µg/ml and a single extract, *Bacillus* sp. strain P_T7 showing activity at 600 – 800 µg/ml (Suppl. Tables S3.1 - S3.2).

Against marine indicator *S. putrefaciens*, seven extracts demonstrated the desired initial adhesion inhibition. A single extract P_E12 exhibited activity at 400 µg/ml – 800 µg/ml, three extracts at 600 – 800 µg/ml and P_T3 at 600 – 800 µg/ml. P_M18 and P_M24 demonstrated bactericidal activity at 800 µg/ml. Two extracts, P_E2, P_E5 demonstrated BFR at 800 µg/ml. A single extract P_T7 demonstrated bactericidal activity at 400 - 800 µg/ml. For mature biofilms, a single *Bacillus* extract (P_E5) demonstrated ≥50% BFR with <40% GI at 800 µg/ml (Suppl. Table S3.3). P_E5 significantly inhibited both initial adhesion and mature biofilm at 800 µg/ml ($p \leq 0.05$).

A single *Phaeobacter* extract, (P_M24) demonstrated the desired initial adhesion inhibition at 600 – 800 µg/ml against *V. coralliilyticus* (Suppl. Table S3.4). Five extracts demonstrated bactericidal activity, with two extracts P_E12, P_E20 demonstrating killing at 200 – 800 µg/ml, and three extracts P_L12, P_T5, P_T7 at 800 µg/ml. For mature biofilms, twelve extracts demonstrated ≥50% BFR. Three extracts (P_E20, P_G1, P_L4) demonstrated activity at 200 – 800 µg/ml and six extracts, (P_E13, P_L12, P_M24, P_S9, P_T3, P_T5) at

400 – 800 µg/ml. Two extracts (P_L4 and P_M18) demonstrated bactericidal activity at 800 µg/ml. Two extracts, (P_M18 and P_T7) demonstrated activity at 600 – 800 µg/ml, and a single extract P_E5 demonstrated activity at 800 µg/ml (Suppl. Table S3.4). More *Bacillus* extracts (P_E5, P_G1, P_T3, P_T5 and P_T7) demonstrated activity at mature biofilms compared to *Phaeobacter* spp. where only three extracts (P_L4, P_M18 and P_M24) showed activity. Two *Halomonas* extracts demonstrated activity at mature biofilms and a single *Staphylococcus* and *Streptomyces* extract exhibited activity (Suppl. Table S3.4). Three extracts, *H. venusta* P_E20, *B. altitudinis* P_G1 and *P. gallaeciensis* P_L4 were most effective as they demonstrated activity at mature biofilms at 200 – 800 µg/ml, with P_L4 demonstrating bactericidal activity at 800 µg/ml (Suppl. Table S3.4). These extracts were statistically significant ($p \leq 0.05$) The *P. gallaecinsis* P_M24 extract inhibited both initial adhesion at 600 – 800 µg/ml and mature biofilms at 400 – 600 µg/ml (Suppl. Table S3.4).

Against *V. parahaemolyticus*, five extracts demonstrated ≥50% BFR for initial adhesion, with two extracts (P_A9 and P_M18) showing activity at 600 µg/ml but P_M18 was bactericidal at 800 µg/ml and two extracts(P_E2 and P_E5) demonstrated activity at 800 µg/ml (Suppl. Table S3.5). Bactericidal activity was demonstrated by eight extracts, with two extracts (P_T3 and T7) showing killing at 400 – 800 µg/ml, two extracts (P_M24 and P_T5) at 600 – 800 µg/ml and four extracts showing killing at 800 µg/ml. No extracts were effective against *V. parahaemolyticus* mature biofilms (Suppl. Table S3.5).

Against *V. shilonii*, seven extracts demonstrated ≥50% BFR for initial adhesion, a single extract (P_L4) demonstrated activity at 200 – 800 µg/ml, 3 extracts P_T3, P_T5 and P_T7 at 200 – 600 µg/ml with P_T5 being bactericidal at 600 – 800 µg/ml and P_T7 at 800 µg/ml. Three extracts (P_G1, P_L12) and P_M18 showed activity at 600 – 800 µg/ml with P_L12 being bactericidal at 800 µg/ml (Suppl. Table S3.6). Five extracts demonstrated bactericidal activity, with two extracts (P_E12 and P_E20) showing activity at 200 – 800 µg/ml, only extract P_T5 showing activity at 600 – 800 µg/ml and two extracts showing activity at 800 µg/ml. For mature biofilms no extracts demonstrated ≥50% BFR, with three extracts demonstrating bactericidal activity, i.e., P_L12 showing killing at 400 – 800 µg/ml, P_E12 at 600 – 800 µg/ml and P_L4 at 800 µg/ml (Suppl. Table S3.6).

For initial adhesion inhibition, 43.75% (7/16) of the extracts demonstrated activity against *S. putrefaciens* (Supp. Table S3.3). A trend of more *Phaeobacter* (n=4) extracts demonstrating inhibition activity at varying concentrations compared to *Bacillus* (n=3) extracts was observed. Against *V. shilonii*, 43.75% (7/16) of the extracts demonstrated initial adhesion inhibition (Suppl. Table S3.6). More *Bacillus* (n=4) extracts significantly ($p \leq 0.05$)

demonstrated inhibition activity compared to *Phaeobacter* (n=2) and *Staphylococcus* (n=1) extracts. Against *V. parahaemolyticus*, 31.25% (5/16) of the extracts demonstrated initial inhibition activity (Suppl. Table S3.5). Both the *Bacillus* (n=2) and *Phaeobacter* (n=2) extracts demonstrated the desired activity, respectively. Against *V. coralliilyticus,* a single *Phaeobacter* sp, strain P_M24 extract (6.25%, 1/16) demonstrated initial adhesion activity (Suppl. Table S3.5). No activity was observed for *Halomonas* and *Streptomyces* extracts at initial adhesion. A single extract, *P. gallaeciensis* P_M18 demonstrated activity against three indicators (Suppl. Tables S3.3, S3.5; S3.6). Five extracts, P_E2, P_E12, P_M24, P_T3 and P_T5 demonstrated activity against two indicators (Suppl. Tables S3.3, S3.5). Five extracts (P_A9, P_E5, P_G1, P_L4 and P_L12) showed activity against one indicator (Suppl. Tables S3.3, S3.6). For initial adhesion inhibition, extracts were most effective against three indicators, *S. putrefaciens, V. parahaemolyticus* and *V. shilonii* and were dose-dependent ($p \leq 0.05$) (Suppl. Tables S3.3, S3.5, S3.6).

For mature biofilms, a single extract demonstrated inhibition activity against *S. putrefaciens* (Suppl. Table S3.3). Against *V. coralliilyticus* 75% (12/16) of the extracts demonstrated activity, however, a trend of more *Bacillus* (n=5) extracts being effective compared to *Phaeobacter* (n=4)*, Halomonas* (n=2) and *Streptomyces* (n=1) extracts. No activity by the extracts was observed against *V. parahaemolyticus* and *V. shilonii* was observed for mature biofilms (Suppl. Tables S3.5 - S3.6). No extracts were effective against three indicators. A single extract, *B. altitudinis* P_E5 was effective against two indicators (Suppl. Table S3.3-S3.4). For mature biofilm, extracts were most effective against *V. coralliilyticus* (Suppl. Table S3.4). Against clinical indicators, extracts were most effective against methicillin-resistant *S. aureus* and against marine indicators, extracts were most effective against *V. coralliilyticus* (Suppl. Tables S3.2-S3.4). However, members of the genus *Bacillus Phaeobacter* were more effective compared to *Halomonas, Staphylococcus* and *Streptomyces* bacterial isolates, as they demonstrated inhibition activity against multiple indicators against both initial adhesion and mature biofilms.

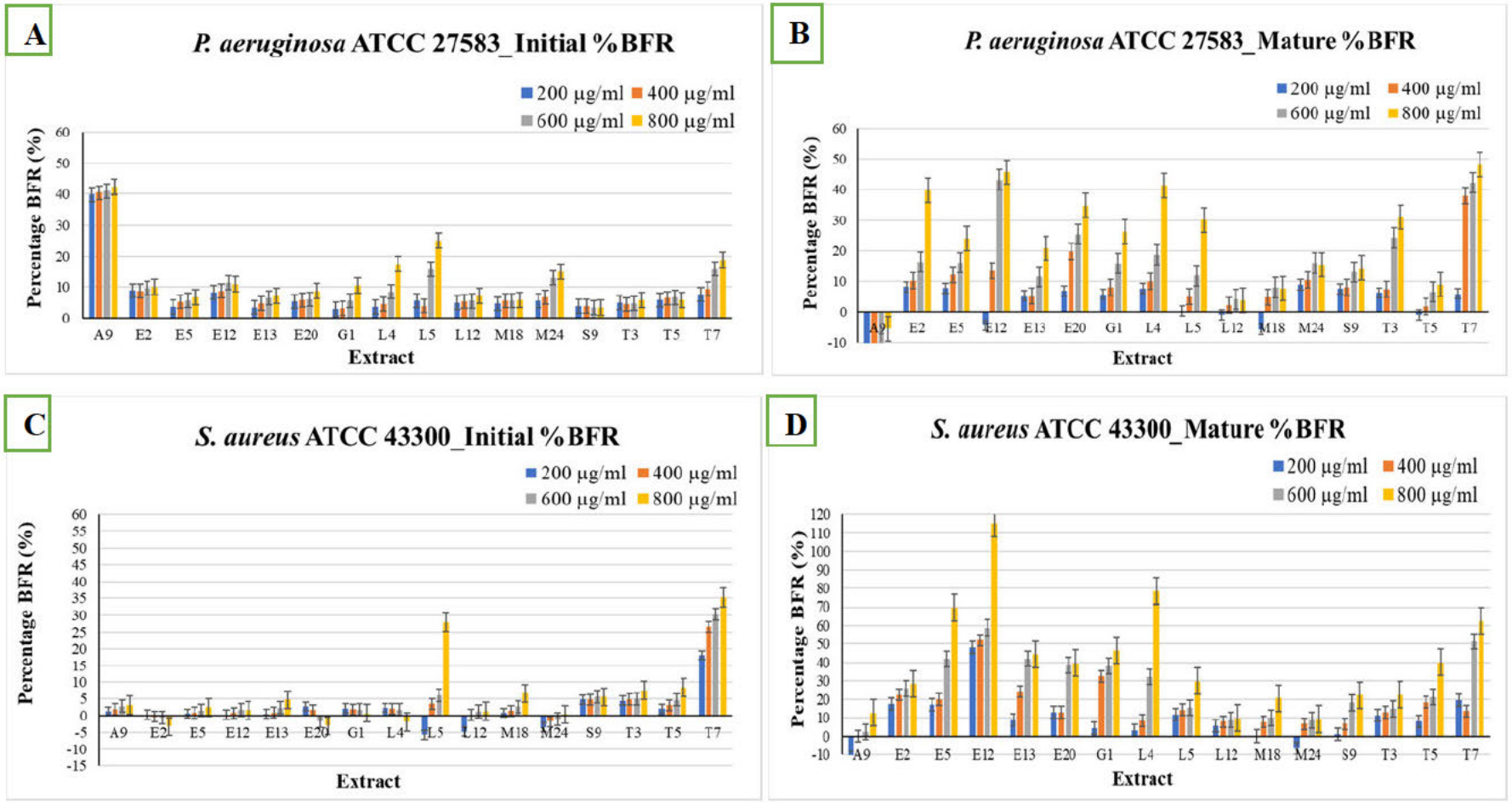


Figure 3.7: Percent biofilm reduction of *Pocillopora* CAB extracts on initial adhesion and mature biofilm of clinical indicators *Pseudomonas aeruginosa* ATCC 27853 (A-B) and *Staphylococcus aureus* ATCC 43300 (C-D), as quantified by the crystal violet staining in the microtitre plate assay. Mean values of two replicates are shown. Differences in mean values following exposure to 200 – 800 µg/ml of extracts were statistically significant ($p \leq 0.05$).

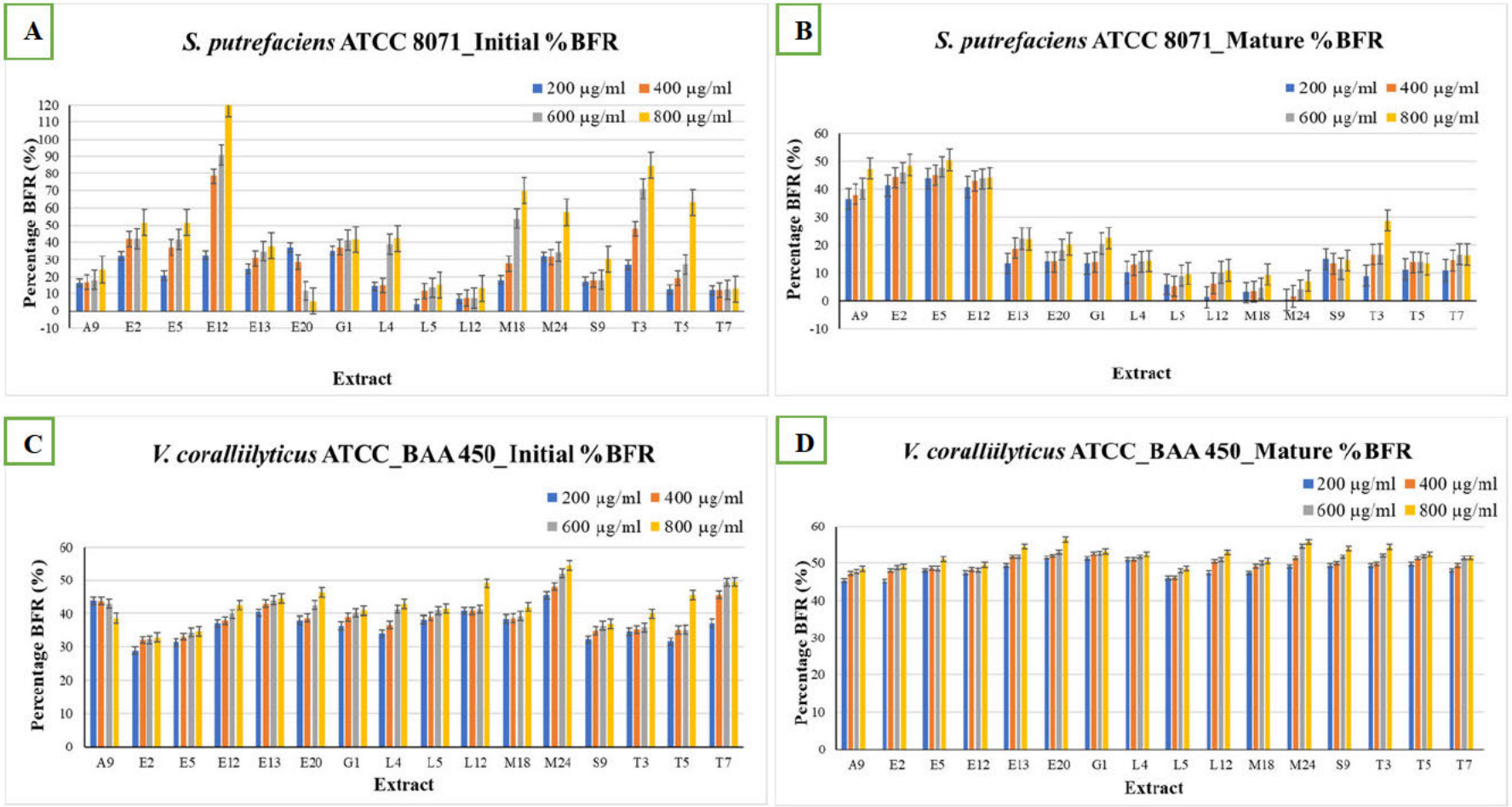


Figure 3.8: The potential effect of % BFR of *Pocillopora* CAB extracts on growth of initial adhesion and mature adhesion of marine bacteria *Shewanella putrefaciens* ATCC 8071 (A - B) and *Vibrio coralliilyticus* ATCC_BAA 450 (C - D), as quantified by the crystal violet staining in microtiter plate assay. Mean values of two replicates are shown Differences in mean values following exposure to 200 – 800 µg/ml of extracts were statistically significant ($p \leq 0.05$).

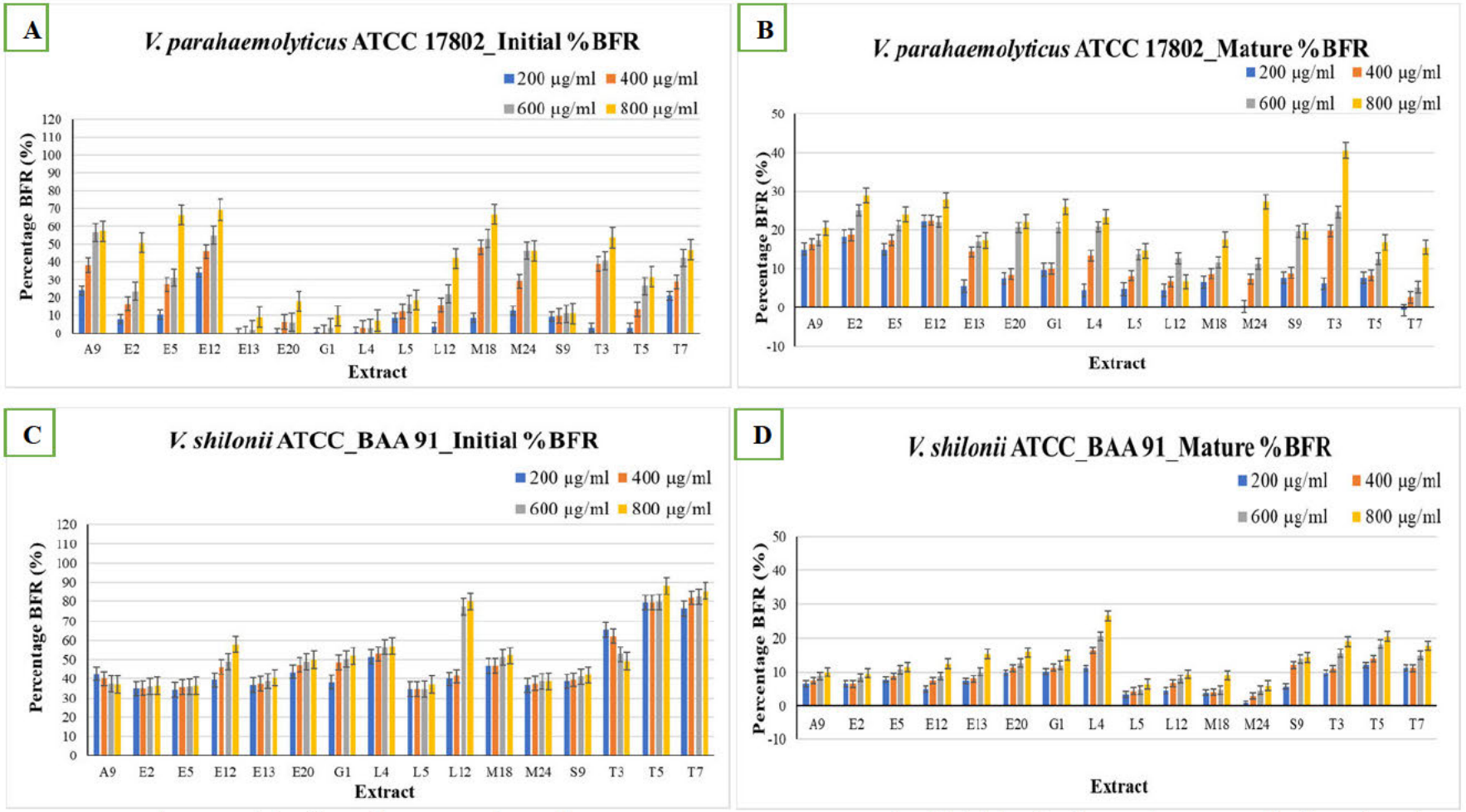


Figure 3.9: The potential effect of % BFR of *Pocillopora* CAB extracts on growth of initial adhesion and mature adhesion of marine pathogens *Vibrio parahaemolyticus* ATCC 17802 (A - B) and *Vibrio shilonii* ATCC_BAA 91 (C - D), as quantified by the crystal violet staining in microtiter plate assay. Mean values of two replicates are shown. Differences in mean values following exposure to 200 – 800 µg/ml of extracts were statistically significant ($p \leq 0.05$).

3.4 Discussion

The marine environment remains among the richest ecosystems whose natural products are esteemed to be an extraordinary source of therapeutics as they exhibit a wide range of bioactive compounds for potential applications in various sectors including pharmaceuticals (Bourne *et al*., 2016; Peixoto *et al.,* 2019). Corals are among marine organisms whose bacterial communities are suggested to be emerging producers of bioactive compounds and new compounds continue to be isolated and identified from coral bacteria (Glasl *et al.,* 2016; Peixoto *et al*., 2021). The ability of these corals to harbor diverse microbial communities is not only beneficial to the external microorganisms but also to the coral host itself as these microbes play an important role in the health and protection of the coral host (Modolon *et al*., 2020). They accomplish this through the production of antimicrobial compounds against a wide spectrum of pathogens, disrupting their cell-to-cell communication, thus completely removing pathogens from coral surfaces (Chen *et al*., 2019; Eskander *et al.,* 2018). Several studies have demonstrated that bacteria isolated from corals are able to inhibit the colonization and growth of many other bacteria through antibacterial activity, including coral pathogens such as *Vibrio* species (Bourne *et al*., 2016; Busch *et al*., 2019; Clerissi *et al*., 2018). This study aimed at assessing the antimicrobial, anti-QS and anti-biofilm potential of selected bacteria isolated from a *Pocillopora* coral species.

When the antimicrobial activity of the 16 CAB was assessed, results demonstrated that antimicrobial activity against both clinical pathogens multidrug-resistant *P. aeruginosa* ATCC 27853 and methicillin-resistant *S. aureus* ATCC 43300 were not effective (Table 3.1; Supp. Fig S3.2). The lack of activity by the extracts against *P. aeruginosa* may be due to *P. aeruginosa* being a multidrug resistant Gram-negative pathogen with a cell envelope consisting of an outer lipid membrane and thin layer of peptidoglycan. The *P. aeruginosa* bacterial cell is thus more difficult to penetrate (Sarmiento-Vizcaíno *et al*., 2017; Yayan *et al*., 2015) as opposed to *S. aureus* which has a thick peptidoglycan cell wall and is more susceptible to antibiotics as they lack an outer membrane (Panawala, 2017). Sharma *et al*. (2019) reported antimicrobial compounds from CAB to be effective against some clinically important strains such as *S. aureus, E. coli* and *B. subtilis*. Coral-associated bacteria are suggested to produce antimicrobial compounds to prevent the growth of pathogenic organisms through antibiosis mechanisms (Busch *et al*., 2019, Sharma *et al*., 2019) to help avoid the development of diseases (Pereira *et al*., 2017).

Extracts were tested for antimicrobial activity against five indicators, however, they only demonstrated activity against three indicators, *S. putrefaciens, V. coralliilyticus* and *V. shilonii*

(Tables 3.2 – 3.3; Suppl. Fig. S3.2). Antimicrobial activity was demonstrated primarily against *V. shilonii*. The result of weak-to-no activity against some indicators such as *V. alginolyticus* is in line with a study by Fang *et al*. (2021), who reported three CAB compounds, indole-derived, uridine-derived and 1H-indole-2-carbaldehyde, which exhibited very low antimicrobial activity against the coral pathogen *V. alginolyticus*. These compounds were suggested to have increased the presence of bacterial compounds which enhance resistance of CAB to pathogens (Fang *et al*., 2019). Coral-associated bacteria are known to possess antagonistic properties against pathogens (Modolon *et al.,* 2020). A CAB strain isolated from the coral *P. damicornis* was observed to exhibit antagonistic properties against *V. coralliilyticus* (Rosado *et al.,* 2019). Another isolate identified from the same coral, *Pseudovibrio* sp. P12 was suggested to metabolize dimethylsulfuniopropionate and more tropodithietic acid (TDA) was produced, resulting in a strong antimicrobial activity against two *Vibrio* species, *V. coralliilyticus* and *V. owensii* (Raina *et al.*, 2016).

Genera identified from the 16S rRNA sequencing included *Bacillus, Halomonas, Phaeobacter, Staphylococcus* and *Streptomyces* species Based on the bioactivity screening data (Chapter 2) *Bacillus* and *Phaeobacter* appeared to be more dominant, however, *P. gallaeciensis* P_M18 showed activity against multiple indicators, making *Phaeobacter* species a better source of antimicrobial compounds compared to *Bacillus* species This is in line with studies by Bourne *et al*. (2016) and Egan and Gardner (2016) who reported that a diversity of potential probiotic bacteria can be found in the genus *Phaeobacter*, with *P. gallaeciensis* taking the lead in the production of tropodithietic acid (TDA). *P. gallaeciensis* has also been observed to be antagonistic against a range of bacteria such as *Vibrio anguillarum, B. subtilis, Halomonas* and *Pseudoalteromonas* species due to its production of TDA (Bourne *et al*., 2016). A study by D'Alvise *et al*. (2013) demonstrated that *P. gallaeciensis* strongly reduced *V. anguillarum* in live feed cultures and protected fish larvae from *Vibrio* species Porsby *et al*. (2011) also reported that members of the *Roseobacter* clade, including *P. gallaeciensis* were effective against a range of human pathogenic bacteria, both Gram-positive and Gram-negative, due to its ability to produce TDA.

Halomonas extracts demonstrated better activity than *Staphylococcus* and *Streptomyces* extracts. Although the *Streptomyces* extract had activity against the *V. shiloni* in the intermediate range. Braña *et al.* (2015) reported antimicrobial activity of CAB *Streptomyces* species against *S. aureus* and *E. coli*, and strong antimicrobial activity was observed against these clinical pathogens. Zhang *et al*. (2013) reported antibacterial activity of 123 coral-associated actinobacteria, including *Streptomyces, Micromonospora, Nocardia,*

Verrucosispora, Rhodococcus and *Agrococcus* species. Over 50% (38) demonstrated strong bioactivity against clinical pathogens *B. subtilis, Bacillus thuringiensis, E. coli* and 28% (20) against marine pathogens *V. alginolyticus, Pseudoalteromonas piscida* and *M. luteus*. When the *Pocillopora* CAB extracts were targeted against marine bacteria *S. putrefaciens*, *V. alginolyticus* and *V. parahaemolyticus*, no-to-weak activity was observed against *V. alginolyticus* and *V. parahaemolyticus.* The *P. gallaeciensis* P_M18 extract showed intermediate activity at 800 µg against *S. putrefaciens*, while the *H. venusta* P_E13 extract demonstrated intermediate-to-strong activity. Ravikumar *et al.* (2016) reported the antibacterial potential of carotenoids of *Halomonas* against antibiotic-resistant pathogens *P. aeruginosa, S. aureus, S. pneumonia* and *Klebsiella* species Maximum antimicrobial activity was observed against *Klebsiella* species and *E. coli*, with minimum activity observed against *P. aeruginosa* and *S aureus.* Carotenoids from the *Halomonas* species. are suggested to exhibit pharmacological properties to cure human diseases caused by microorganisms (Ravikumar *et al.,* 2016).

The *P. gallaeciensis* P_M18 extract demonstrated to be more effective against indicators compared to other extracts as a trend of antimicrobial activity was observed against four of the five bacterial indicators tested. Previous studies reported a diversity of potential probiotic bacteria in the *P. gallaeciensis* group due to its production of the compound (TDA) (Bourne *et al*., 2016; Egan and Gardiner, 2016). *P. gallaeciensis* has also been observed to be antagonistic against a range of bacteria (D'Alvise *et al*., 2013; Porsby *et al*., 2011). *Phaeobacter* species inhibited isolates including *Halomonas* species, *Pseudomonas* species and *Cobetia marina*. This is in agreement with other studies as Alphaproteobacteria can inhibit diverse marine bacterial isolates (Ravikumar *et al*., 2016). Results of this study may suggest that CAB from the genus *Phaeobacter* may protect the coral host against marine pathogens through the production of antimicrobial compounds.

The understanding of the interaction between CAB and other microbial communities are important to detect population densities and manage their behaviors (Certner and Vollmer, 2018). Coral-associated bacteria especially pathogens make use of QS signals to destabilize the population structure and health of the coral host (Gutiérrez-Almada *et al*., 2020; Torres *et al*., 2019). Quorum sensing can control a wide range of biological functions such as the production of virulence factors, biofilm formation, antibiotic production and plays a role in the pathogenicity of these bacterial communities (Zhou *et al*., 2020). Several studies have demonstrated that the QS system can be disrupted to minimize the production of virulence factors and biofilm formation by CAB and can be an advantageous way to inhibit bacterial

infections in corals (Başaran *et al*., 2020; Reina *et al*., 2021). Furthermore, QS inhibitors have been observed from marine organisms such as corals and were observed to inhibit QS in various pathogens (Bakkiyaraj *et al*., 2010, 2013; Torres *et al*., 2019).

Extracts demonstrated QS inhibition activity against both *C. subtsugae* CV017 and *C. violaceum* ATCC 12472 significantly ($p \leq 0.05$) (Suppl. Fig. S3.4). However, 56.25% (9/16) of the extracts demonstrated QS inhibition ≥50% VI with <40% GI, against *C. violaceum* ATCC 12472 compared to (37.5%) extracts against *C. subtsugae* CV017. Five extracts demonstrated broad-spectrum activity as they exhibited QS inhibition activity against both biosensors. Majority of isolates identified in this study belonged to *Bacillus* (n=6) and *Phaeobacter* species (n=6). Interestingly, *Bacillus* extracts demonstrated better short and long-chain inhibition, followed by *Phaeobacter* and *Halomonas* extracts. *Bacillus* extracts demonstrated a broad-spectrum activity against both biosensors and 50% of the extracts tested demonstrated this activity (Suppl. Fig. S3.4). *Phaeobacter* extracts were less effective in comparison to *Bacillus* extracts with the exception of *P. gallaeciensis* P_M18 which was the best broad-spectrum extract demonstrating antimicrobial activity, but *Bacillus* extracts demonstrated better activity in the QS inhibition assays (Suppl. Fig. S3.4). Zhang and Chu (2017) have highlighted that CAB have a great potential to produce bioactive compounds that can be used as QS inhibitors, though some of these may be species-specific. This was also observed in the current study as bioactivities differed with test extracts. The current study demonstrated that the CAB were more effective against long-chain AHL producer, *C. violaceum* ATCC 12472 and were dose-dependent ($p \leq 0.05$). This is in line with El-Kurdi *et al*. (2021) who reported anti-QS activity of CAB extracts including *Bacillus* species against biosensor *C. violaceum* ATCC 12472. They observed that 66.2% of the screened extracts demonstrated anti-QS activity where the degradation of the purple pigment of the bioreporter strain was observed, however, cell growth was not affected. Their study also reported CAB to have exhibited greater anti-QS activity than other bacteria obtained from other marine organisms. Bakkiyaraj *et al.* (2013) screened forty-one CAB and nine of those demonstrated anti-QS activity against *C. violaceum* ATCC 12472, with two extracts, CAB23 and 41 demonstrating about 90% anti-QS activity against this biosensor strain and no reduction in growth was observed. Ma *et al*. (2020) reported that 15% (30/200) of their CAB exhibited anti-QS activity against *C. violaceum* ATCC 12472. Among the tested isolates was *B. cereus* and *S. hominis*, which was reported to have strongly inhibited QS by reducing the purple pigment of *C. violaceum* ATCC 12472. They also reported that *Bacillus* species were among the

identified QS inhibitors. Goldberg *et al*. (2013) reported QS inhibition activity of CAB extracts against *A. tumefaciens, C. violaceum* CV026 and *E. coli.*

In the current study, the six *Phaeobacter* extracts tested demonstrated minimal QS inhibition with the P_M18 extract demonstrating broad-spectrum potential and P_L4 long-chain inhibition. A trend of minimal anti-QS activity against both biosensors across all concentrations was observed for all the *Phaeobacter* extracts with the exception of *P. gallaeciensis* P_M18. It is also suggested that the production of TDA and some secondary metabolites is controlled by AHL-mediated QS in *Phaeobacter* species (Berger *et al*., 2011). The limited QS inhibition activity of *Phaeobacter* against *C. violaceum* 12472 may be because they produce long chain AHLs, more than 10 and sometimes up to 8 different AHLs by one strain. Therefore, further assessment of each of the six *Phaeobacter* extracts may be required to determine the AHLs they produce. The presence of these AHLs will be detected by the inhibition of violacin production or inhibition AHL-dependent QS reporters (Singh *et al*., 2016). Therefore, more QS inhibition by the *Phaeobacter* extracts will be observed. Zhou *et al*. (2020) demonstrated the presence of AHL- producing bacteria isolated from *P. damicornis* coral with some of the bacterial isolates demonstrating QSI activity. The Alphaproteobacteria clade possessed QS inhibitory compounds reduced the production virulence factors of pathogens although bacterial cells were not killed (Zhou *et al*., 2020).

The search of potential compounds with anti-QS activity continues due to clinical and environmental applications (Ma *et al*., 2018). Therefore, an improved understanding of the role played by QS systems in the pathogenicity of bacteria may help improve coral and human health. This activity is related to both enzymes and secondary metabolite production by CAB as the production of QQ and QSI enzymes could be responsible for degrading AHL signals and shutting down QS (Singh *et al*., 2016). This can occur by the inhibition of the biosynthesis of autoinducer peptides (Singh *et al*., 2016).

Autoinducer-2 is a molecule detected in a variety of Gram-negative and Gram-positive bacteria, including those that lack a QS response to the LuxS gene (Grandclément *et al*., 2016; Zimmer *et al*., 2014). It is also involved in the induction of bioluminescence in *V. harveyi* BB120. *Vibrio harveyi* regulates bioluminescence using QS by using three channels, autoinducer 2 hydroxybutanoyl-L-homoserine lactone, *(S)*-3-hydroxytridecan-4-one (Lorenz *et al*., 2017; Teasdale *et al*., 2011). It was observed that 68% (11/16) of the *Pocillopora* CAB extracts interfered with *V. harveyi* bioluminescence, with 31.25% (5/16) exhibiting killing effects across all concentrations (Table 3.7; Suppl. Fig. S3.5). This is expected as the AI-2 molecule is suggested to be an interspecies signal molecule and is in line with Teasdale *et al.*

(2011) who observed that six marine *Bacillus* isolates and *Halobacillus salinus* were able to inhibit both *V. harveyi* bioluminescence and violacein production of *C. violaceum* ATCC 12472. Results from the present study also identified extracts that are able to inhibit both AI-2 and AHLs, P_A9, P_E12, P_E13, P_E20, P_G1, P_L4, P_L5, P_L12, P_M18, P_S9, P_T5 belonging to genera *Bacillus, Halomonas, Phaeobacter, Staphylococcus* and *Streptomyces* (Tables 3.5-3.7; Suppl. Fig. S3.5). The present study is in line with Teasdale *et al*. (2011) as 50% of the *Bacillus* extracts inhibited both AI-2 and AHLs (Table 3.7), in addition to four *P. gallaeciensis*, two *H. venusta* and an *S. griseus* isolate. *Bacillus* species are known to produce quorum quenching (AiiA) lactonases capable of degrading AHL signals and it was suggested that the AiiA lactonase was responsible for some of the observed bioluminescence inhibition (Teasdale *et al.,* 2011), however, specific small molecule antagonists that block the QS-controlled phenotype were identified, i.e., cyclo-L-proline-L-tyrosine from *Bacillus* and N-(2-phenylethyl)-isobutyramide from *Halobacillus*. Interference with QS is a promising potential therapeutic approach to treat bacterial infection, and *V. harveyi* is a suitable organism for the discovery of QS antagonists produced by marine bacteria due to multisensing signals.

Biofilm formation is a serious global problem and there is growing interest especially in the marine environment, on sustainable and non-toxic biofilm control strategies (Divya *et al*., 2018; Gehman *et al*., 2019; Hernandez-Agreda *et al.,* 2018). Biofilm formation is suggested to be an important factor of virulence in pathogens and QS molecules have been shown to be crucial for biofilm formation (Fordyce *et al*., 2021; Pernice *et al*., 2020; Song *et al*., 2018). Biofilm formation starts with initial attachment, followed by formation of microcolonies then the maturation of these microcolonies leading into a three-dimentional structure, enclosed by extrapolymeric substances which play an important role in enabling microorganisms to initially attach to a surface and form biofilms (Sangsawang *et al*., 2017; Tello *et al*., 2012). Quorum sensing, however, is a driver of biofilm formation and its inhibition is a potential strategy to mitigate biofilm associated infections (Couradeau *et al.,* 2017; Ricci *et al*., 2019).

In the present study, the anti-biofilm potential of bacterial extracts was assessed against clinical and marine pathogens. When assessing initial adhesion, no extracts were effective against clinical multi-resistant *P. aeruginosa* and methicillin-resistant *S. aureus*, with only bactericidal activity by a single extract (P_T7) at varying concentrations, i.e., 400 – 800 µg/ml (Fig. 3.7 (A-D); Suppl. Tables S3.1 – S3.2). For mature biofilms, extracts demonstrated no BFR activity against *P. aeruginosa*, but bactericidal activity, however, three extracts demonstrated ≥50% BFR activity against *S. aureus* (Fig. 3.7 (A-D); Suppl. Tables S3.1 – S3.2). This is in line with several studies that demonstrated CAB strongly inhibit *S. aureus*

(Gowrishankar *et al*., 2012; Kaur *et al*., 2018; Nithya and Pandian, 2010). Anti-biofilm CAB are reported to be strong candidates for controlling clinically important pathogens (Pham *et al.,* 2016). *Bacillus firmus* extract isolated from a coral demonstrated biofilm reduction of *S. aureus* by 83% (Gowrishankar *et al*., 2012). Other CAB identified to produce anti-biofilm compounds include *Streptomyces* and *V. parahaeomolyticus* (Gowrishankar *et al*., 2012). They further reported two CAB extracts, *Bacillus firmus* and *V. parahaemolyticus* to have strongly inhibited biofilm formation against *S. aureus* as they reduced the exopolysaccharide layer. Bakkiyaraj and Pandian (2010) demonstrated CAB to be a potential source of anti-biofilm compounds for clinical pathogens. Bakkiyaraj and Pandian (2010) reported a coral-associated actinomycete to have inhibited *in vitro* biofilm formation of methicillin-resistant *S. aureus*.

Extracts demonstrated better anti-biofilm activity at initial adhesion inhibition compared to mature biofilm inhibition. This because initial biofilms are easy to inhibit, whereas mature biofilms are difficult to penetrate due to the presence of the extracellular polymeric substance layer (Kaur *et al.,* 2019). These results are not surprising as most studies have demonstrated that extracts perform better at initial adhesion inhibition compared to the mature stage (Kaur *et al*., 2018; Nithya and Pandian, 2010). For initial adhesion, the most dominant extracts were from *Bacillus* and *Phaeobacter* species as they demonstrated a trend of biofilm inhibition and bactericidal activity against marine pathogens *S. putrefaciens, V. parahaemolyticus* and *V. shilonii*, while only bactericidal activity was demonstrated against *P. aeruginosa*, *S. aureus* and *V. coralliilyticus.* This is an expected result as *P. gallaeciensis* is known to be antagonistic to various pathogens including those of *Vibrio* species (Prado *et al.,* 2009). However, a trend of *Bacillus* extracts exhibiting greater biofilm inhibition against marine pathogen *V. coralliilyticus* was observed compared to *Phaeobacter* extracts. This is in line with a Nithya and Pandian (2010) who reported that CAB *B. pumilus* and *B. indicus* inhibited initial adhesion, biofilm formation and dispersed mature biofilm of *Vibrio* species which threatens human and animal lives. In summary, at initial adhesion 43.75% of the extracts demonstrated better BFR activity against *S. putrefaciens*, 43.74% showed activity against *V. shilonii*, 31.25% against *V. parahaeomolyticus* and 6.25% of the extracts showed activity against *V. coralliilyticus*. For mature biofilms, 75% of extracts demonstrated activity against *V. coralliilyticus* and 6.25% showed activity against *S. putrefaciens*. No initial adhesion inhibition activity was demonstrated by the extracts against *V. parahaemolyticus* and *V. shilonii* (Figs. 3.7 -3.9 (A-D); Suppl. Tables S3.3 – 3.6). The *Bacillus* and *Phaeobacter* genera, however, were more effective compared to *Halomonas, Staphylococcus* and *Streptomyces* bacterial isolates, as they demonstrated inhibition activity against multiple indicators against both initial adhesion and

mature biofilms. These results are in line with Song *et al*. (2018) who demonstrated that CAB from *P. damicornis* inhibited biofilm formation and 15% of the extracts demonstrated activity against a wide range of *Vibrio* species and *C. violaceum* ATCC 12472. For clinical indicators, *P. aeruginosa* and *S. aureus*, if they were to be applied in a clinical setting, the concentration range could be adjusted to inhibit biofilm formation.

The present study is in line with these studies as *Bacillus* species produce bioactive compounds with anti-biofilm properties thus inhibiting clinical and environmental pathogens. Gowrishankar *et al*. (2012) also reported extracts from CAB to contain anti-biofilm properties as many bacteria are now known to be producers of many bioactive compounds. The results of the current study also demonstrate that CAB bioactive compounds are important potential anti-biofilm agents.

3.5 Conclusion

The results from this study have demonstrated that *Porcillopora*-associated bacteria are a valuable potential bioresource pool for developing antimicrobial, anti-quorum sensing and anti-biofilm compounds. These bacterial extracts were, however, less effective against clinical indicators than marine indicator organisms. This suggests that they can be useful in environmental applications rather than clinical applications. The *Phaeobacter* sp. strain P_M18 extract which displayed a wide spectrum of activity against selected test indicators may be a promising probiotic candidate as it has shown antagonistic characteristics against a wide range of indicators. More effective strategies on the application of these probiotics need to be designed especially in clinical and physical environment settings.

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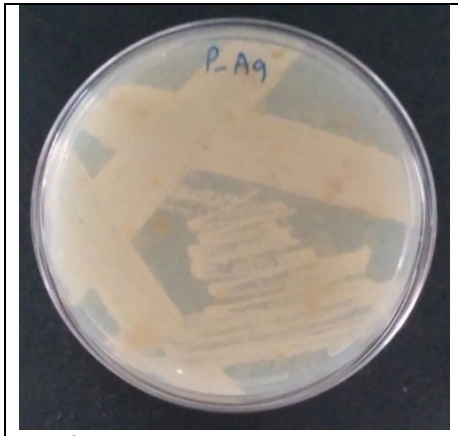
3.7 Supplementary information

3.7.1 Selected bacterial cultures



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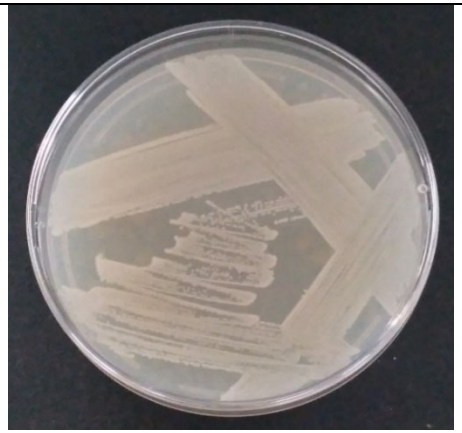
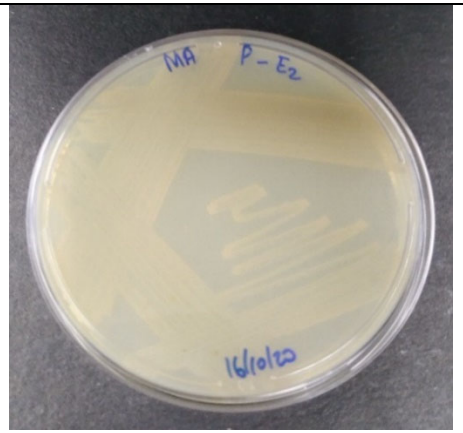
Bacillus altitudinis





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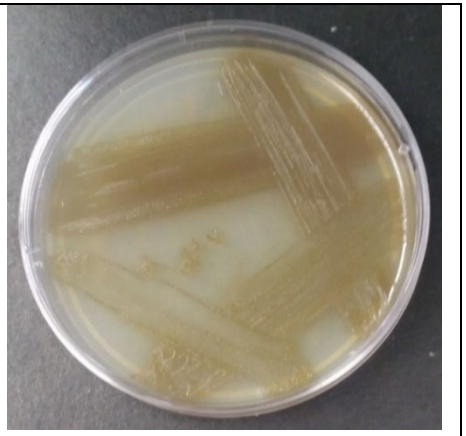
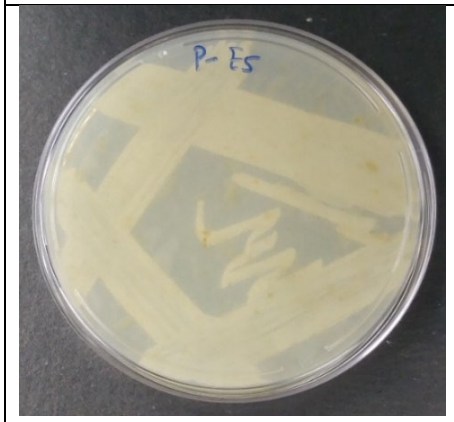
Phaeobacter gallaeciensis





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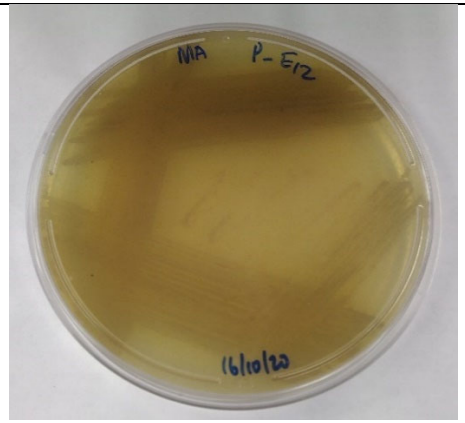

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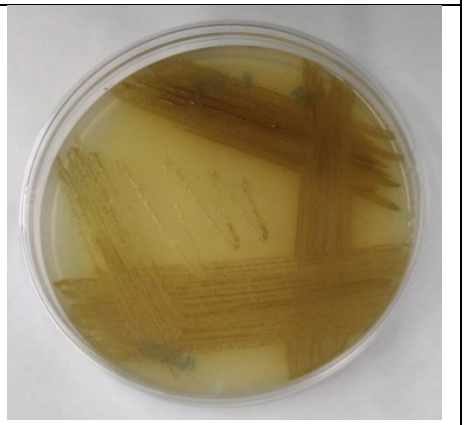

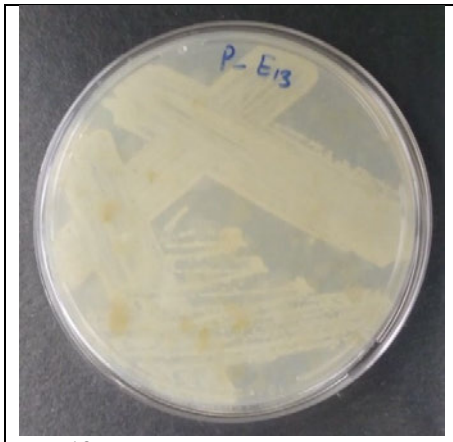

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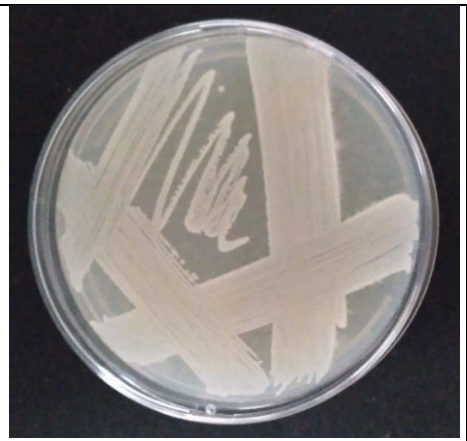

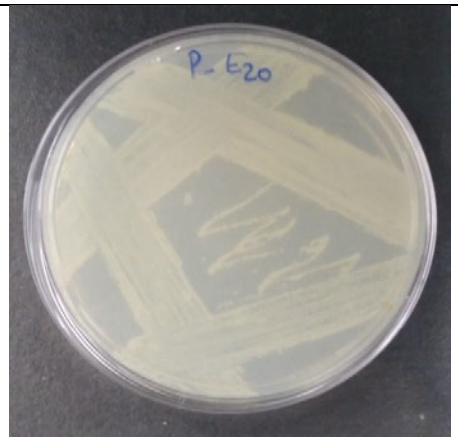

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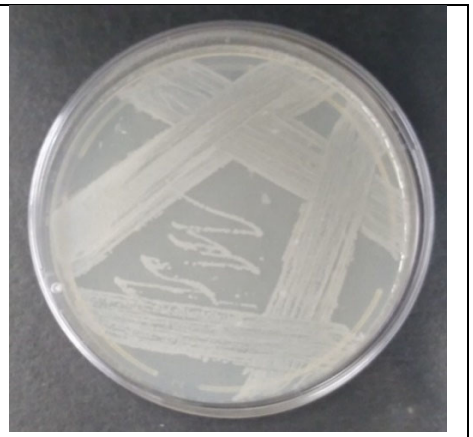

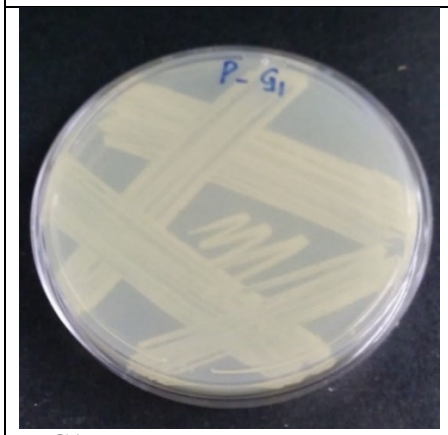

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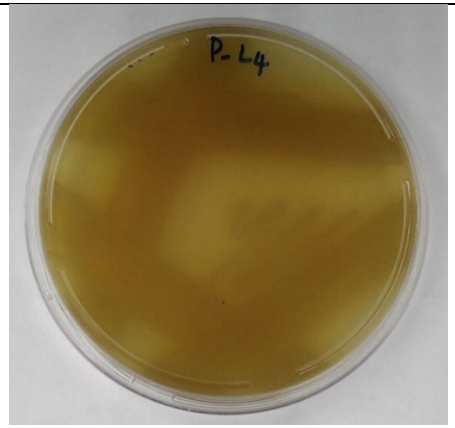

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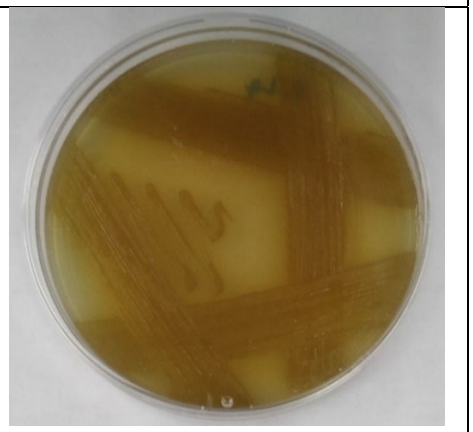

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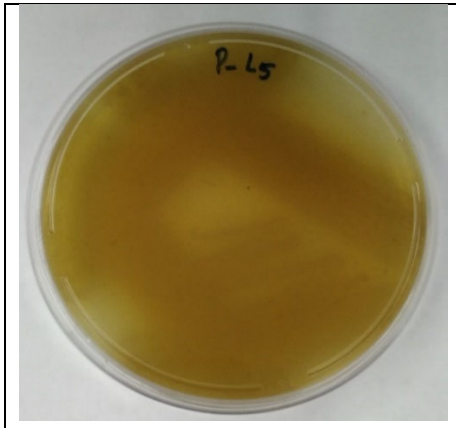
<td> 

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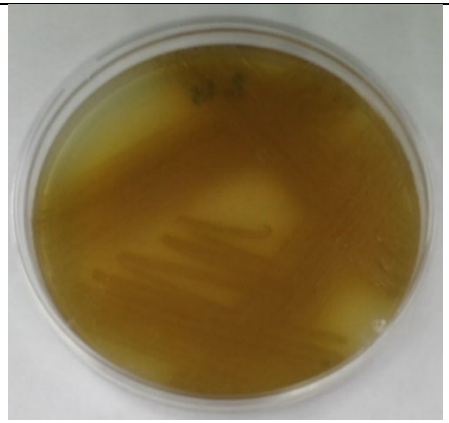
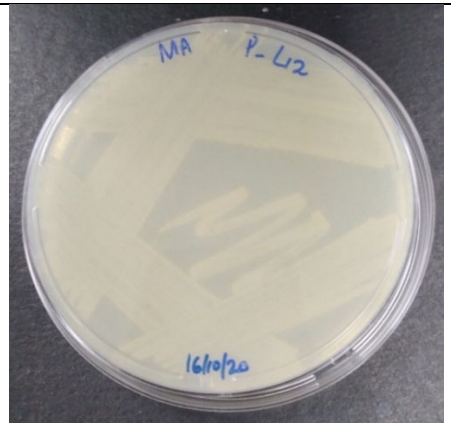
<td>  </td>
<td> 

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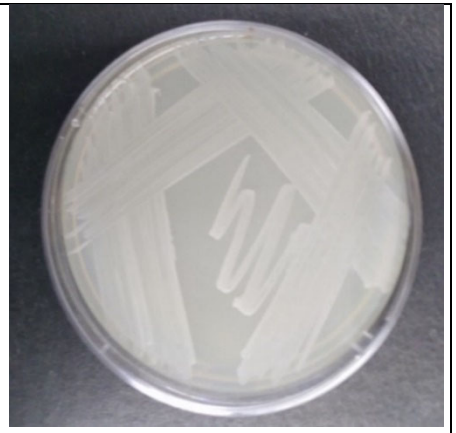
<td>  </td>
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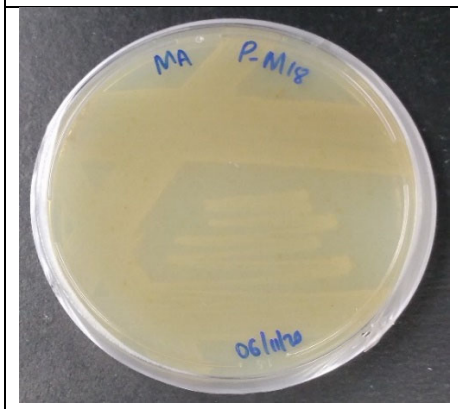
<td> 

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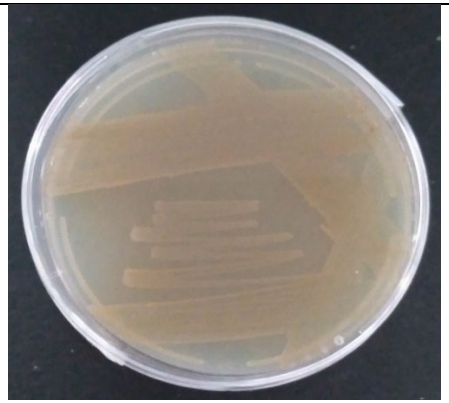
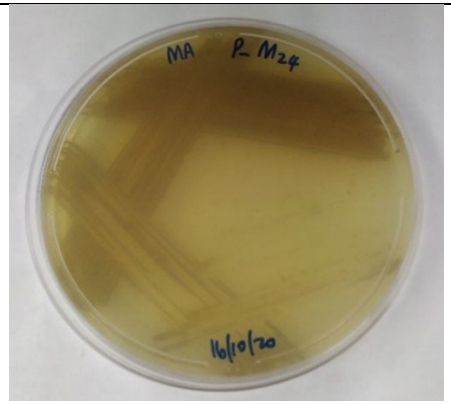
<td>  </td>
<td> 

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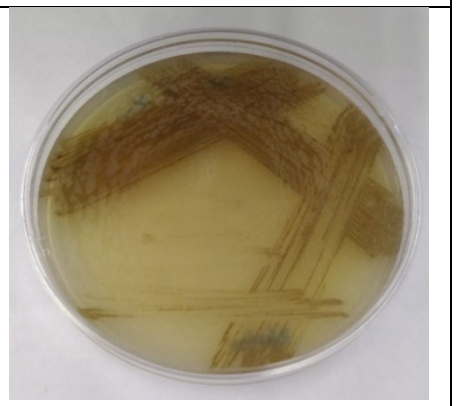
<td>  </td>
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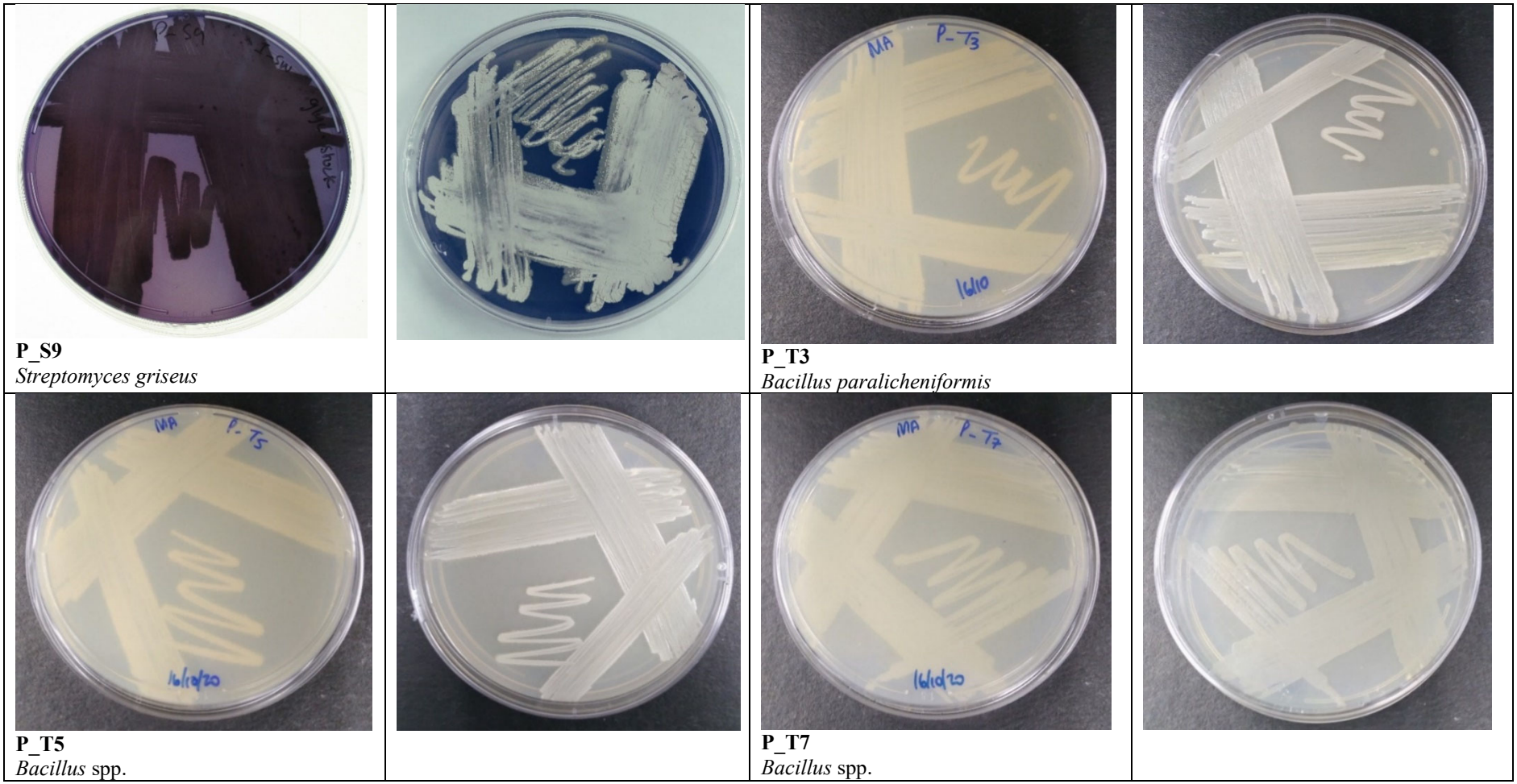
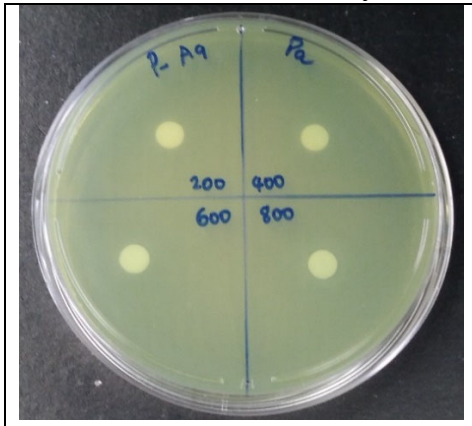
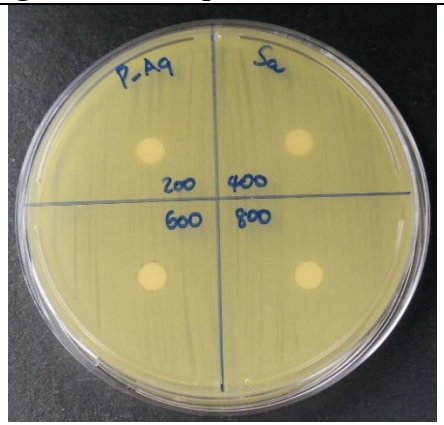


Figure S3.1: Sixteen *Pocillopora* CAB isolates were selected from primary screening for fermentation and secondary metabolite extraction.

3.7.2 Antimicrobial activity screening of 16 *Pocillopora* CAB extracts.



P_A9_*Pseudomonas aeruginosa*



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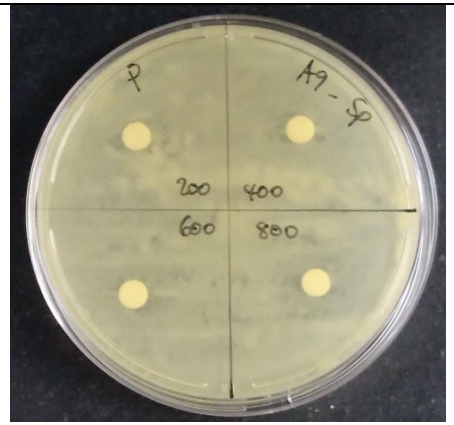
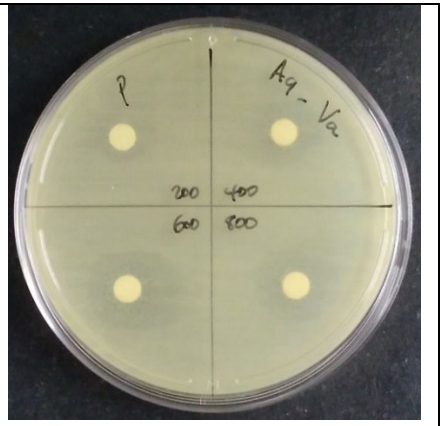


P A9 *Shewanella putrefaciens*



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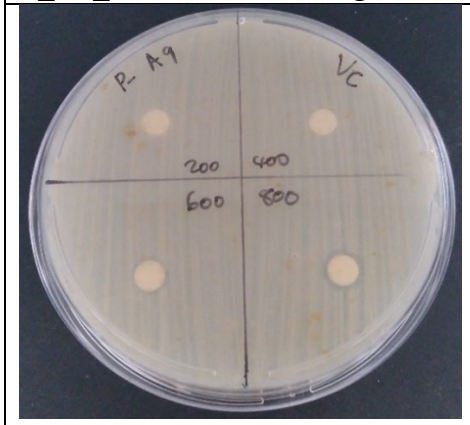

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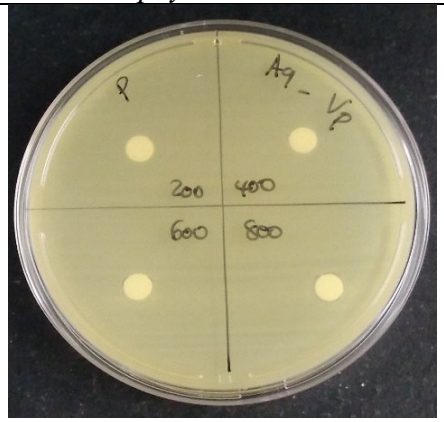

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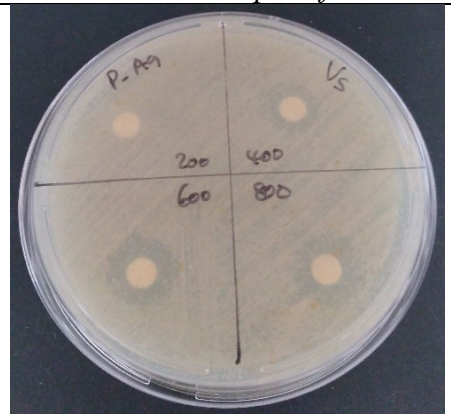

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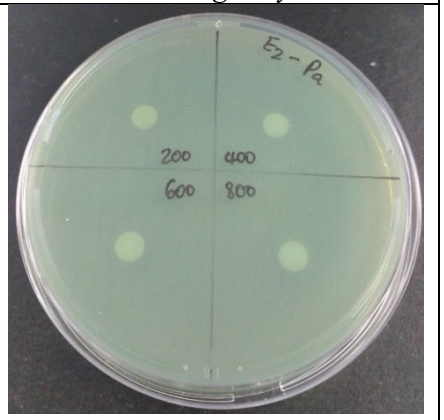

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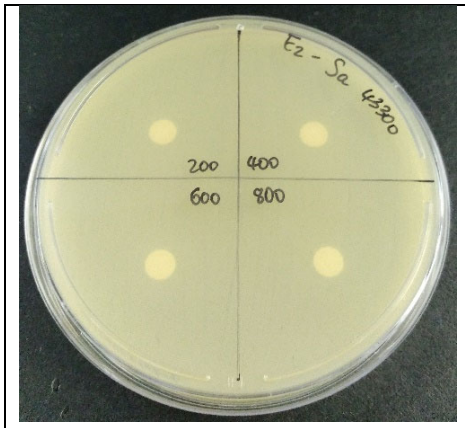

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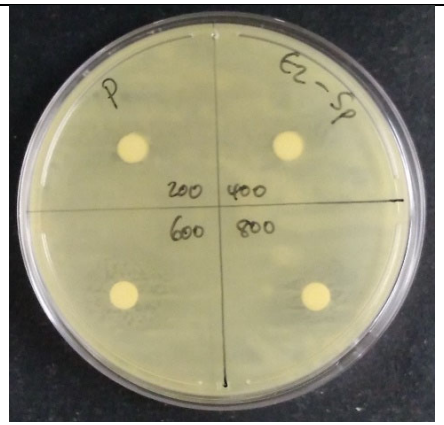

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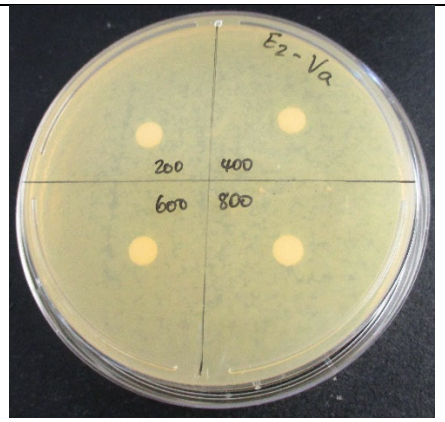
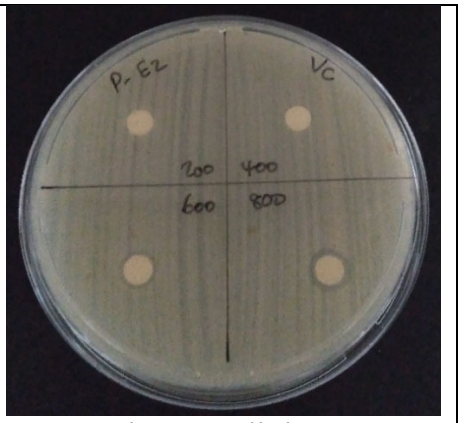
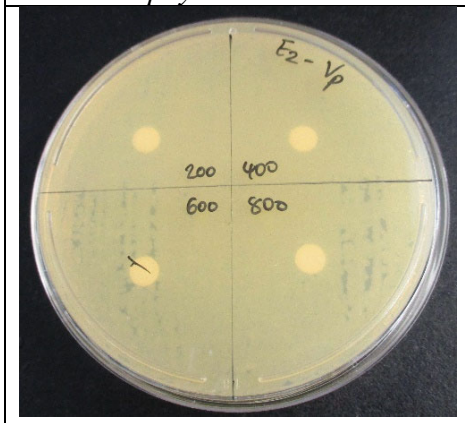


P_E2_*Vibrio alginolyticus*



P_E2_*Vibrio coralliilyticus*



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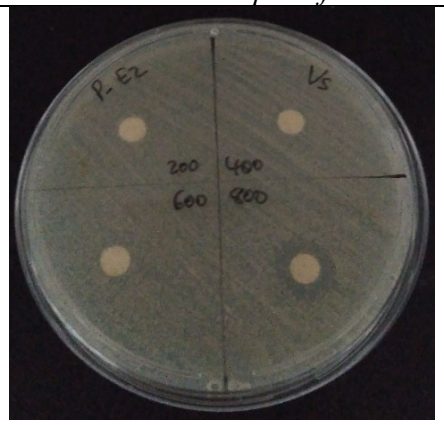

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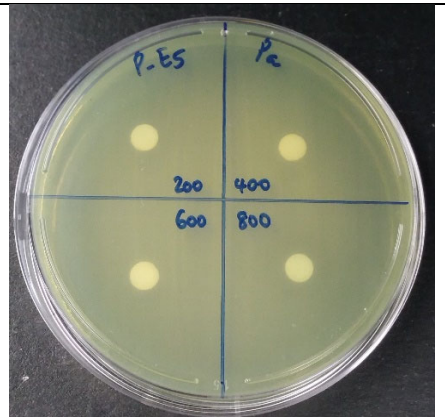

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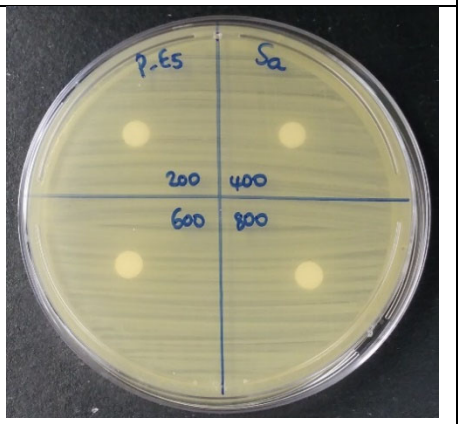
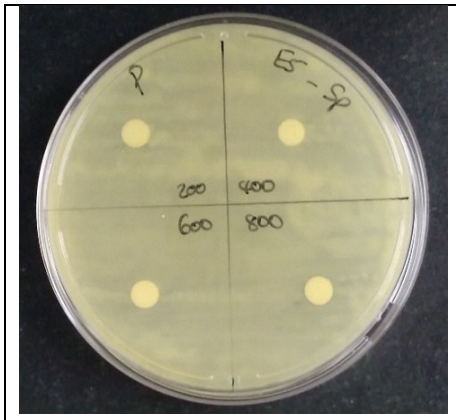
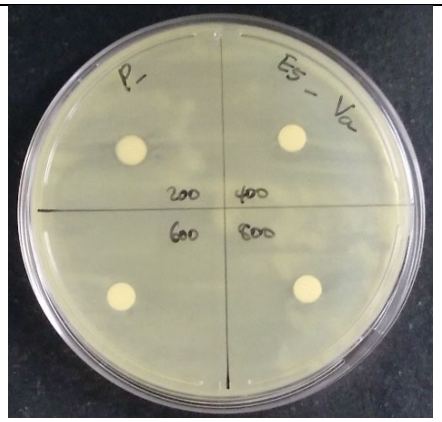
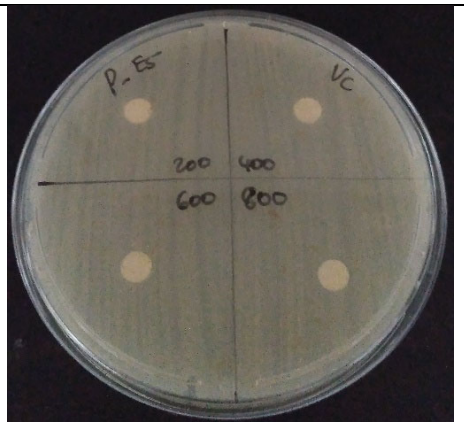
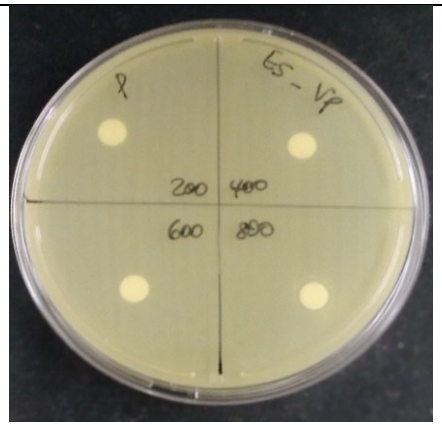
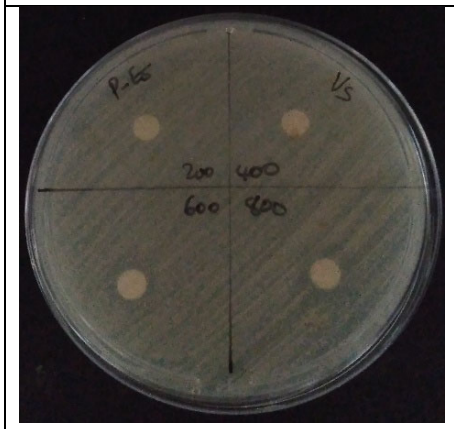
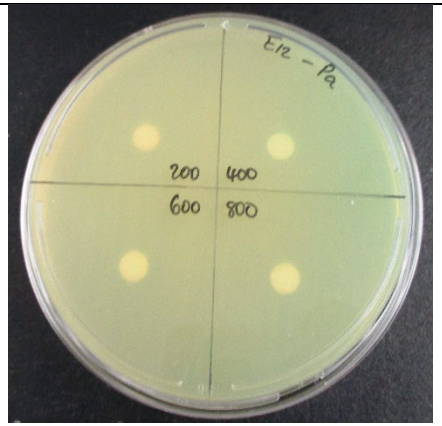
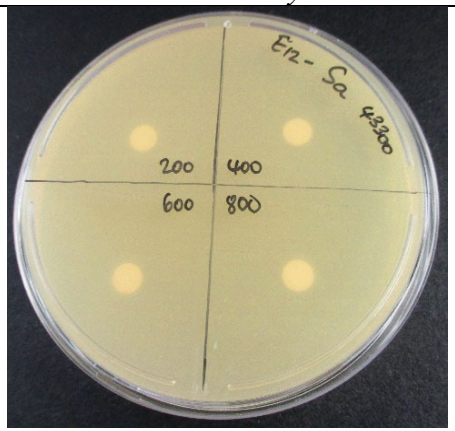
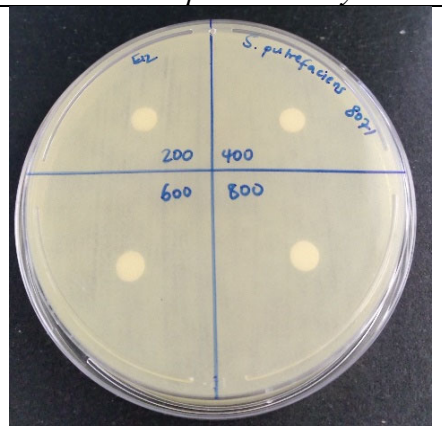
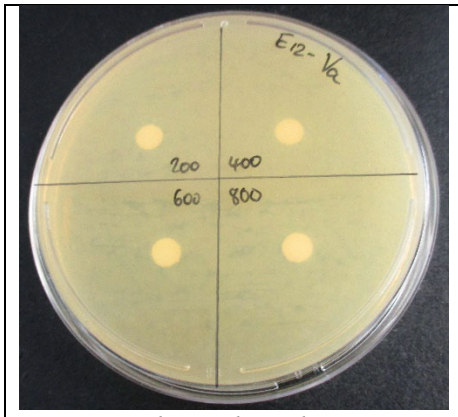
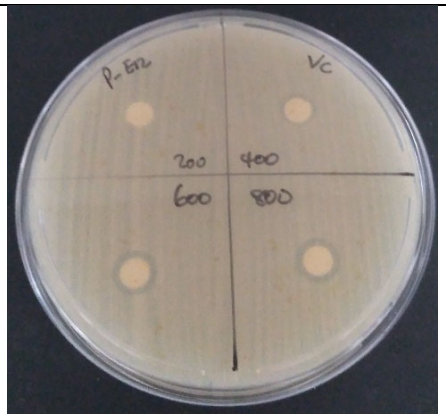
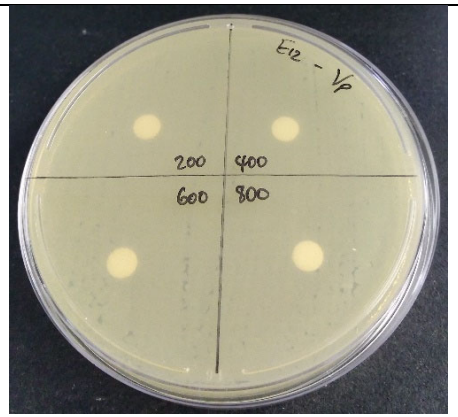
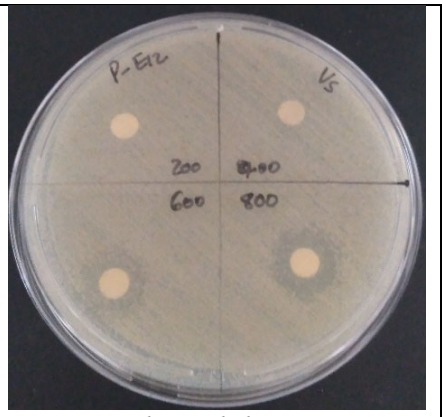
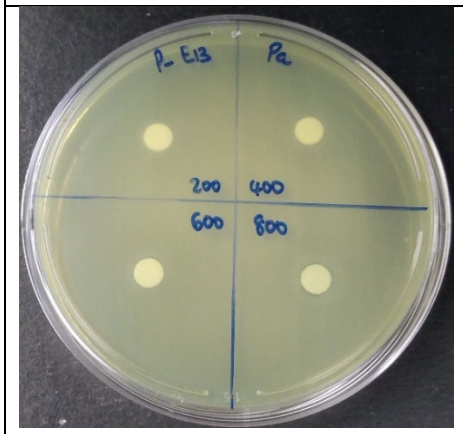
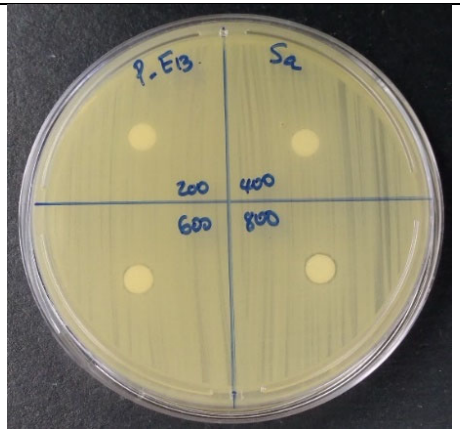
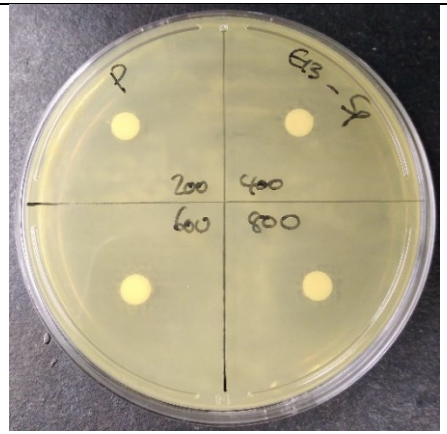
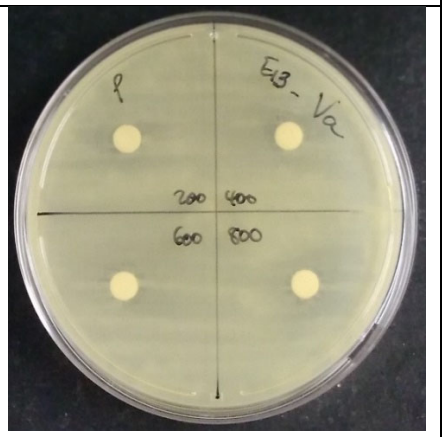
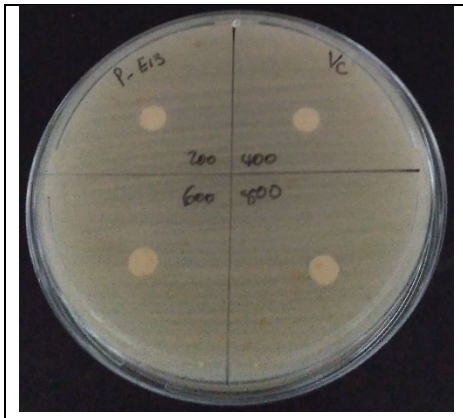


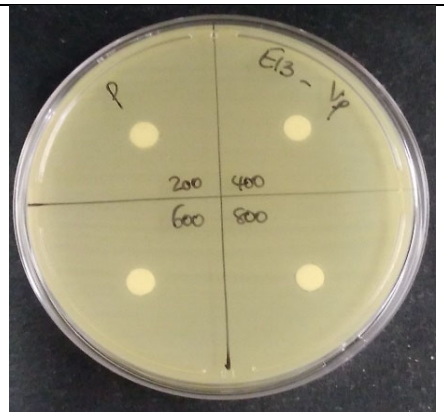
P E5 *Staphylococcus aureus*

 **P_E5**_*Shewanella putrefaciens*	 **P_E5**_*Vibrio alginolyticus*	 **P_E5** *Vibrio coralliilyticus*	 **P_E5** *Vibrio parahaemolyticus*
 **P_E5**_*Vibrio shilonii*	 **P_E12**_*Pseudomonas aeruginosa*	 **P_E12**_*Staphylococcus aureus*	 **P_E12**_*Shewanella putrefaciens*

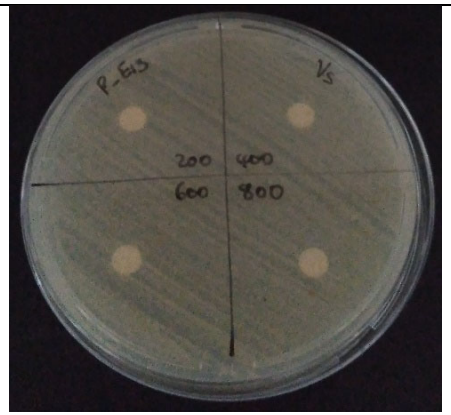
 **P_E12**_*Vibrio alginolyticus*	 **P_E12**_*Vibrio coralliilyticus*	 **P_E12**_*Vibrio parahaemolyticus*	 **P_E12**_*Vibrio shilonii*
 **P_E13**_*Pseudomonas aeruginosa*	 **P_E13**_*Staphylococcus aureus*	 **P_E13**_*Shewanella putrefaciens*	 **P_E13**_*Vibrio alginolyticus*



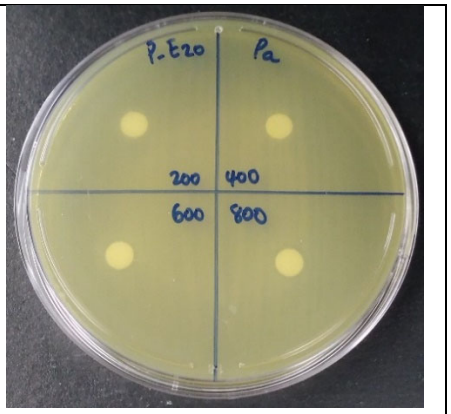
P_E13_*Vibrio coralliilyticus*



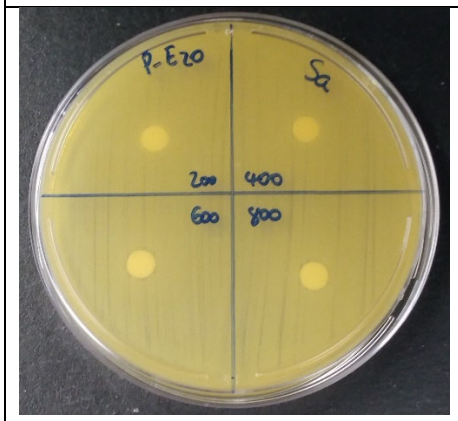
P_E13_*Vibrio parahaemolyticus*



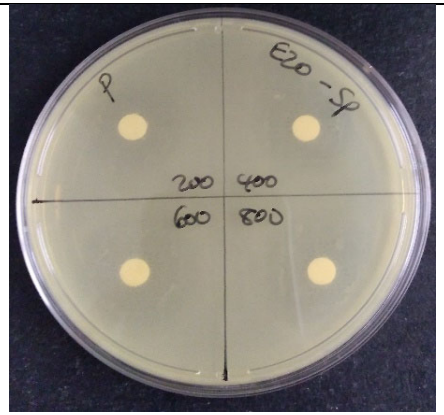
P_E13_*Vibrio shilonii*



P_E20_*Pseudomonas aeruginosa*

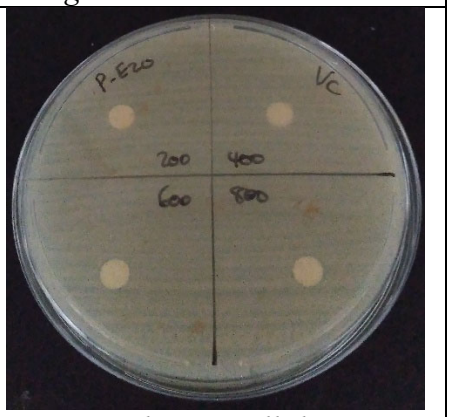


P E20 *Staphylococcus aureus*

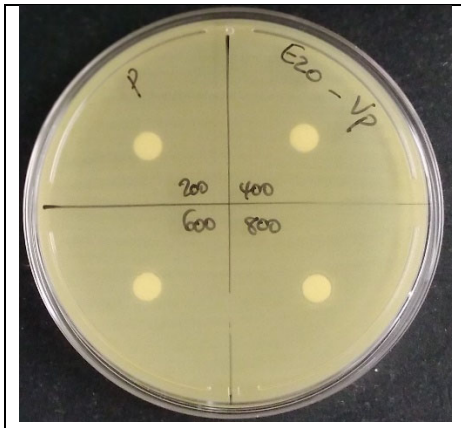


P E20 *Shewanella putrefaciens*

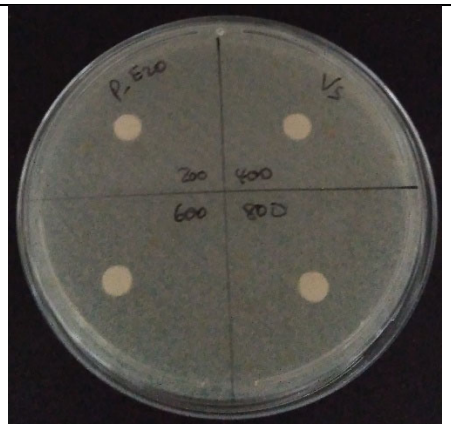
P_E20_*Vibrio alginolyticus*



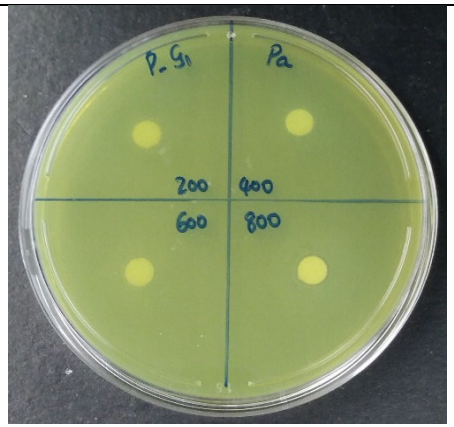
P_E20_*Vibrio coralliilyticus*



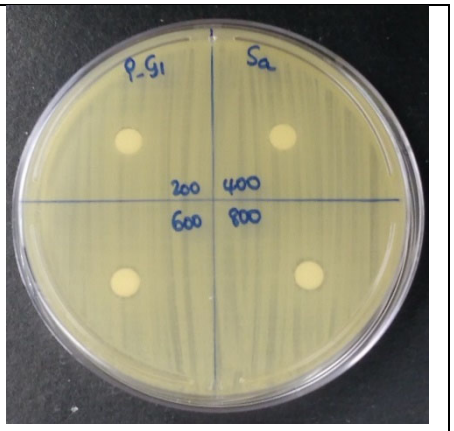
P_E20_*Vibrio parahaemolyticus*



P_E20_*Vibrio shilonii*

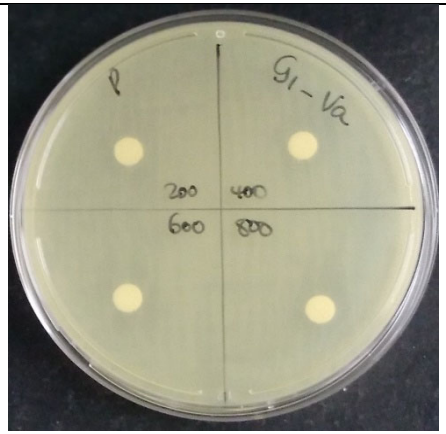


P_G1_*Pseudomonas aeruginosa*

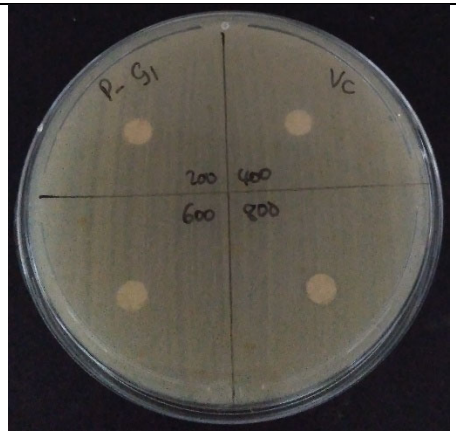


P_G1_*Staphylococcus aureus*

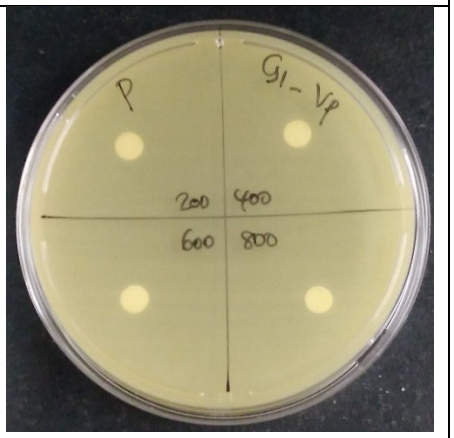
P_G1_*Shewanella putrefaciens*



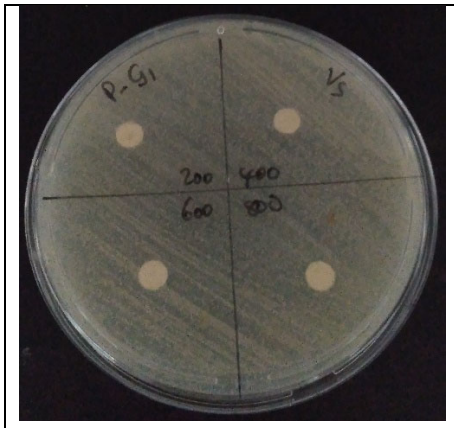
P_G1_*Vibrio alginolyticus*



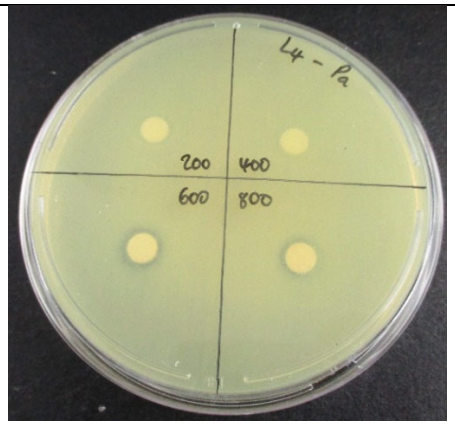
P_G1_*Vibrio coralliilyticus*



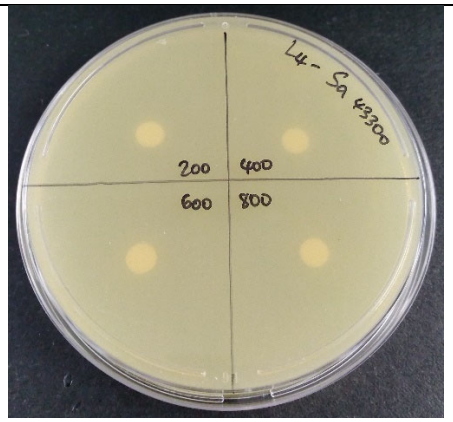
P_G1_*Vibrio parahaemolyticus*



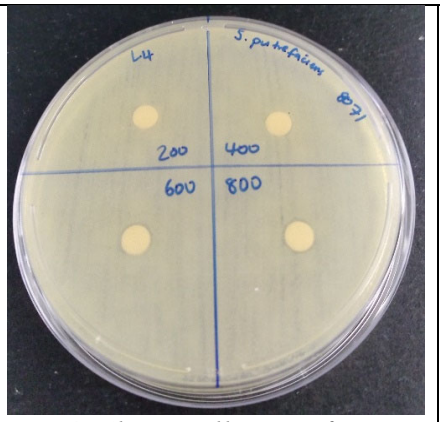
P G1 *Vibrio shilonii*



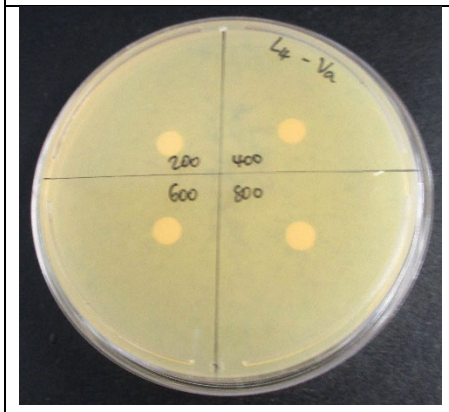
P L4 *Pseudomonas aeruginosa*



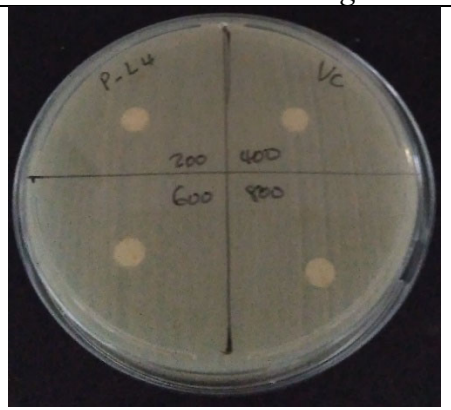
P_L4_*Staphylococcus aureus*



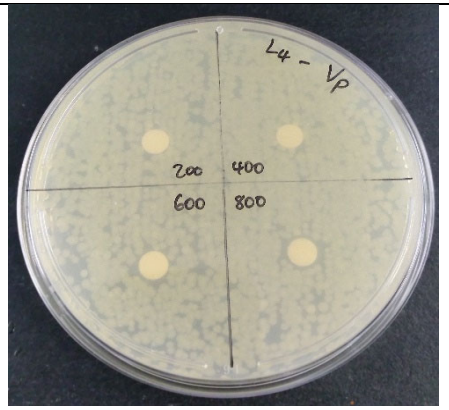
P_L4_*Shewanella putrefaciens*



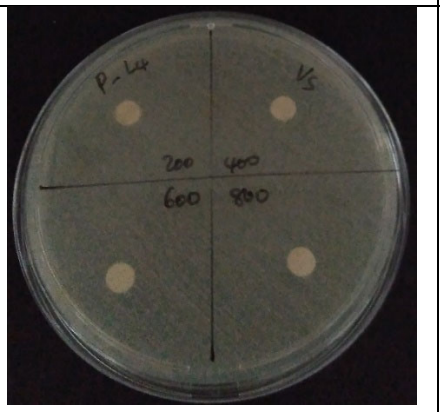
P_L4_*Vibrio alginolyticus*



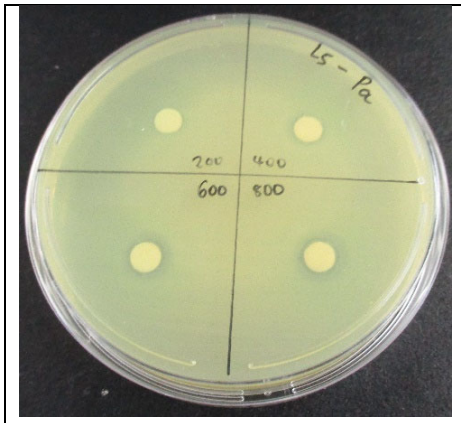
P L4 *Vibrio coralliilyticus*



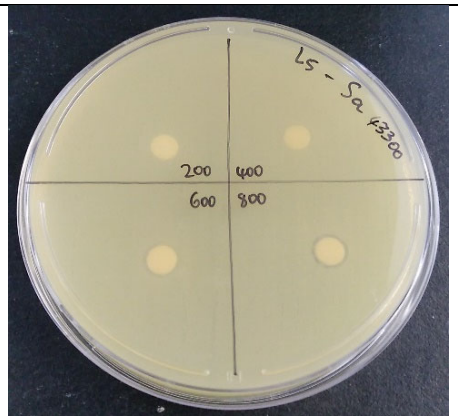
P L4 *Vibrio parahaemolyticus*



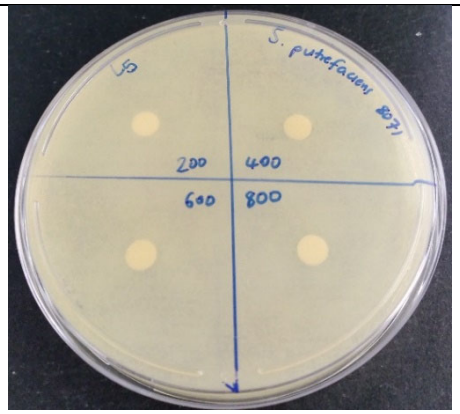
P_L4_*Vibrio shilonii*



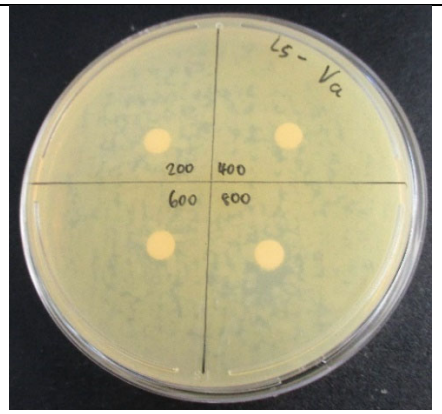
P L5 *Pseudomonas aeruginosa*



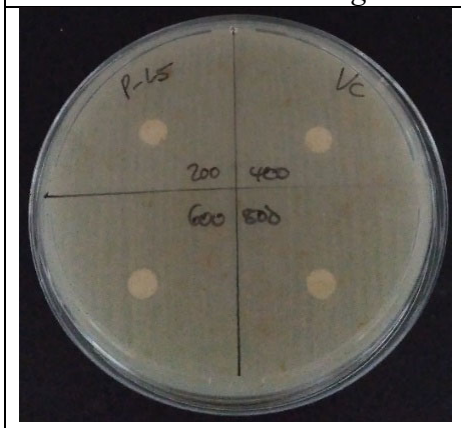
P L5 *Staphylococcus aureus*



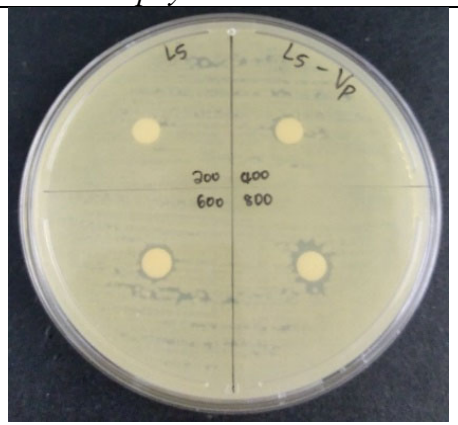
P_L5_*Shewanella putrefaciens*



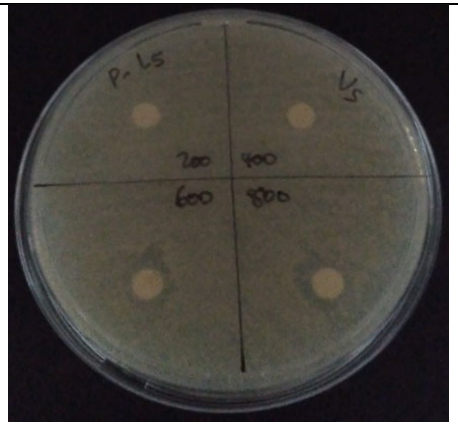
P_L5_*Vibrio alginolyticus*



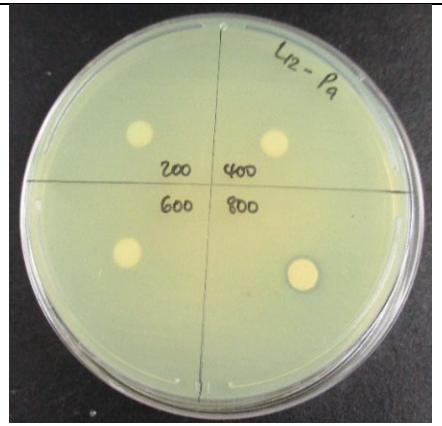
P_L5_*Vibrio coralliilyticus*



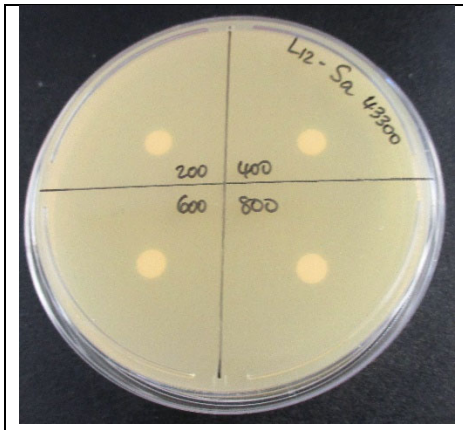
P_L5_*Vibrio parahaemolyticus*



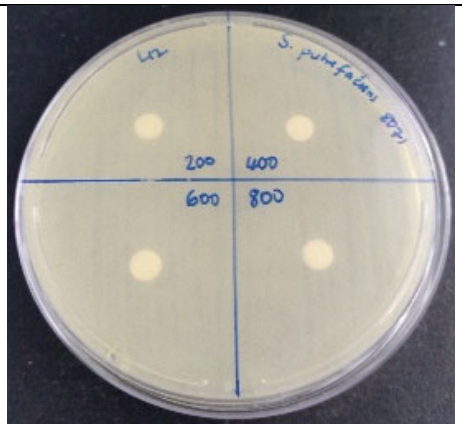
P_L5_*Vibrio shilonii*



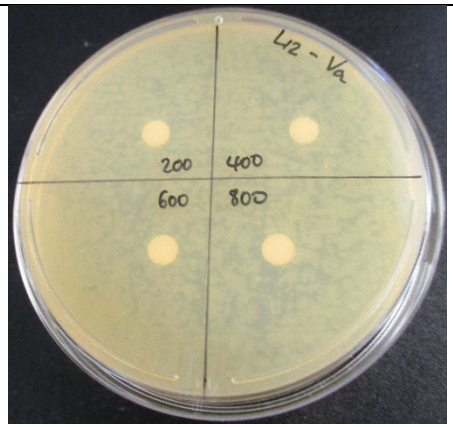
P_L12_*Pseudomonas aeruginosa*



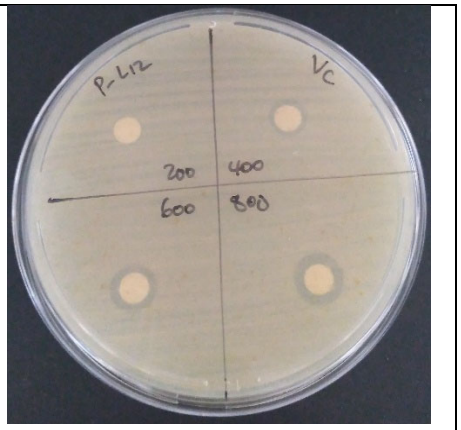
P L12 *Staphylococcus aureus*



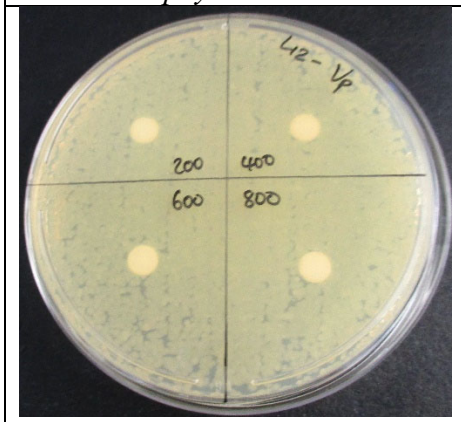
P L12 *Shewanella putrefaciens*



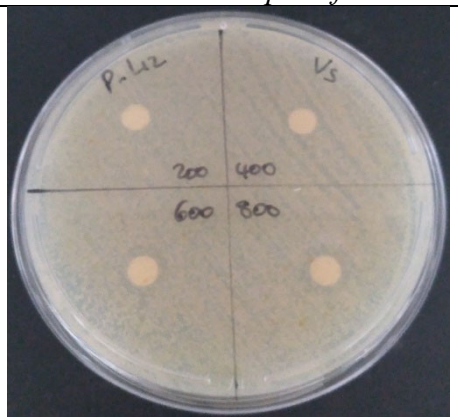
P L12 *Vibrio alginolyticus*



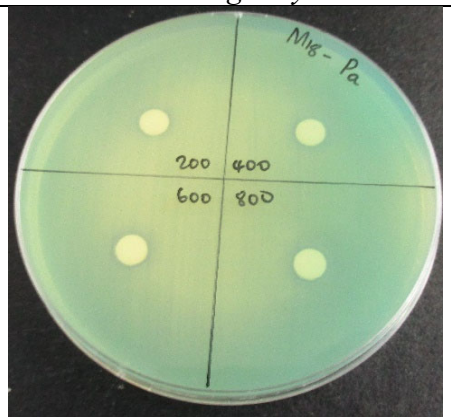
P L12 *Vibrio coralliilyticus*



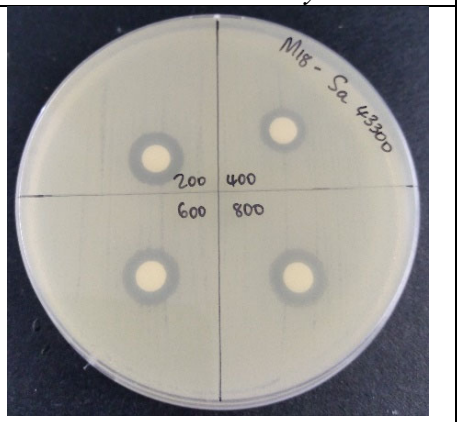
P_L12_*Vibrio parahaemolyticus*



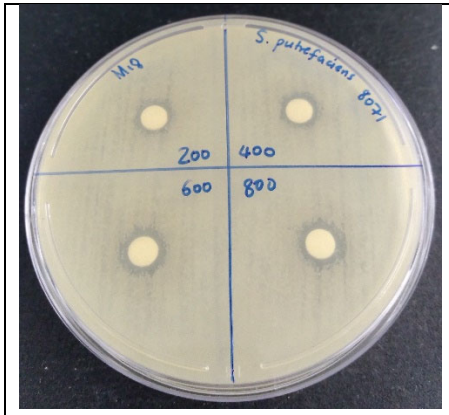
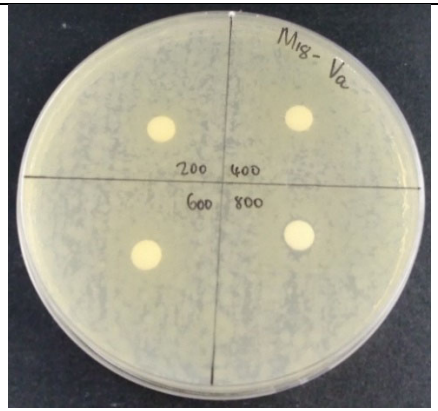
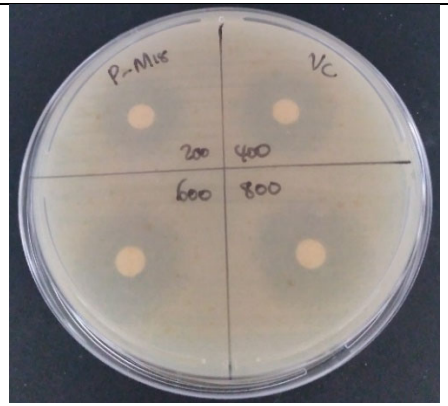
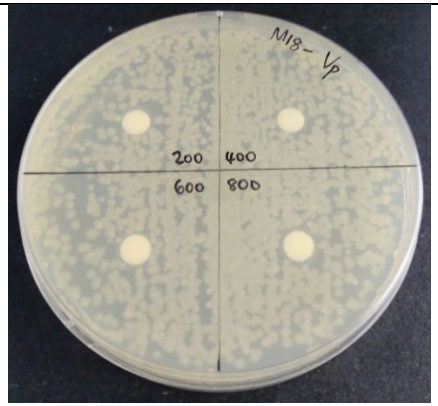
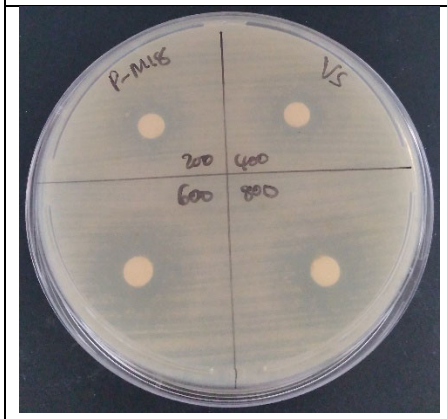
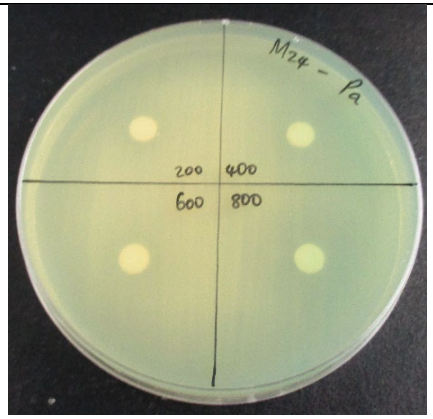
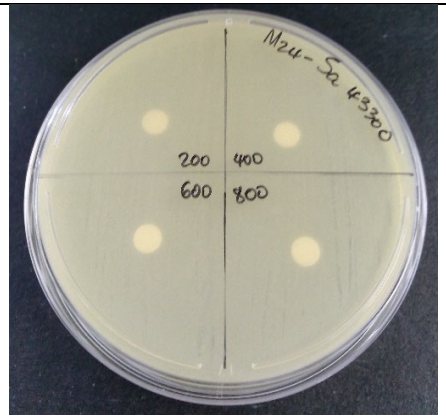
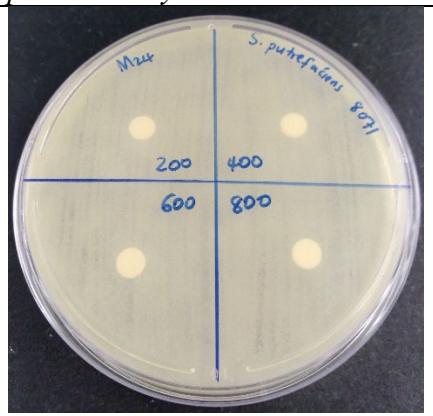
P_L12_*Vibrio shilonii*

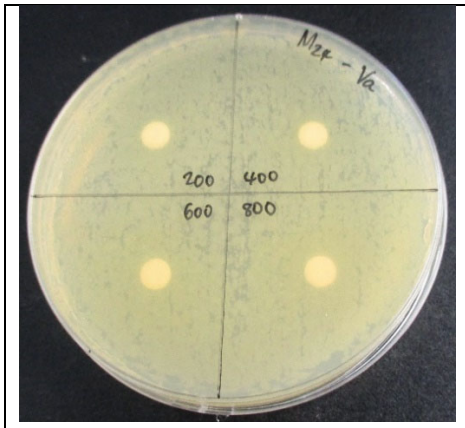


P_M18_*Pseudomonas aeruginosa*

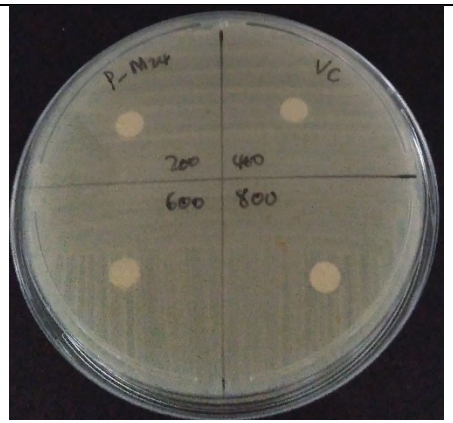


P_M18_*Staphylococcus aureus*

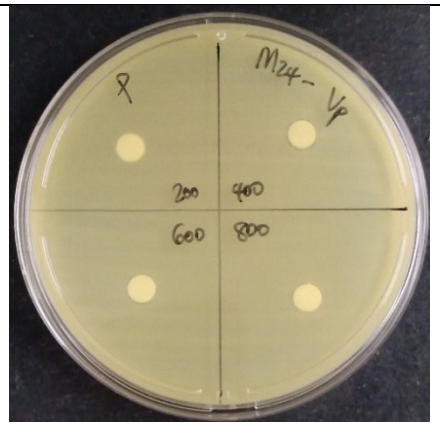
 **P_M18**_*Shewanella putrefaciens*	 **P_M18**_*Vibrio alginolyticus*	 **P_M18**_*Vibrio coralliilyticus*	 **P_M18**_*Vibrio parahaemolyticus*
 **P_M18**_*Vibrio shilonii*	 **P_M24**_*Pseudomonas aeruginosa*	 **P_M24**_*Staphylococcus aureus*	 **P_M24**_*Shewanella putrefaciens*



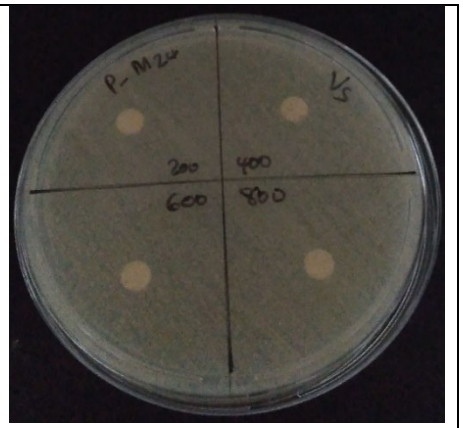
P_M24_*Vibrio alginolyticus*



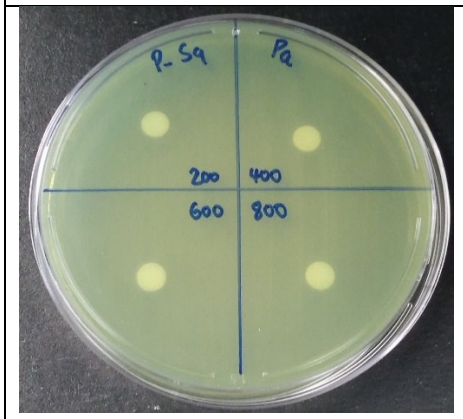
P_M24_*Vibrio coralliilyticus*



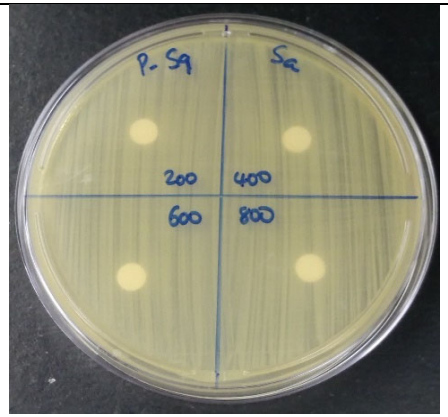
P_M24_*Vibrio parahaemolyticus*



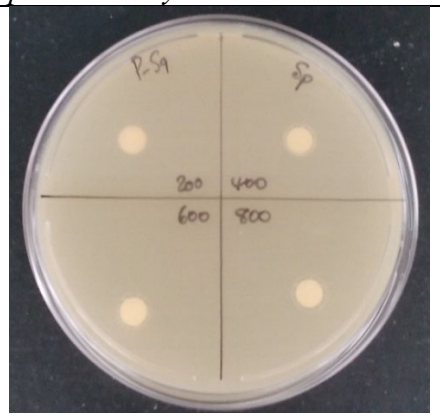
P_M24_*Vibrio shilonii*



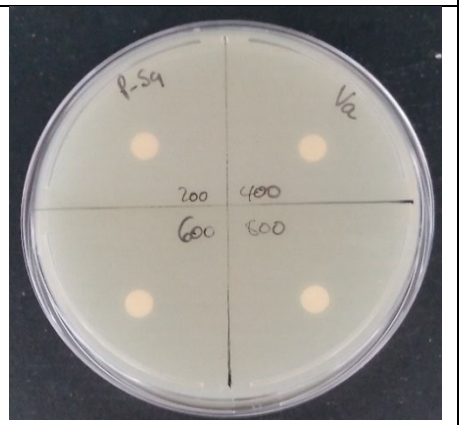
P_S9_*Pseudomonas aeruginosa*



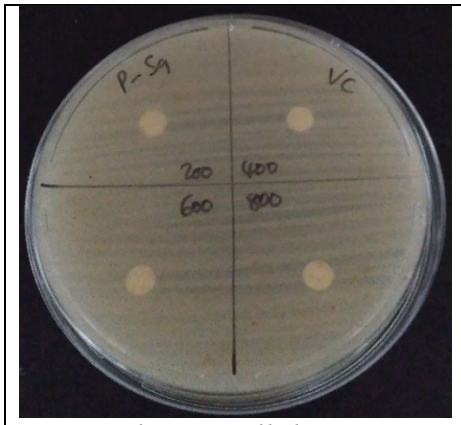
P_S9_*Staphylococcus aureus*



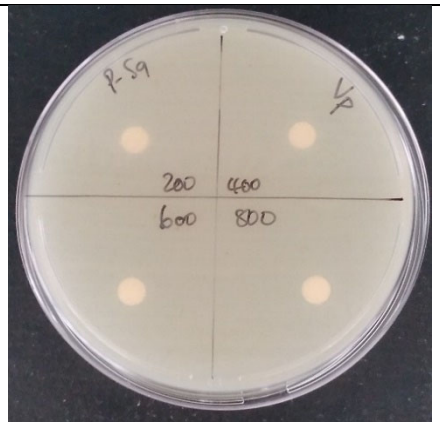
P_S9_*Shewanella putrefaciens*



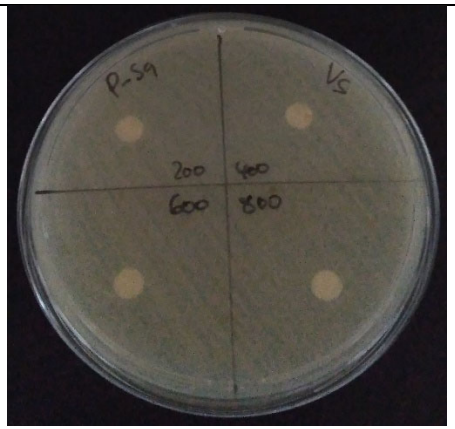
P_S9_*Vibrio alginolyticus*



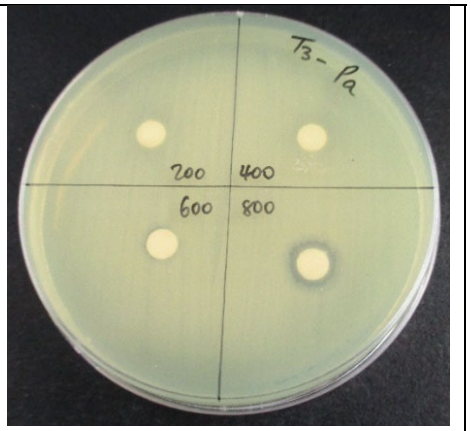
P_S9_*Vibrio coralliilyticus*



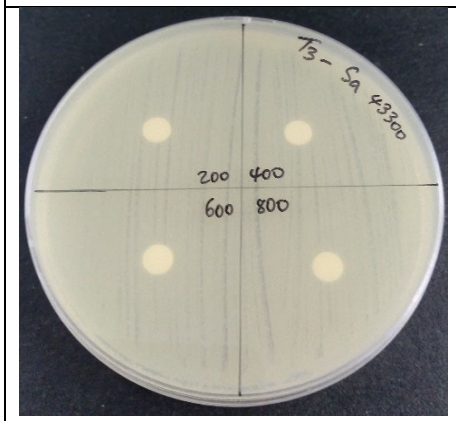
P_S9_*Vibrio parahaemolyticus*



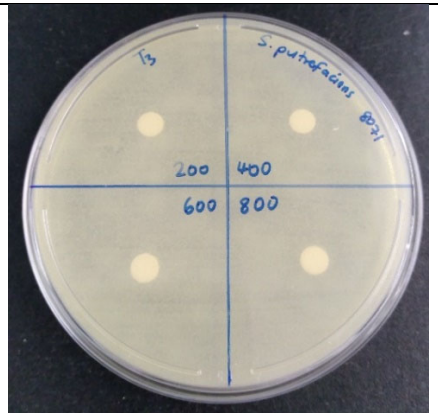
P_S9_*Vibrio shilonii*



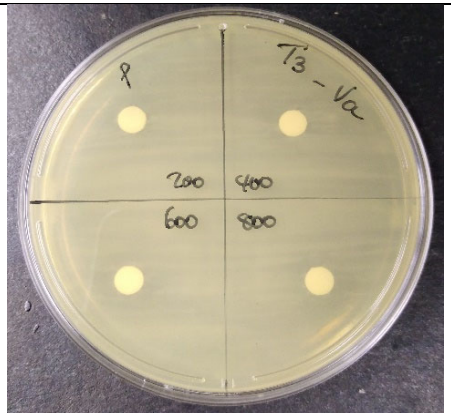
P_T3_*Pseudomonas aeruginosa*



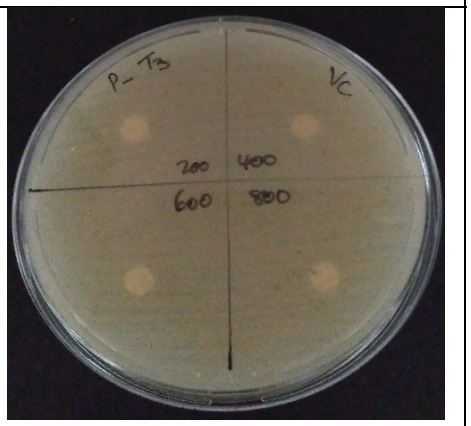
P_T3_*Staphylococcus aureus*



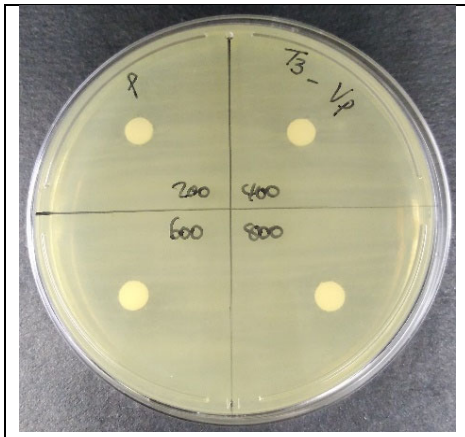
P_T3_*Shewanella putrefaciens*



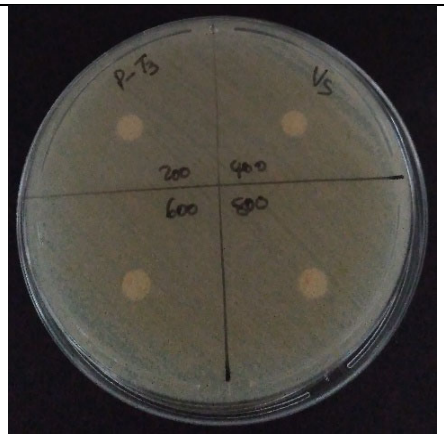
P_T3_*Vibrio alginolyticus*



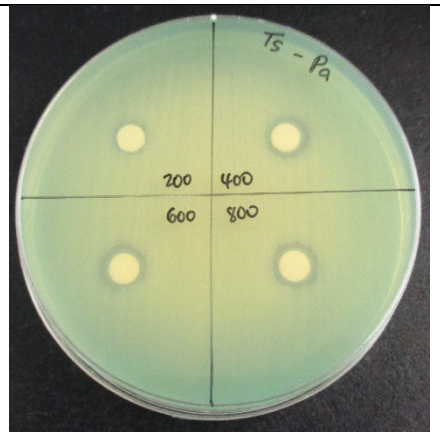
P_T3_*Vibrio coralliilyticus*



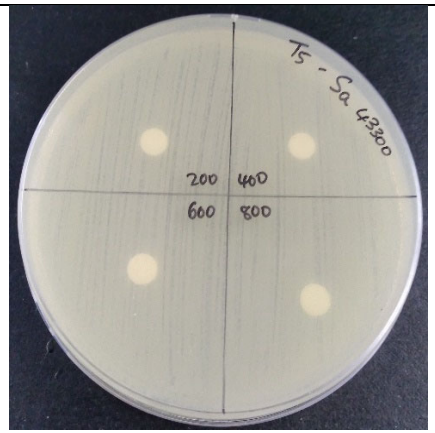
P T3 *Vibrio parahaemolyticus*



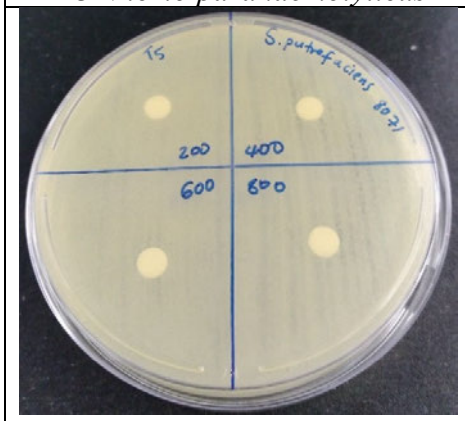
P T3 *Vibrio shilonii*



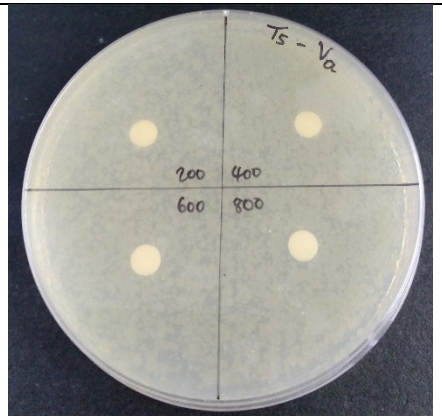
P_T5_*Pseudomonas aeruginosa*



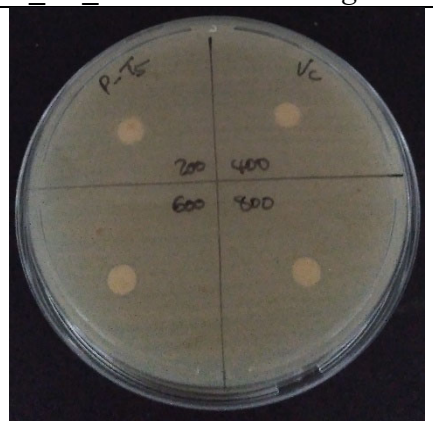
P_T5_*Staphylococcus aureus*



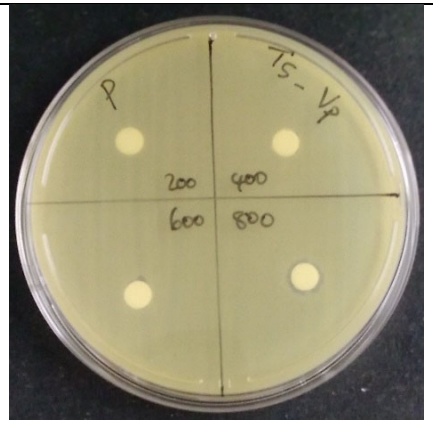
P_T5_*Shewanella putrefaciens*



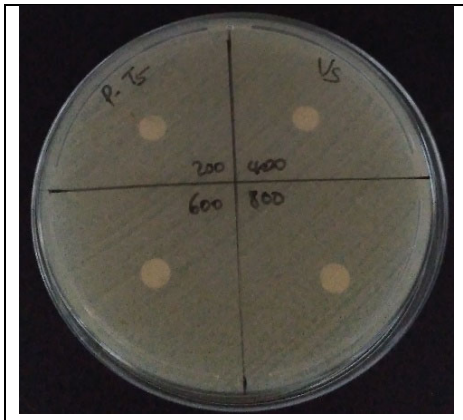
P_T5_*Vibrio alginolyticus*



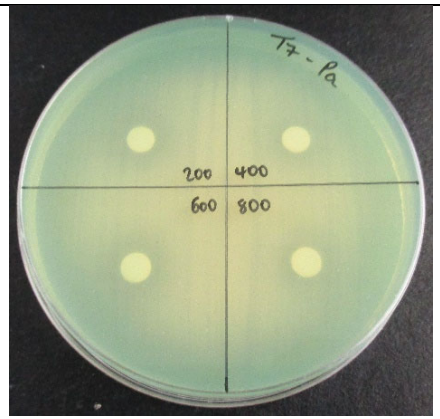
P_T5_*Vibrio coralliilyticus*



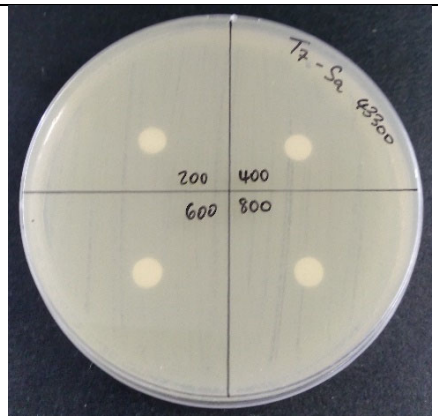
P_T5_*Vibrio parahaemolyticus*



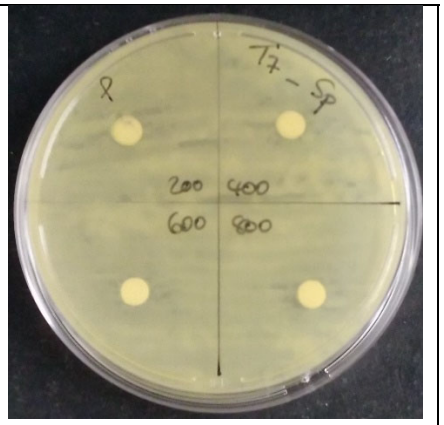
P_T5_*Vibrio shilonii*



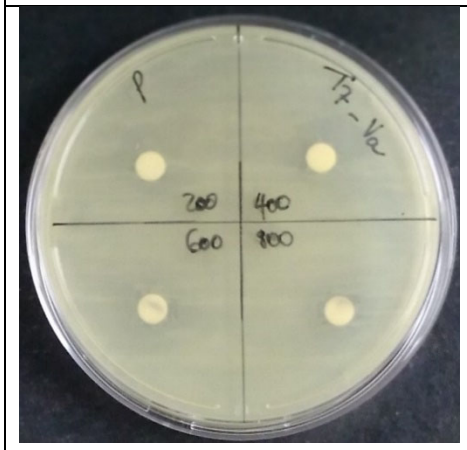
P_T7_*Pseudomonas aeruginosa*



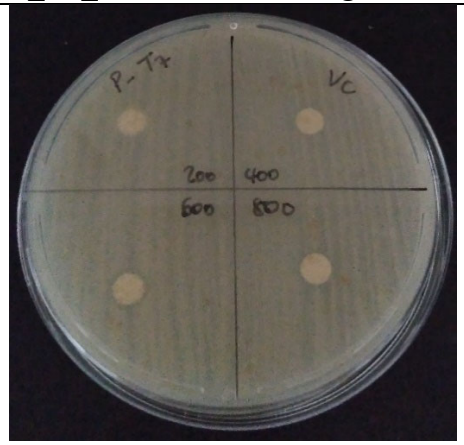
P_T7_*Staphylococcus aureus*



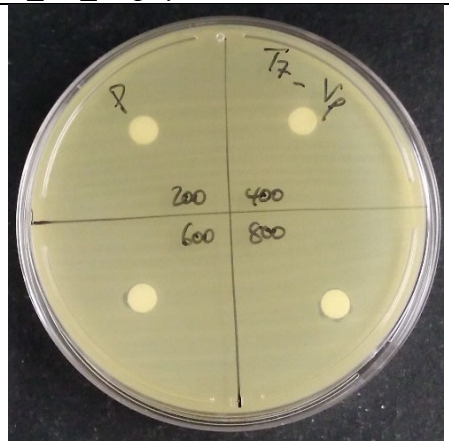
P T7 *Shewanella putrefaciens*



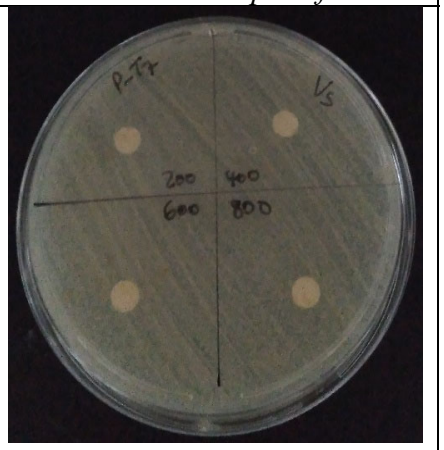
P T7 *Vibrio alginolyticus*



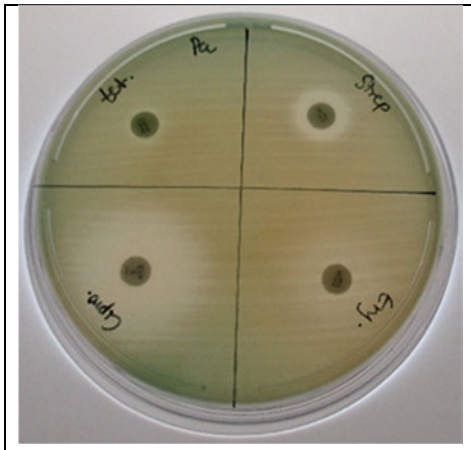
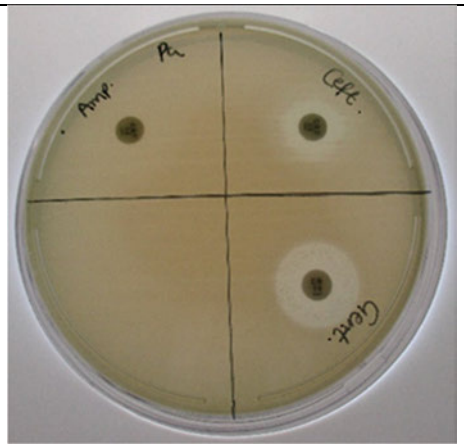
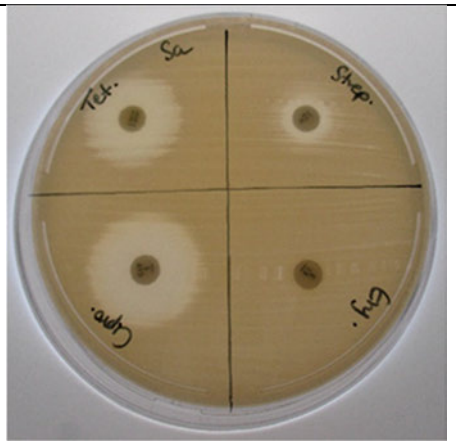
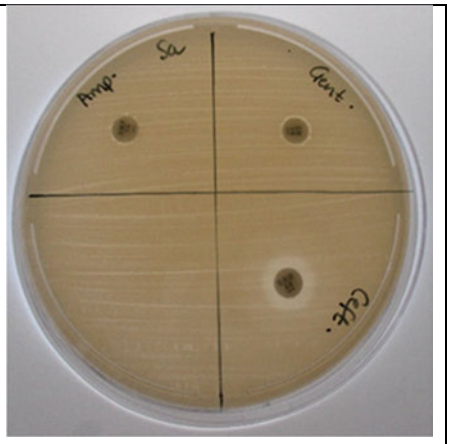
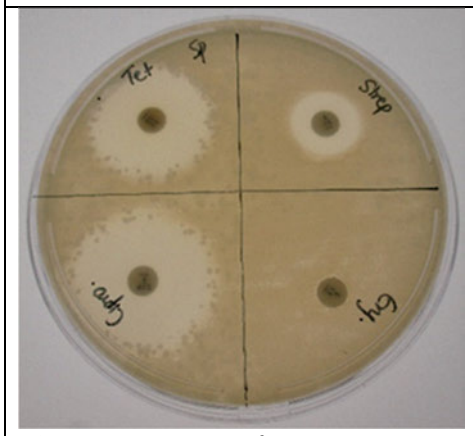
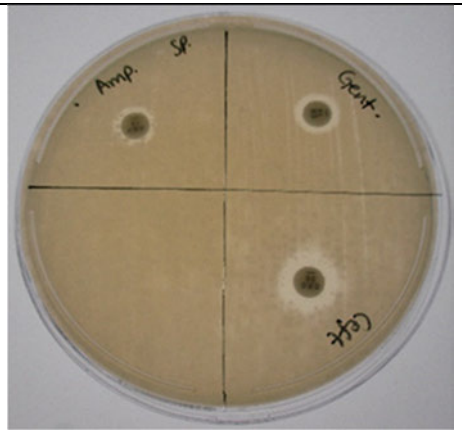
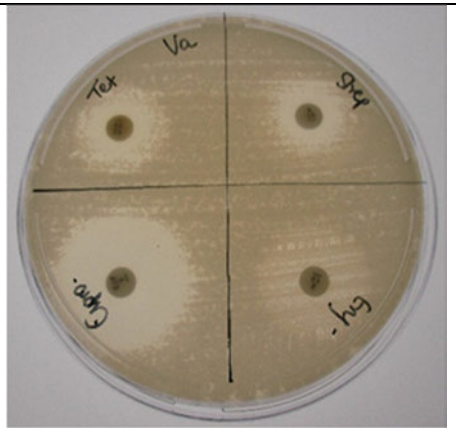
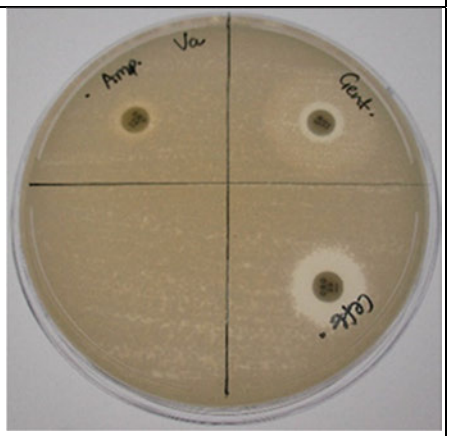
P T7 *Vibrio coralliilyticus*

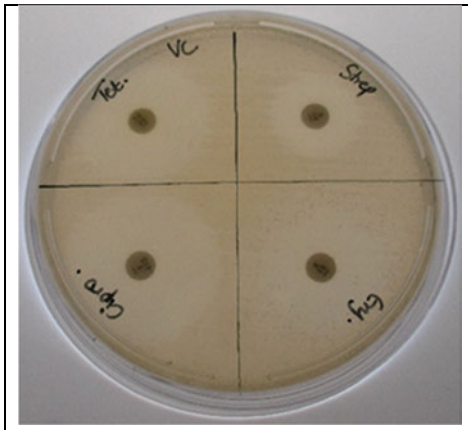


P T7 *Vibrio parahaemolyticus*

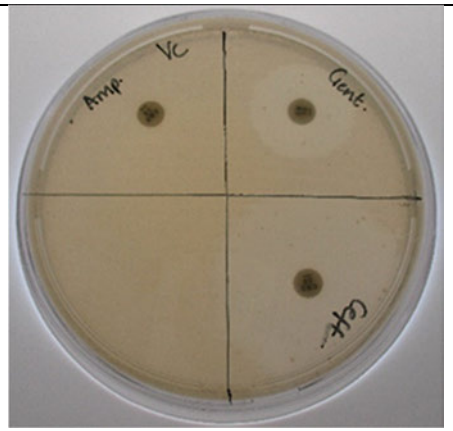


P T7 *Vibrio shilonii*

 **Control_***P. aeruginosa*	 **Control_** *P. aeruginosa*	 **Control_** *S. aureus*	 **Control_** *S. aureus*
 **Control_** *S. putrefaciens*	 **Control_** *S. putrefaciens*	 **Control_***V. alginolyticus*	 **Control_***V. alginolyticus*

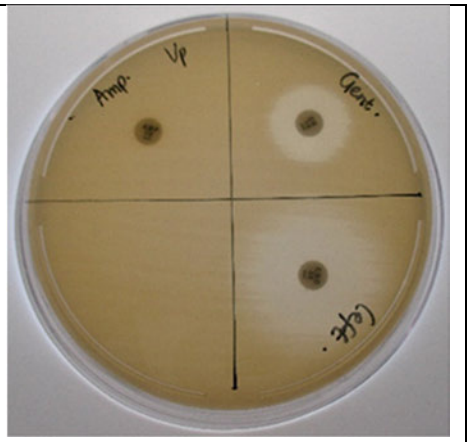


Control_ *V. corallilyticus*

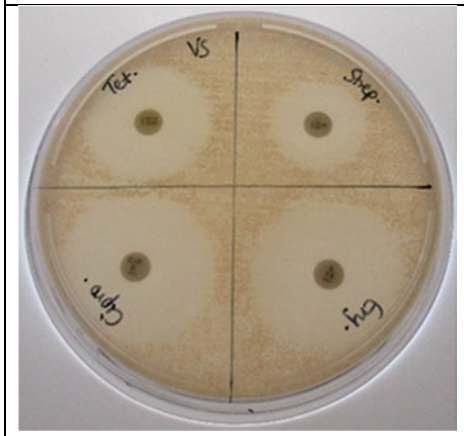


Control_ *V. corallilyticus*

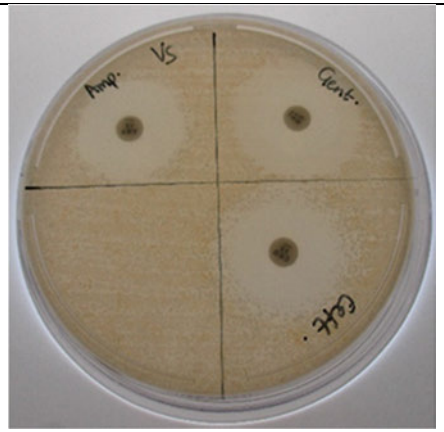
Control_*V. parahaemolyticus*



Control_*V. parahaemolyticus*



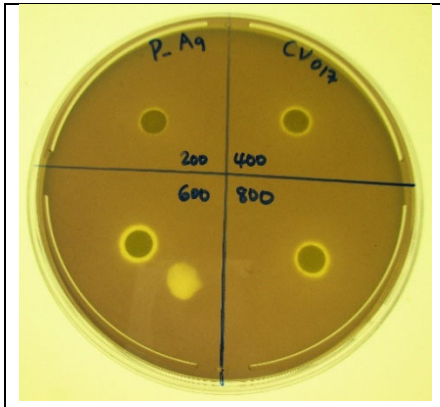
Control *V. shilonii*



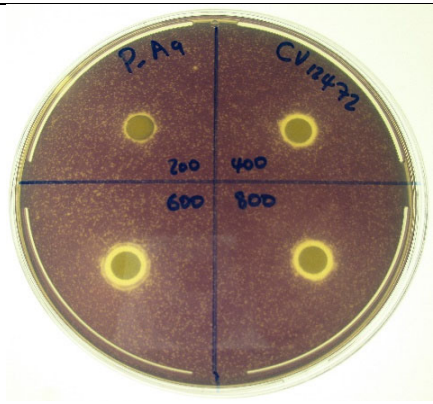
Control *V. shilonii*

Figure S3.2: Sixteen *Pocillopora* CAB extracts were subjected to secondary screening using the disc diffusion assay against clinical indicators, multidrug-resistant *Pseudomonas aeruginosa* ATCC 27853, methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300, marine bacteria *Shewanella putrefaciens* ATCC 8071, *Vibrio alginolyticus* ATCC 17749, *Vibrio parahaemolyticus* ATCC 17802, and marine pathogens *Vibrio coralliilyticus* ATCC_BAA 450, *Vibrio shilonii* ATCC_BAA 91. Antimicrobial activity was denoted by the appearance of clear and opaque zones around the discs, while clear zones were indicative of killing activity.

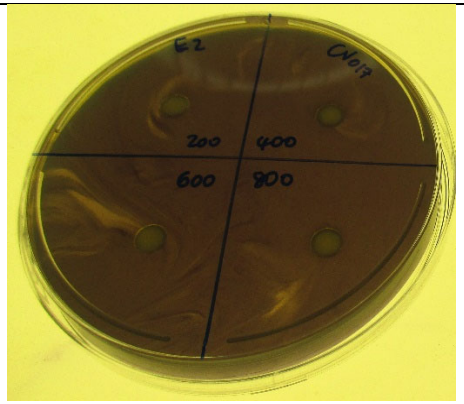
3.6.3 Anti-quorum sensing screening of 16 *Pocillopora* CAB extracts.



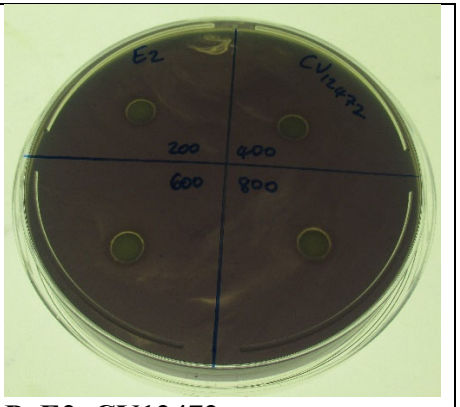
P A9 CV017



P A9 CV12472



P E2 CV017



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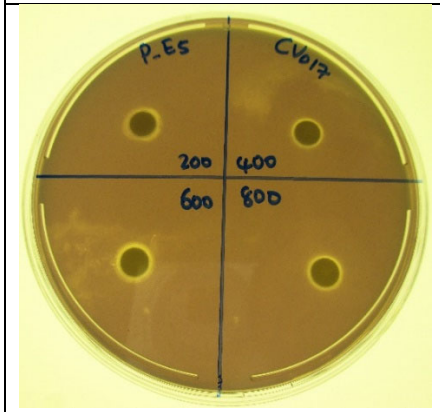
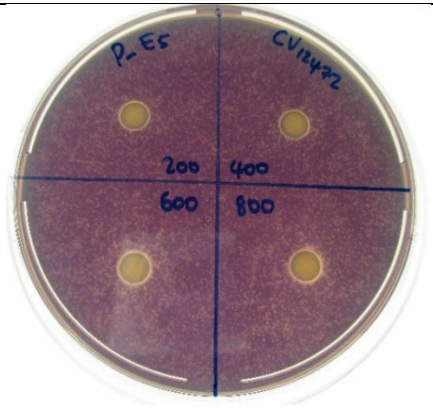


P E5 CV017



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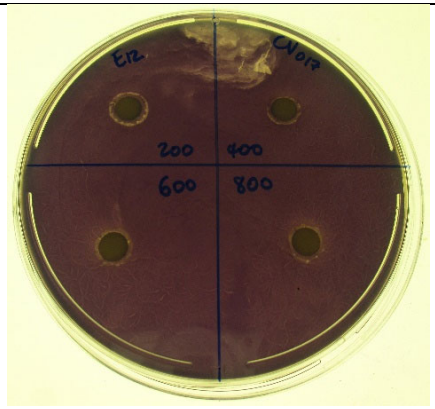

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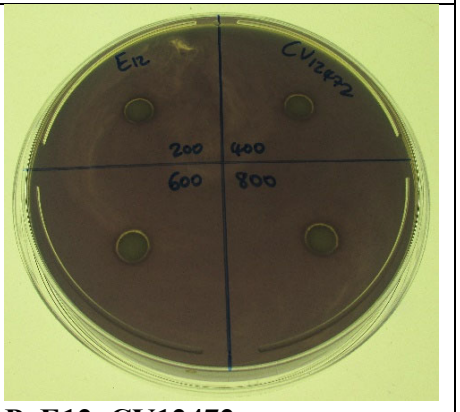

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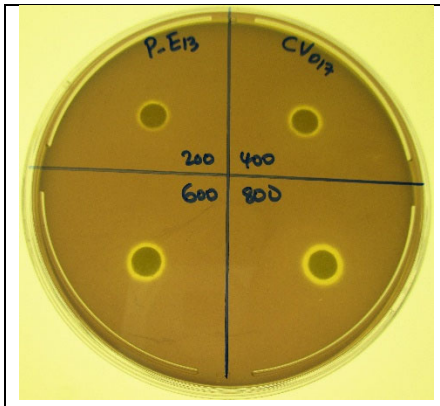

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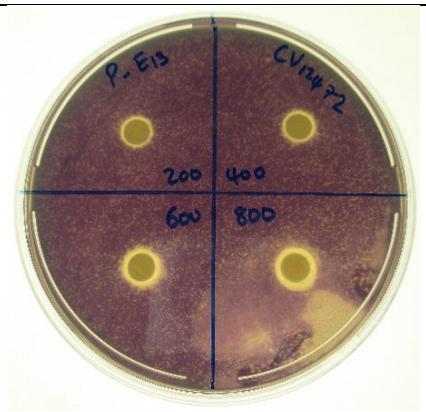

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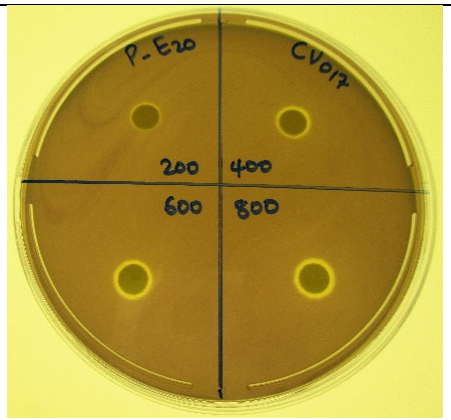

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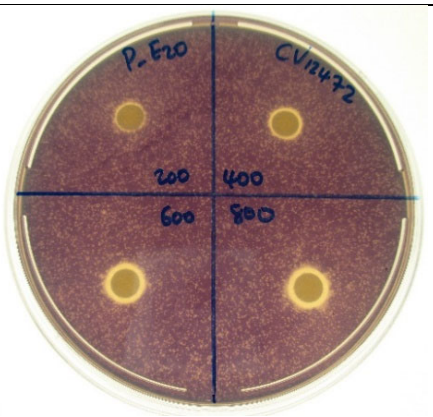

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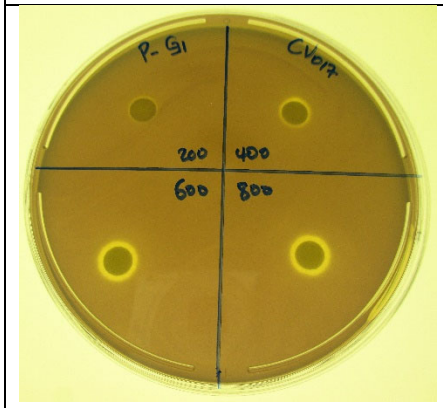
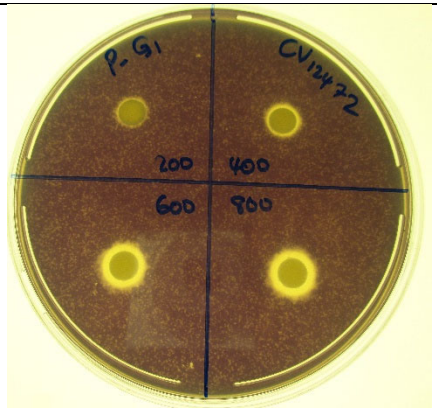


P_G1_CV017



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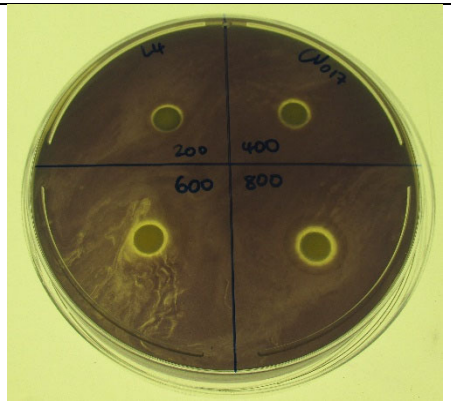

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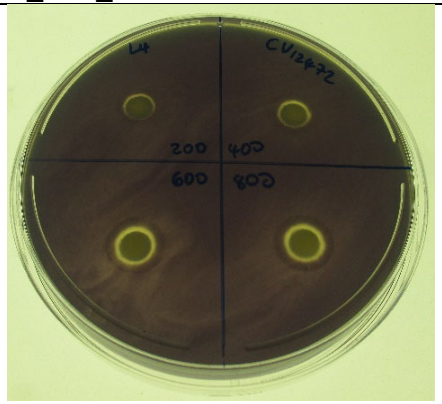
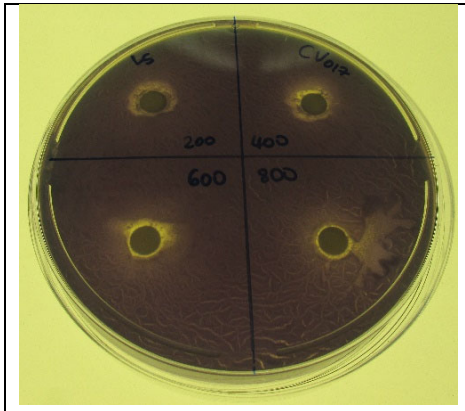
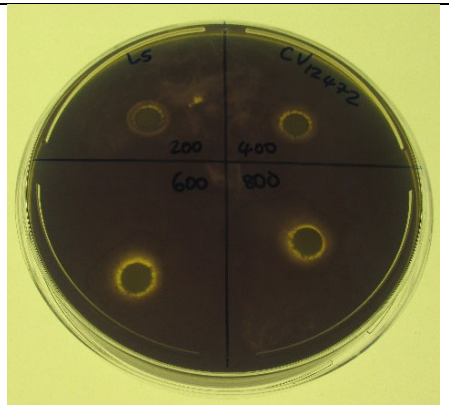
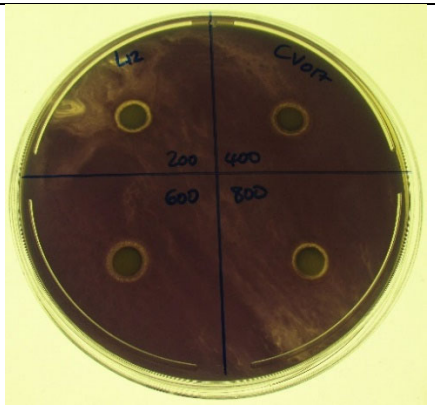
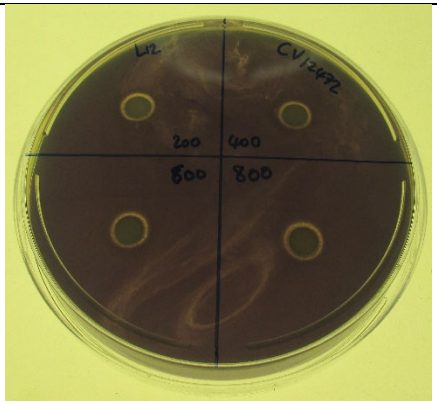
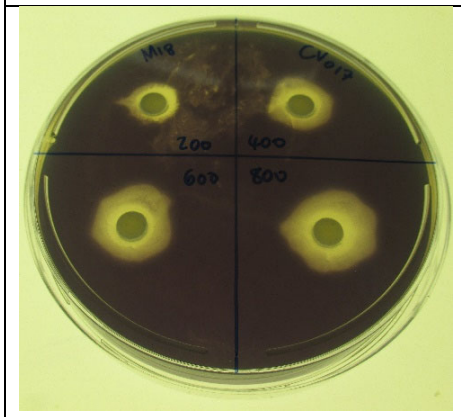
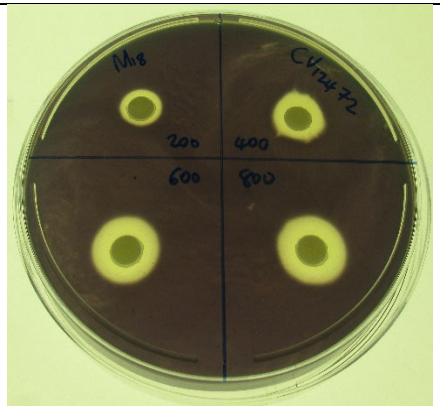
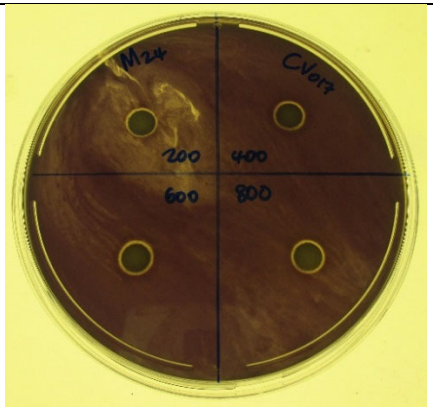
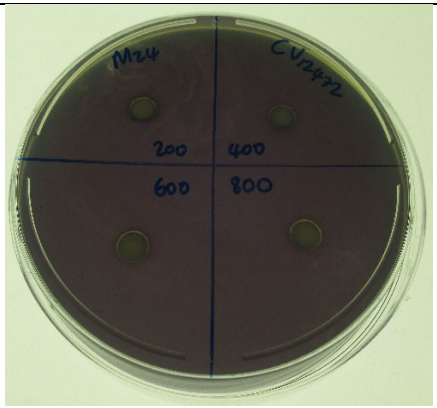
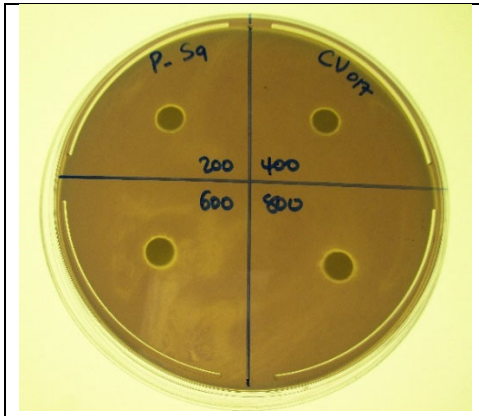
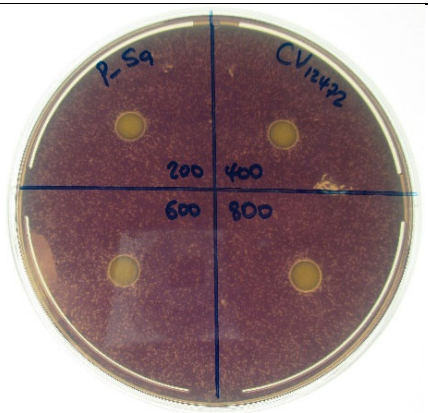
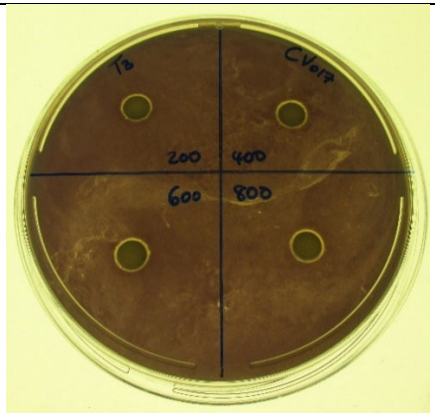
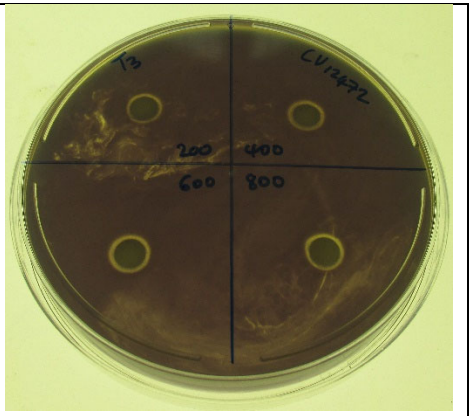
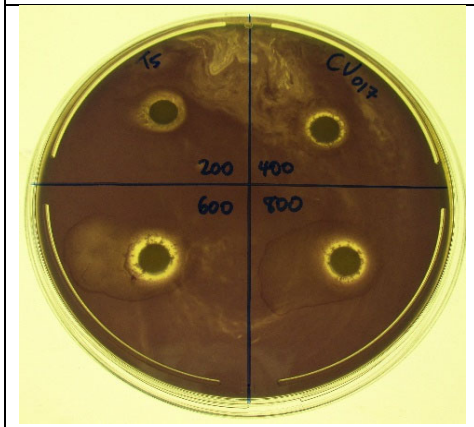
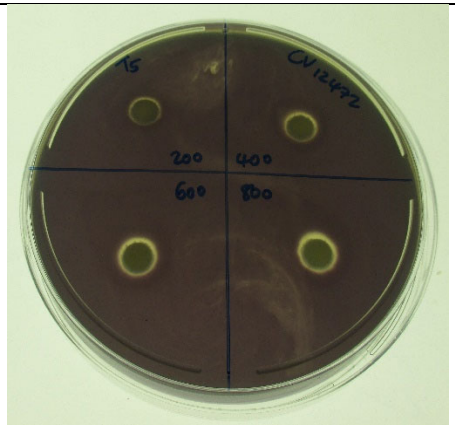
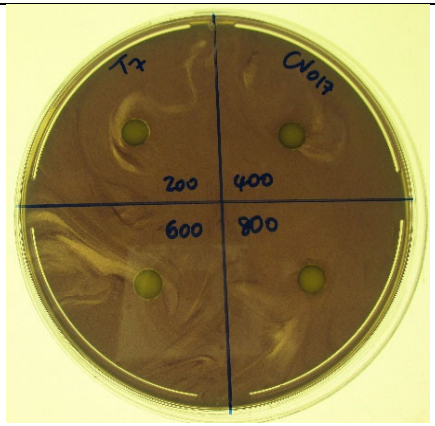
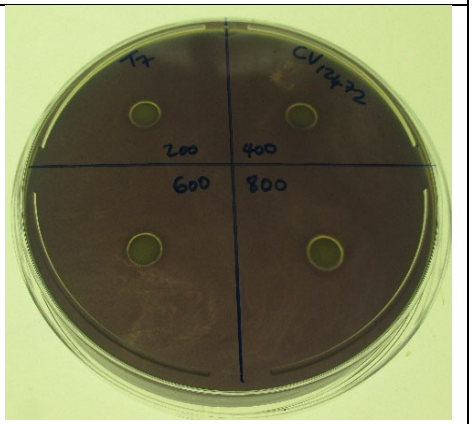
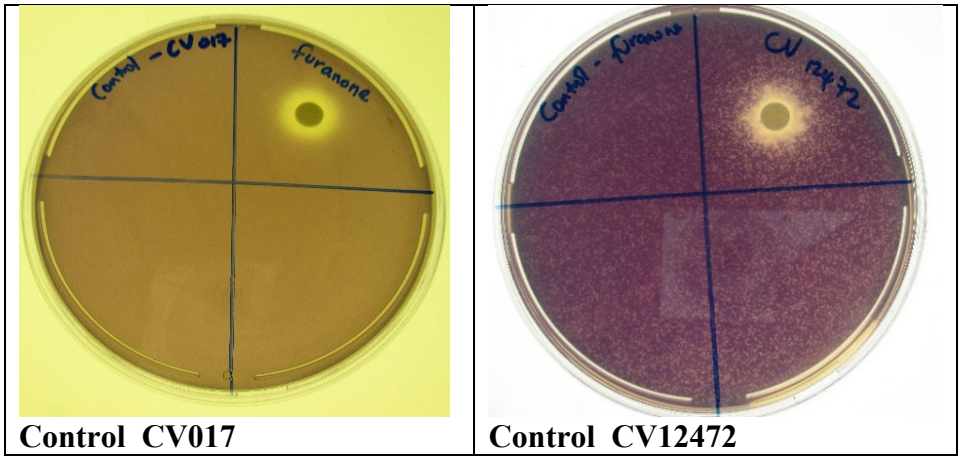


P_L4_CV12472

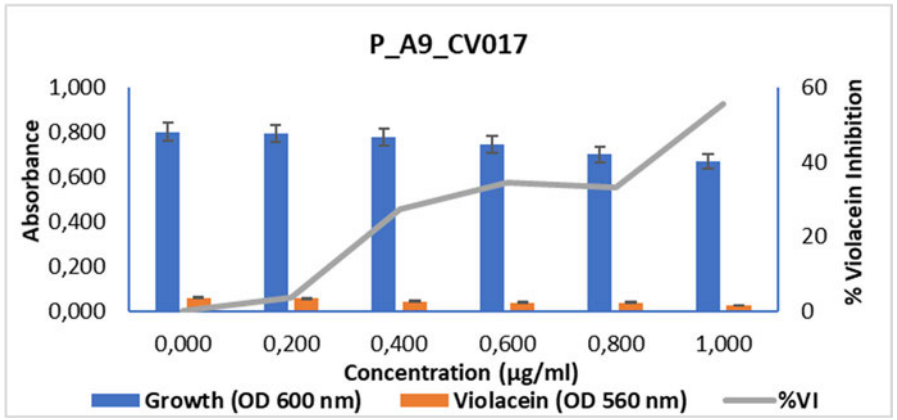
			
P L5 CV017	**P L5 CV12472**	**P L12 CV017**	**P_L12_CV12472**
			
P M18 CV017	**P_M18_CV12472**	**P_M24_CV017**	**P_M24_CV12472**

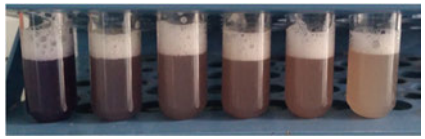
 **P_S9_CV017**	 **P_S9_CV12472**	 **P_T3_CV017**	 **P_T3_CV12472**
 **P_T5_CV017**	 **P_T5_CV12472**	 **P_T7_CV017**	 **P_T7_CV12472**

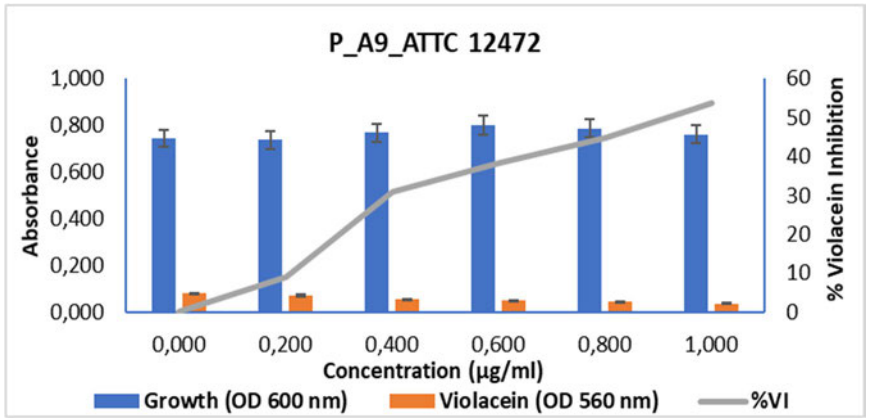


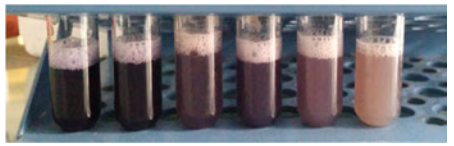
Control CV017 **Control CV12472**

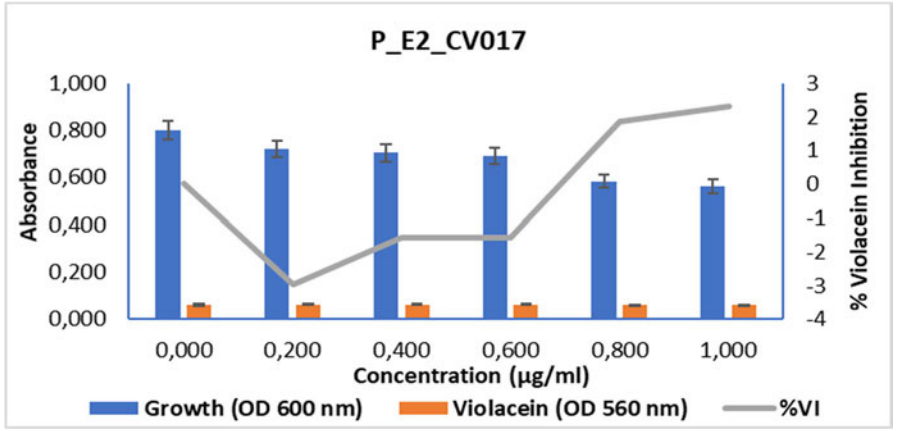
Figure S3.3: Anti-quorum sensing (QS) inhibition of selected *Pocillopora* CAB extracts against *Chromobacterium substugae* CV017 and *Chromobacterium violaceum* ATCC 12472. The inhibition of the violacein pigment production appearing as opaque zones was indicative of QS inhibition and clear zones around the discs were indicative of killing.

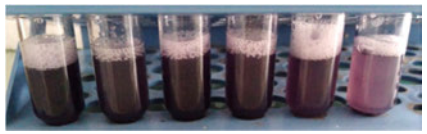

P_A9_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

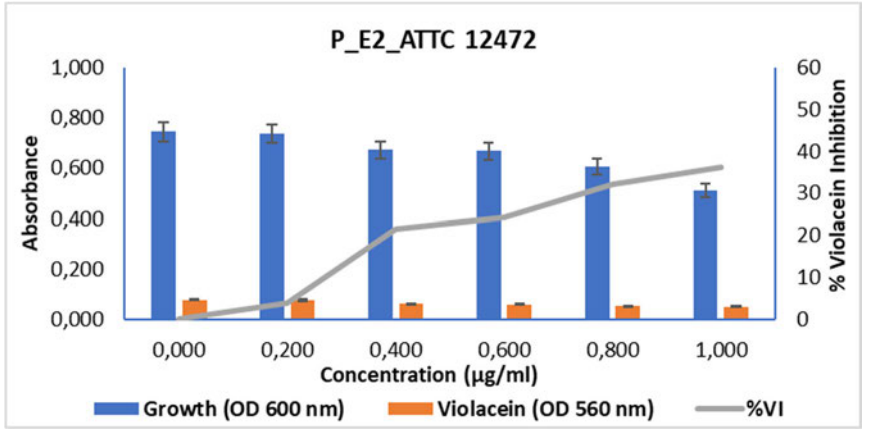


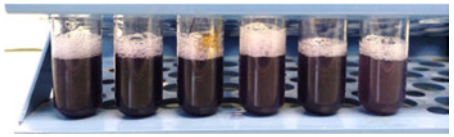

P_A9_ATTC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

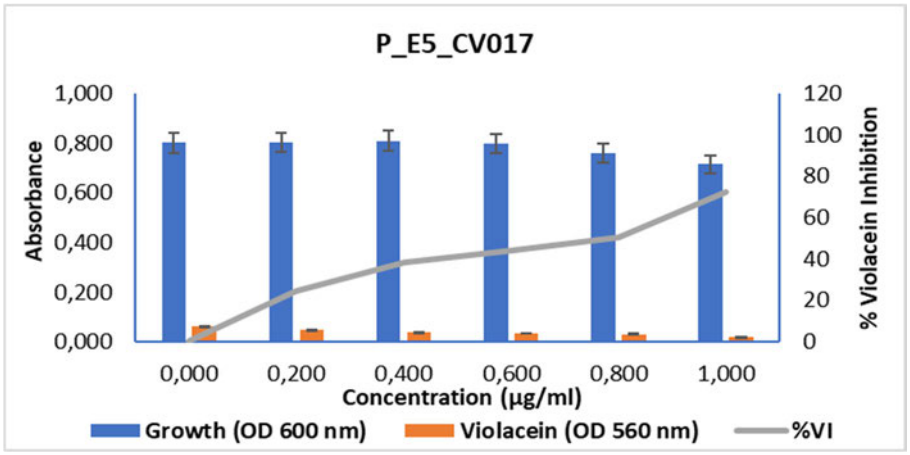


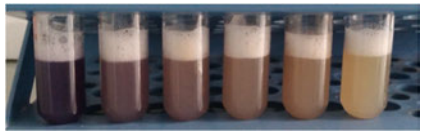

P_E2_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

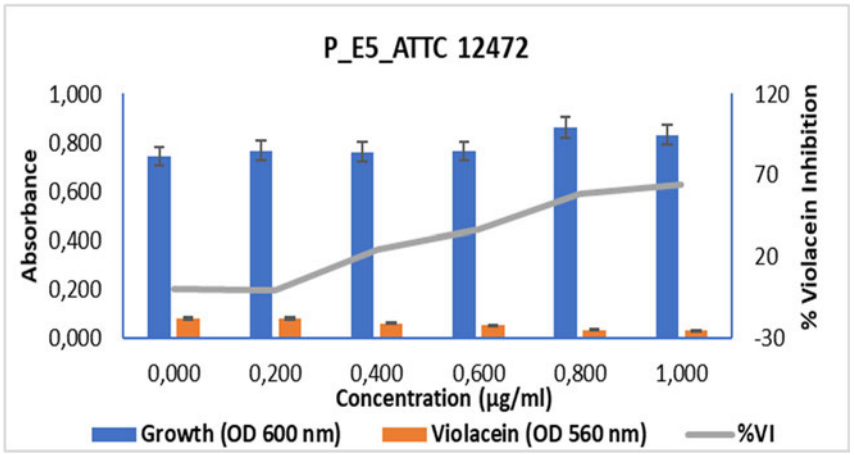


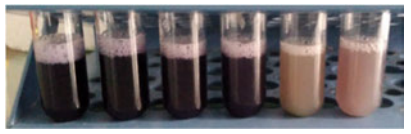

P_E2_ATTC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

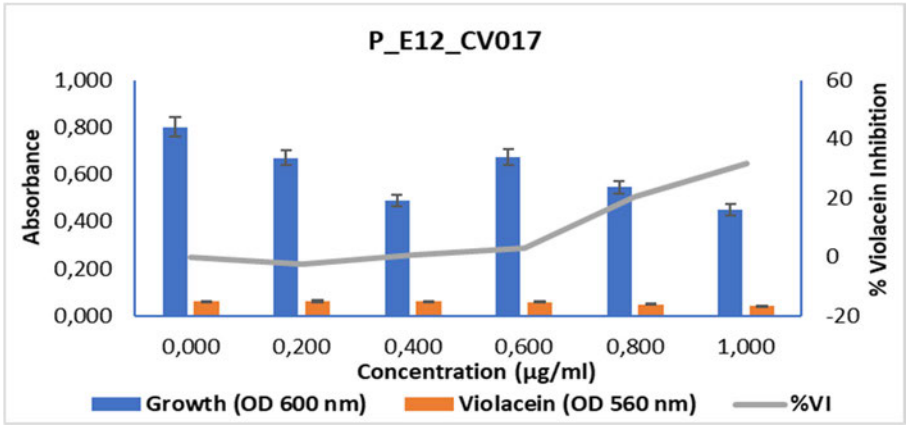


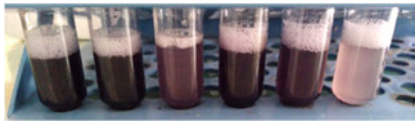

P_E5_CV017
Absorbance
1,000
0,800
0,600
0,400
0,200
0,000
% Violacein Inhibition
120
100
80
60
40
20
0
0,000
0,200
0,400
0,600
0,800
1,000
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

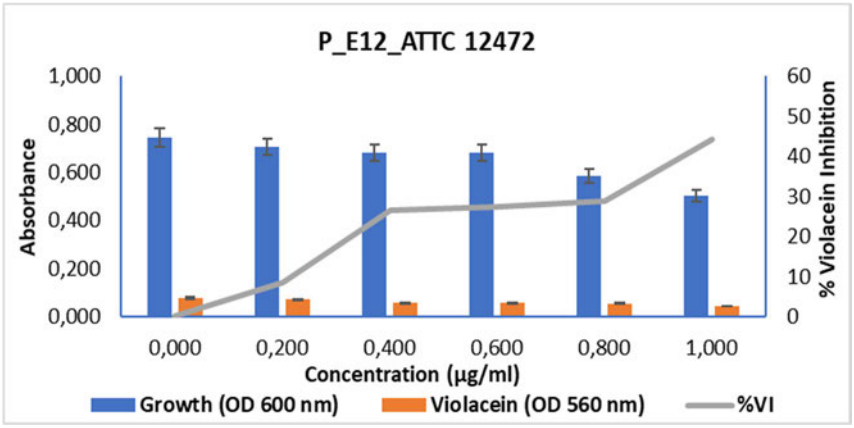


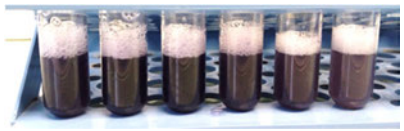

P_E5_ATTC 12472
Absorbance
1,000
0,800
0,600
0,400
0,200
0,000
% Violacein Inhibition
120
70
20
-30
0,000
0,200
0,400
0,600
0,800
1,000
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

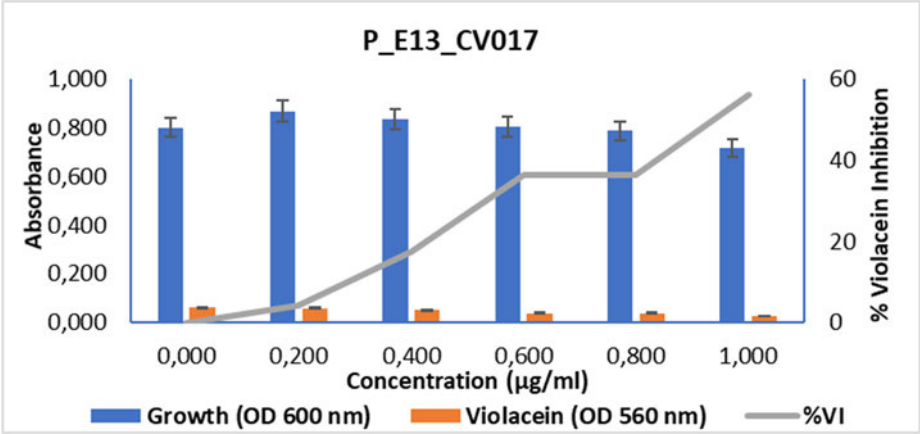


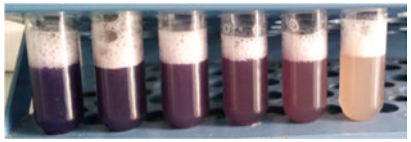

P_E12_CV017
Absorbance
1,000
0,800
0,600
0,400
0,200
0,000
% Violacein Inhibition
60
40
20
0
-20
0,000
0,200
0,400
0,600
0,800
1,000
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

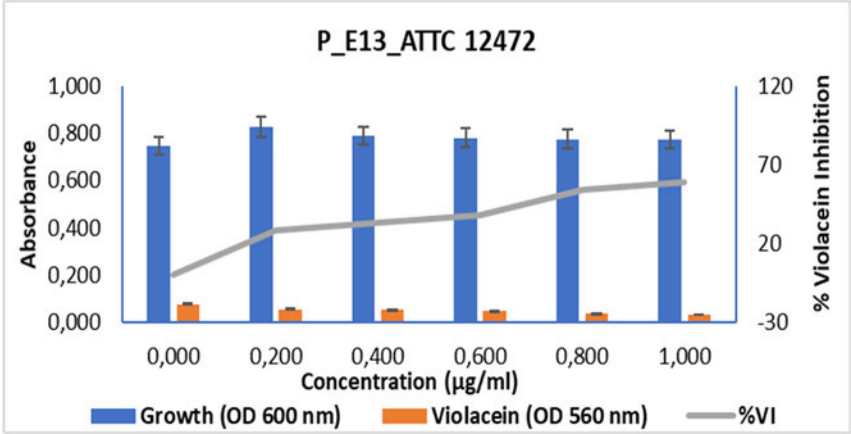


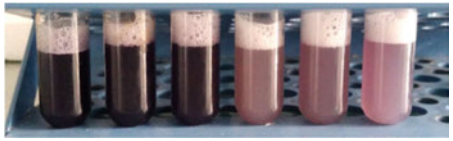

P_E12_ATTC 12472
Absorbance
1,000
0,800
0,600
0,400
0,200
0,000
% Violacein Inhibition
60
50
40
30
20
10
0
0,000
0,200
0,400
0,600
0,800
1,000
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

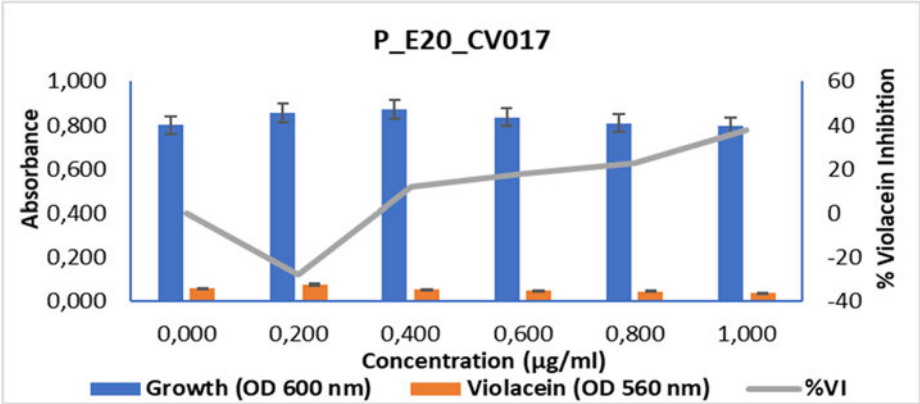


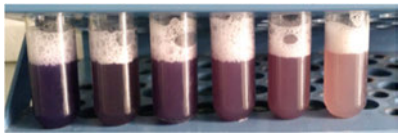

P_E13_CV017
Absorbance
1,000
0,800
0,600
0,400
0,200
0,000
60
40
20
0
% Violacein Inhibition
0,000
0,200
0,400
0,600
0,800
1,000
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

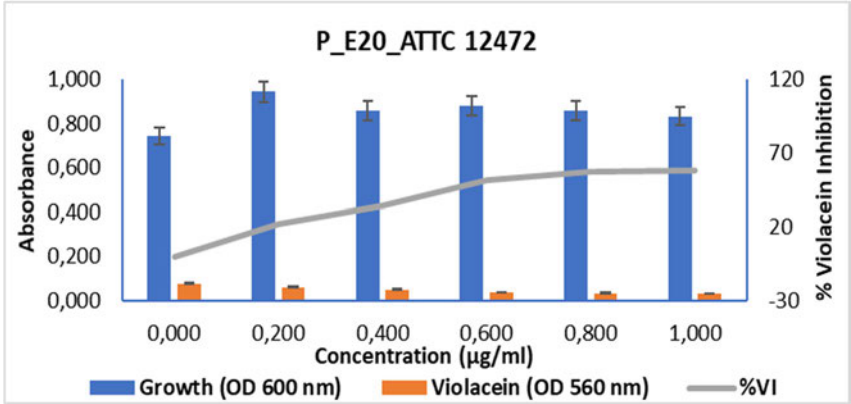


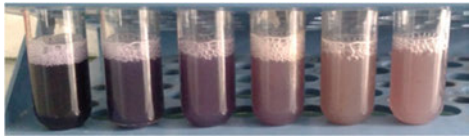

P_E13_ATTC 12472
Absorbance
1,000
0,800
0,600
0,400
0,200
0,000
120
70
20
-30
% Violacein Inhibition
0,000
0,200
0,400
0,600
0,800
1,000
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

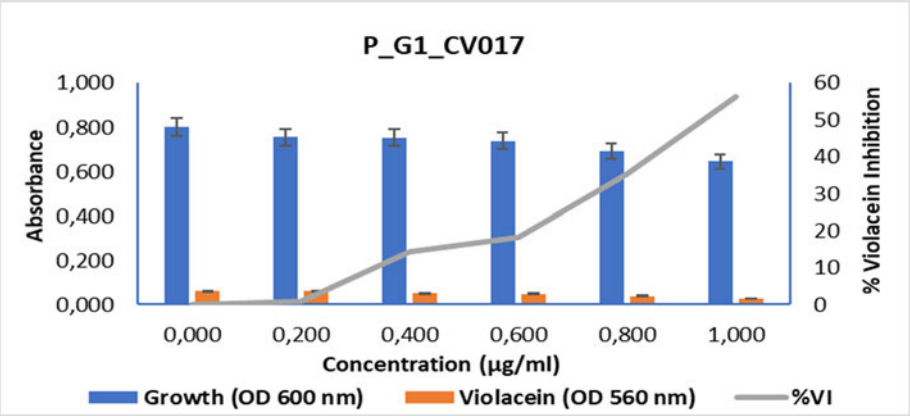


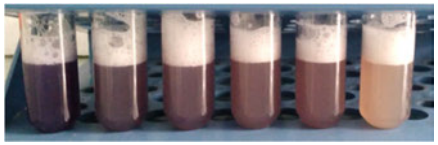

P_E20_CV017
Absorbance
1,000
0,800
0,600
0,400
0,200
0,000
60
40
20
0
-20
-40
% Violacein Inhibition
0,000
0,200
0,400
0,600
0,800
1,000
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

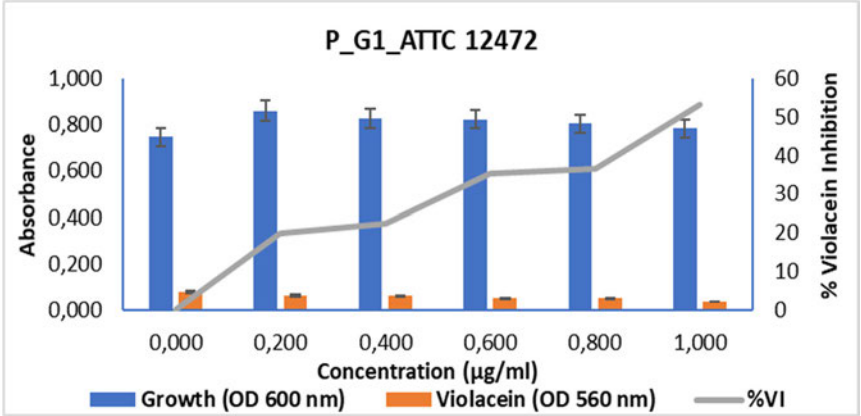


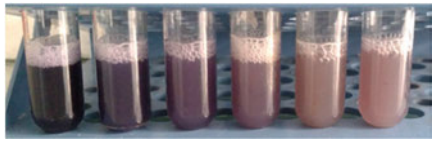

P_E20_ATTC 12472
Absorbance
1,000
0,800
0,600
0,400
0,200
0,000
120
70
20
-30
% Violacein Inhibition
0,000
0,200
0,400
0,600
0,800
1,000
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

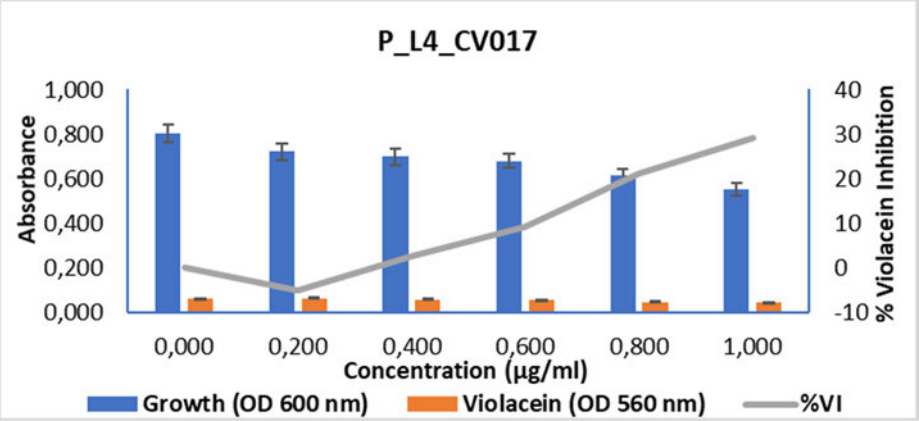


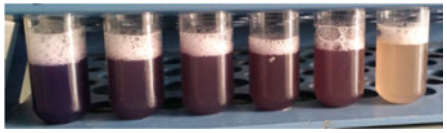

P_G1_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

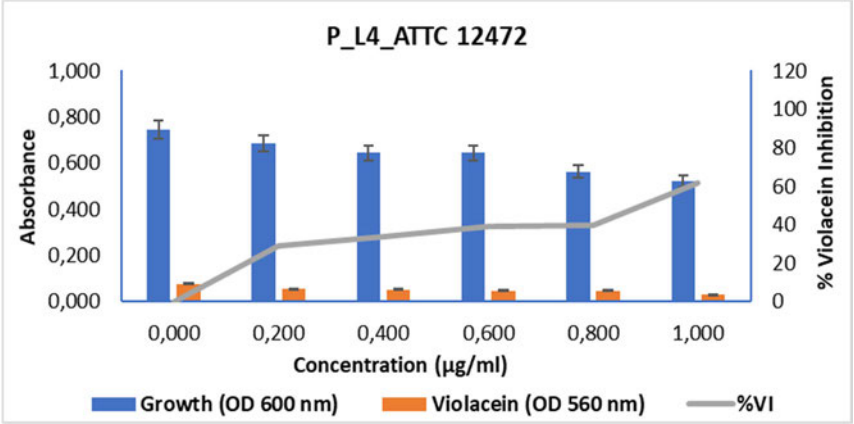


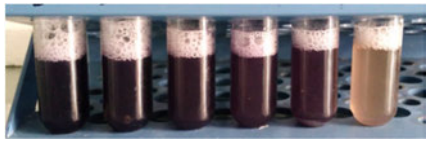

P_G1_ATTC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

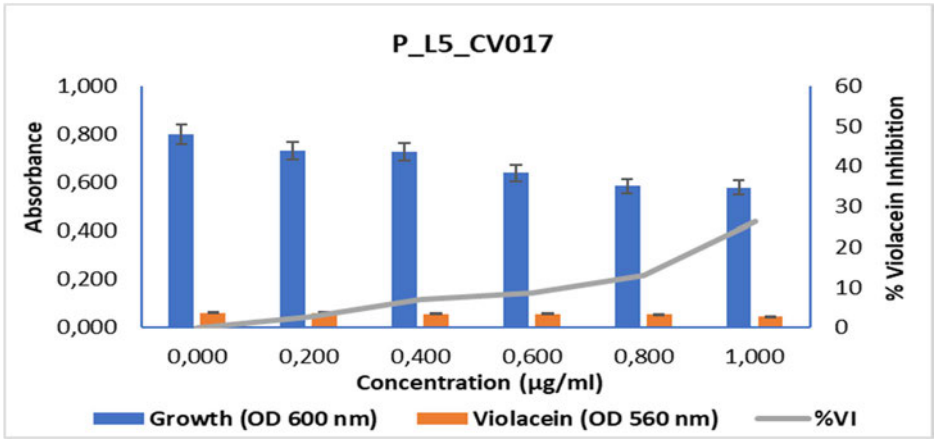


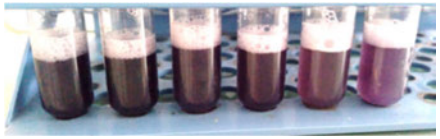

P_L4_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

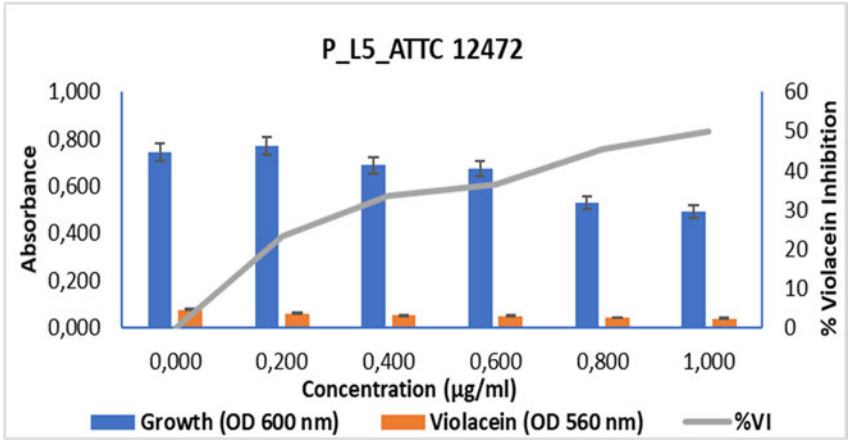


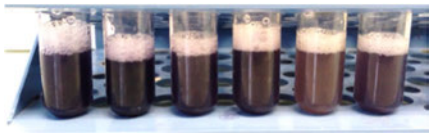

P_L4_ATTC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

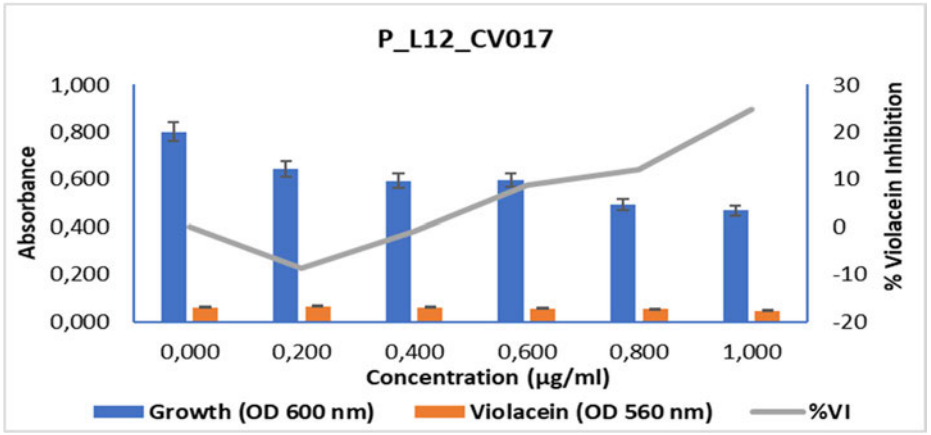


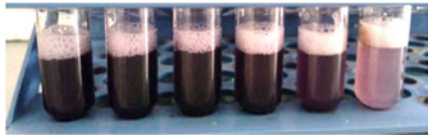

P_L5_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

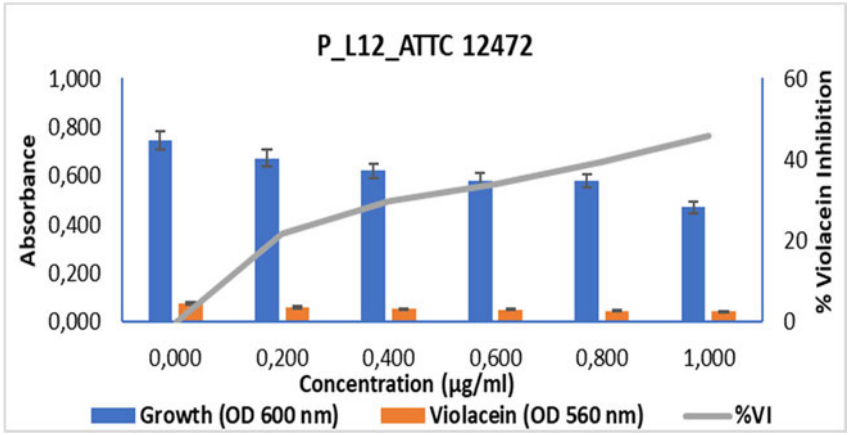


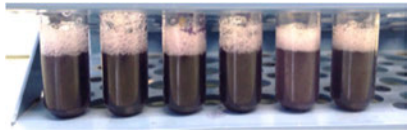

P_L5_ATTC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

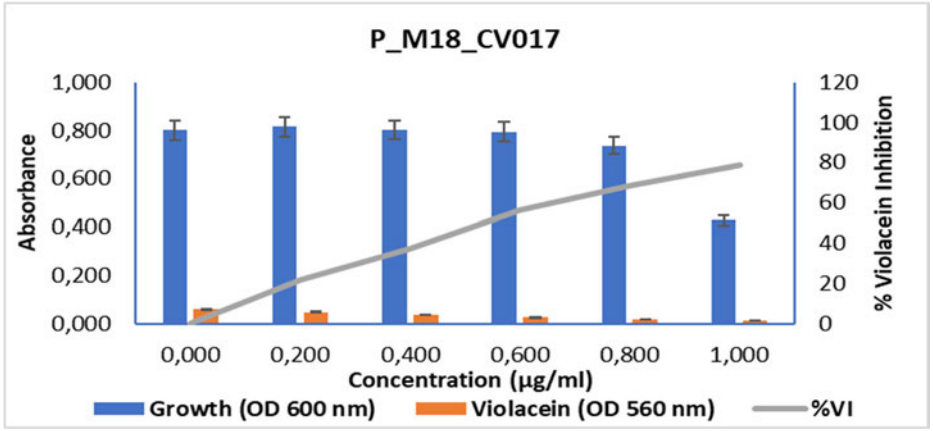


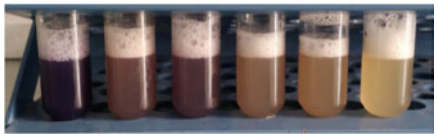

P_L12_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

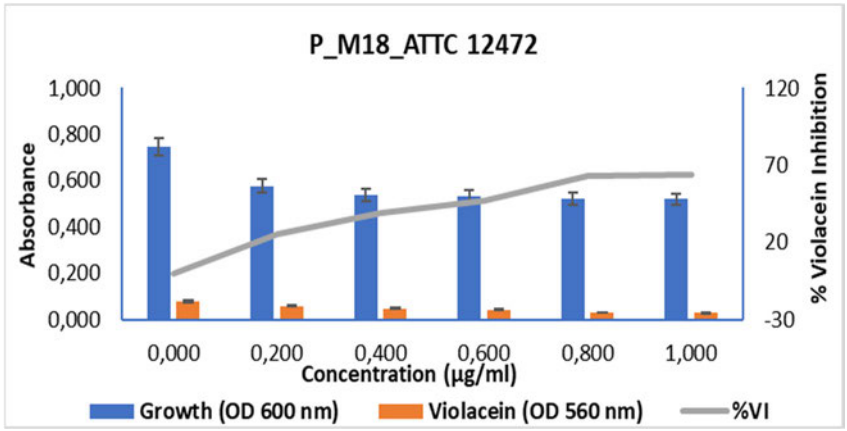


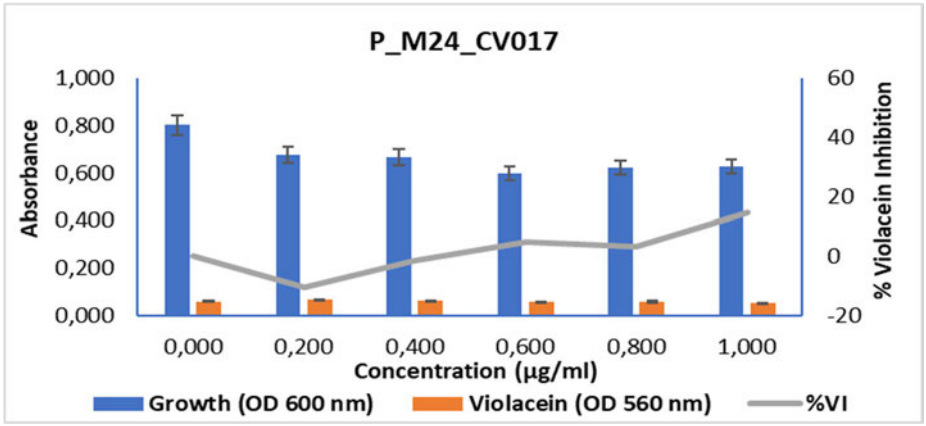

P_L12_ATTC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

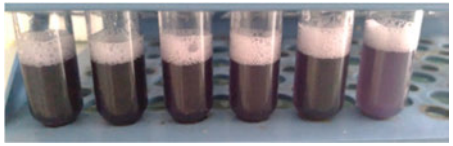


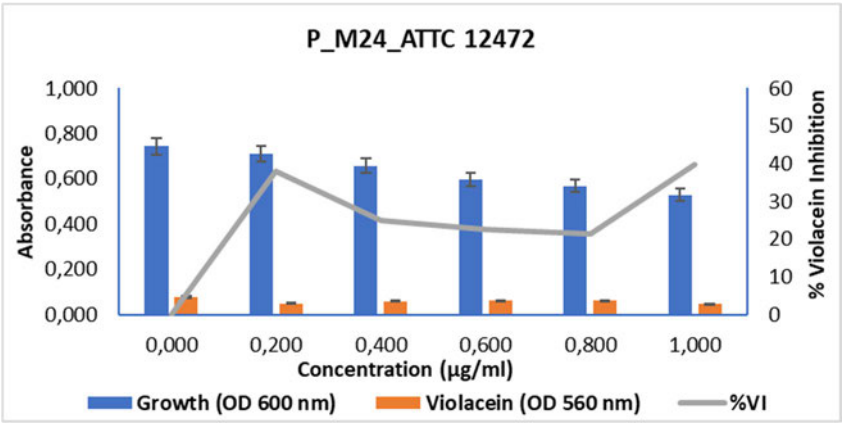

P_M18_CV017
Absorbance
1,000
0,800
0,600
0,400
0,200
0,000
120
100
80
60
40
20
0
% Violacein Inhibition
0,000
0,200
0,400
0,600
0,800
1,000
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

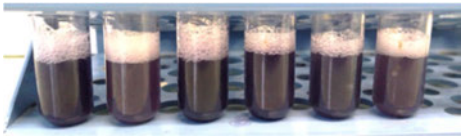


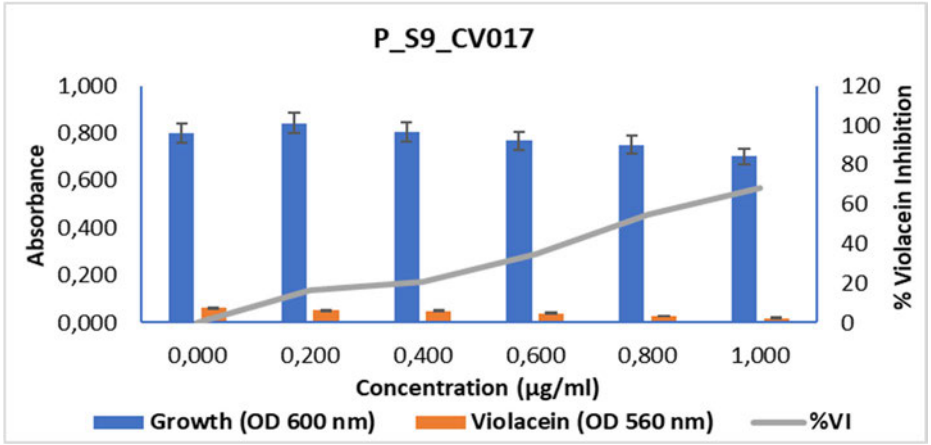

P_M18_ATTC 12472
Absorbance
1,000
0,800
0,600
0,400
0,200
0,000
120
70
20
-30
% Violacein Inhibition
0,000
0,200
0,400
0,600
0,800
1,000
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

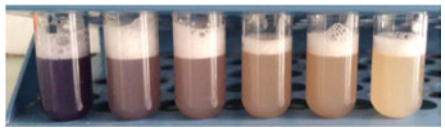

P_M24_CV017
Absorbance
1,000
0,800
0,600
0,400
0,200
0,000
60
40
20
0
-20
% Violacein Inhibition
0,000
0,200
0,400
0,600
0,800
1,000
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

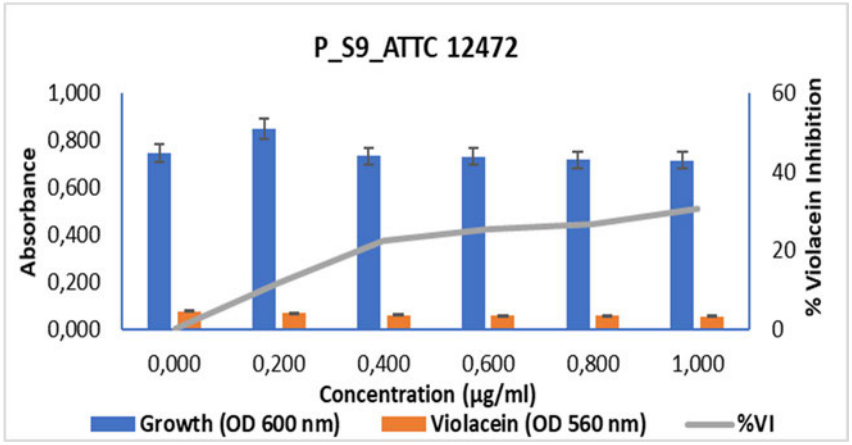


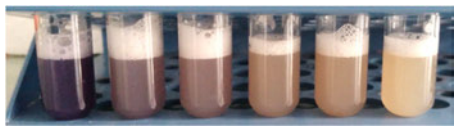

P_M24_ATTC 12472
Absorbance
1,000
0,800
0,600
0,400
0,200
0,000
60
50
40
30
20
10
0
% Violacein Inhibition
0,000
0,200
0,400
0,600
0,800
1,000
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

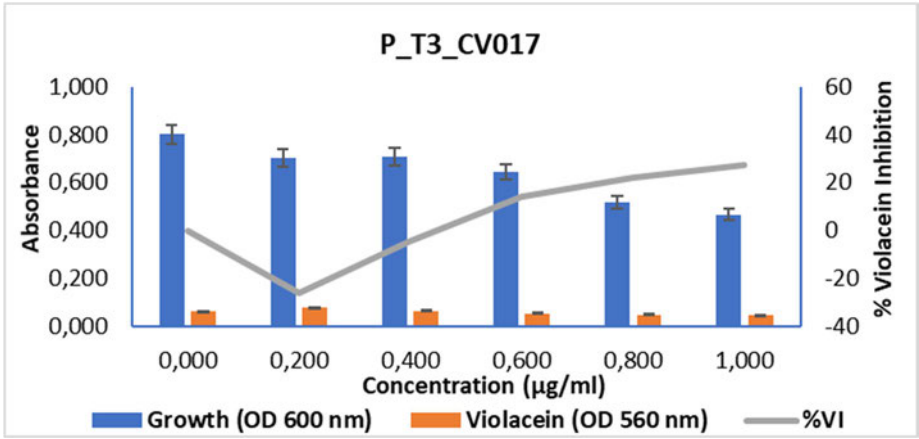


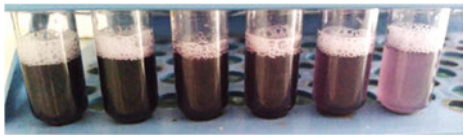

P_S9_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

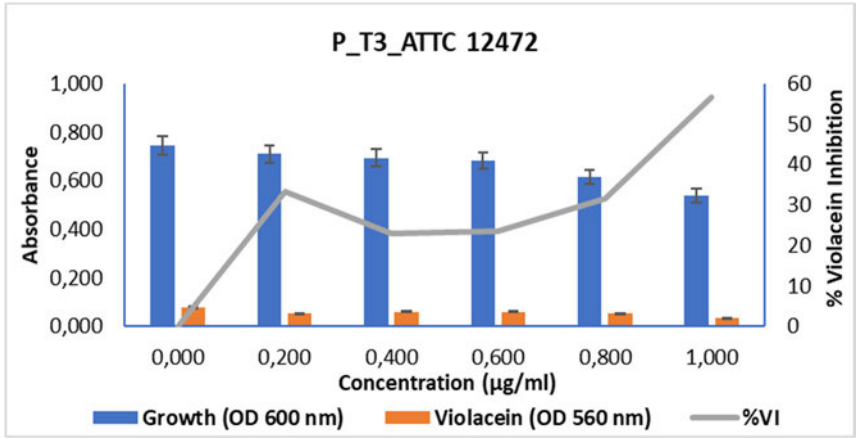


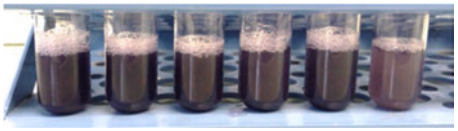

P_S9_ATTC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI




P_T3_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI




P_T3_ATTC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI



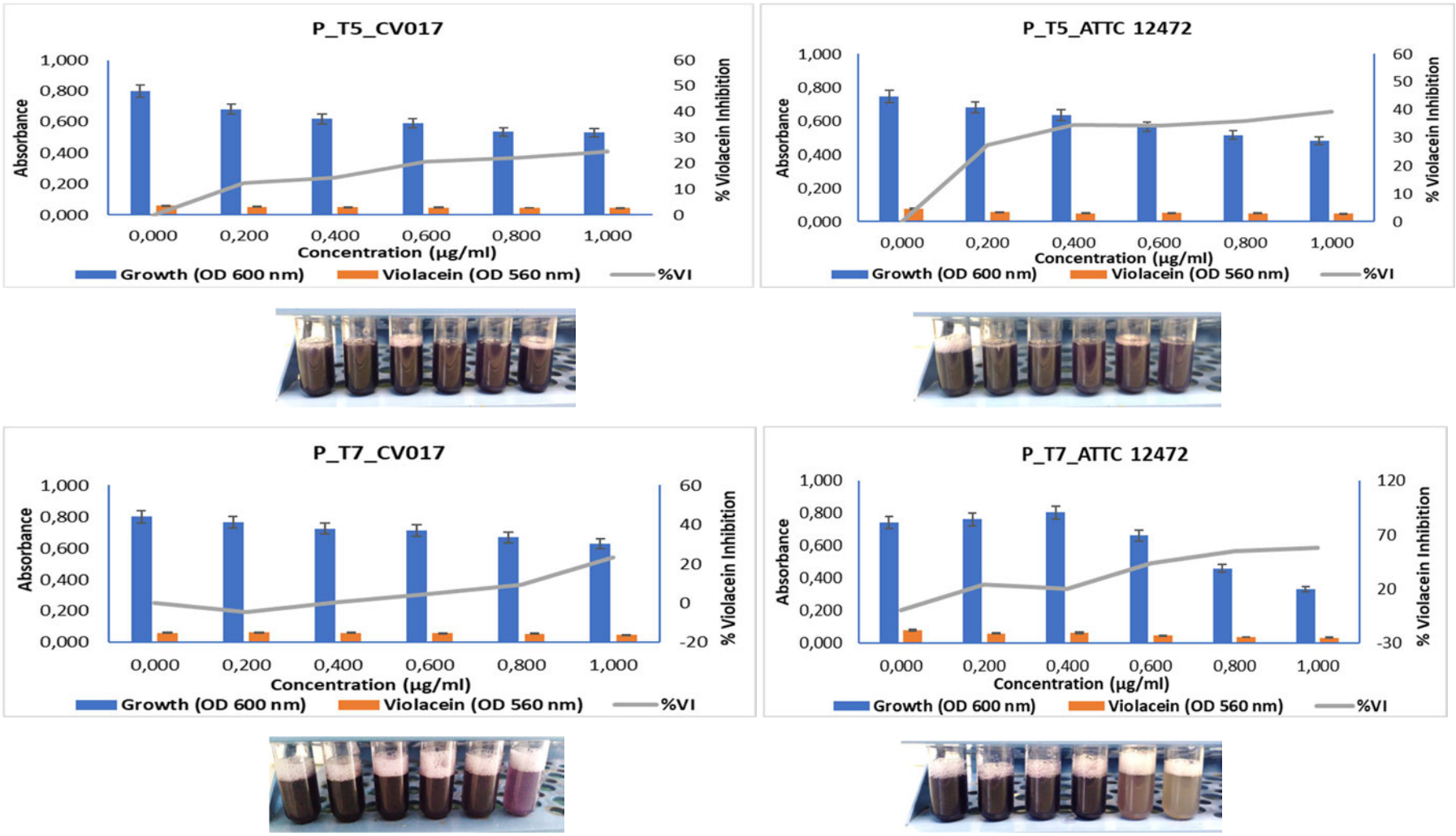
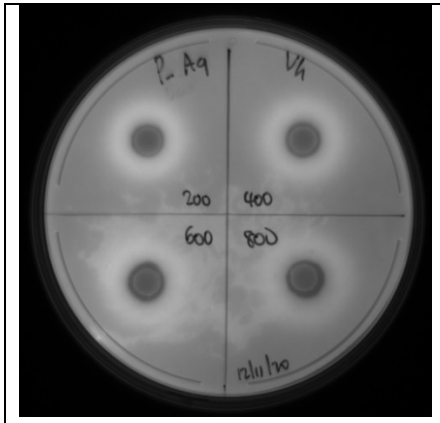
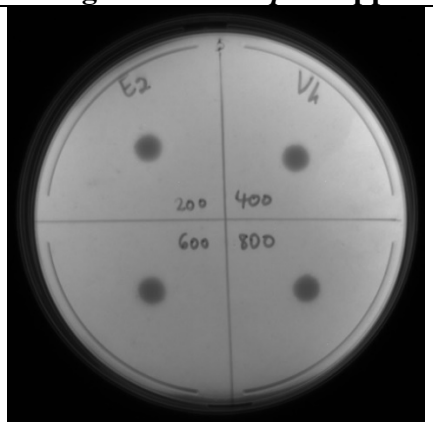


Figure S3.4: Quantitative analysis of violacein inhibitory effects of 16 *Pocillopora* CAB extracts against *Chromobacterium subtsugae* CV017 and *Chromobacterium violaceum* ATCC 12472. Bacterial growth measured at $OD_{600\ nm}$ and violacein production at $OD_{560\ nm}$ are indicated as bar graphs, while the percentage violacein inhibition (%VI) is indicated by the line graph. Data represent the mean of two independent experiments done in triplicate**.**

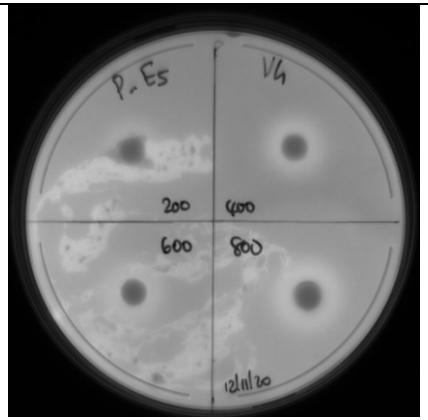
3.6.4 Autoinducer-2 inhibition screening of 16 *Pocillopora* spp. bacterial extracts.



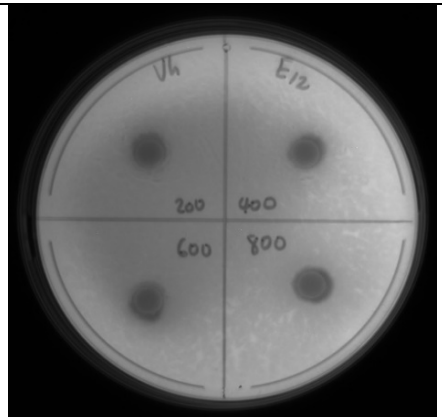
P_A9_*Vibrio harveyi*



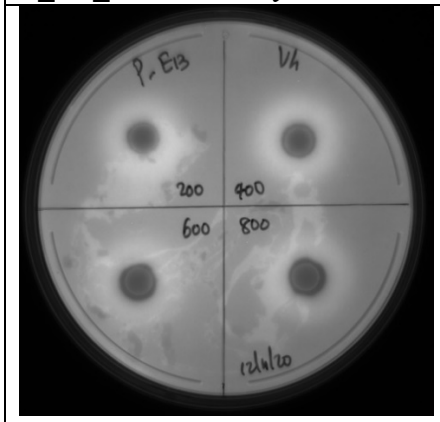
P E2 *Vibrio harveyi*



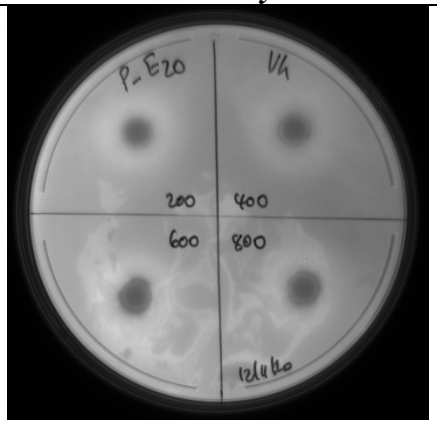
P_E5_*Vibrio harveyi*



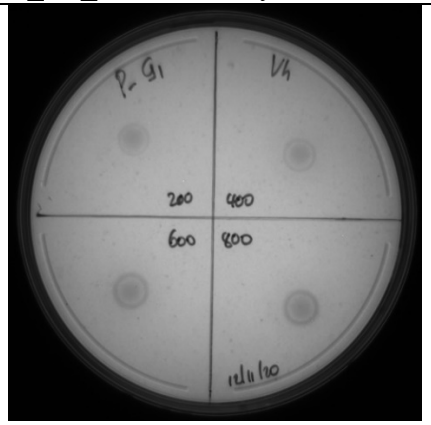
P_E12_*Vibrio harveyi*



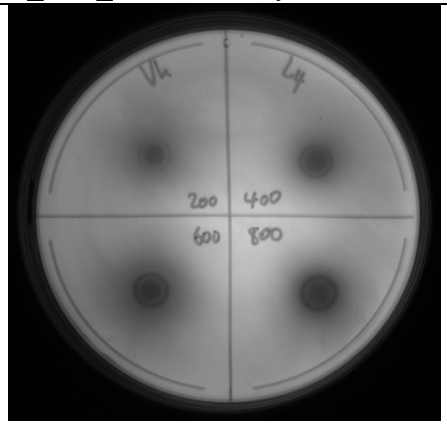
P_E13_*Vibrio harveyi*



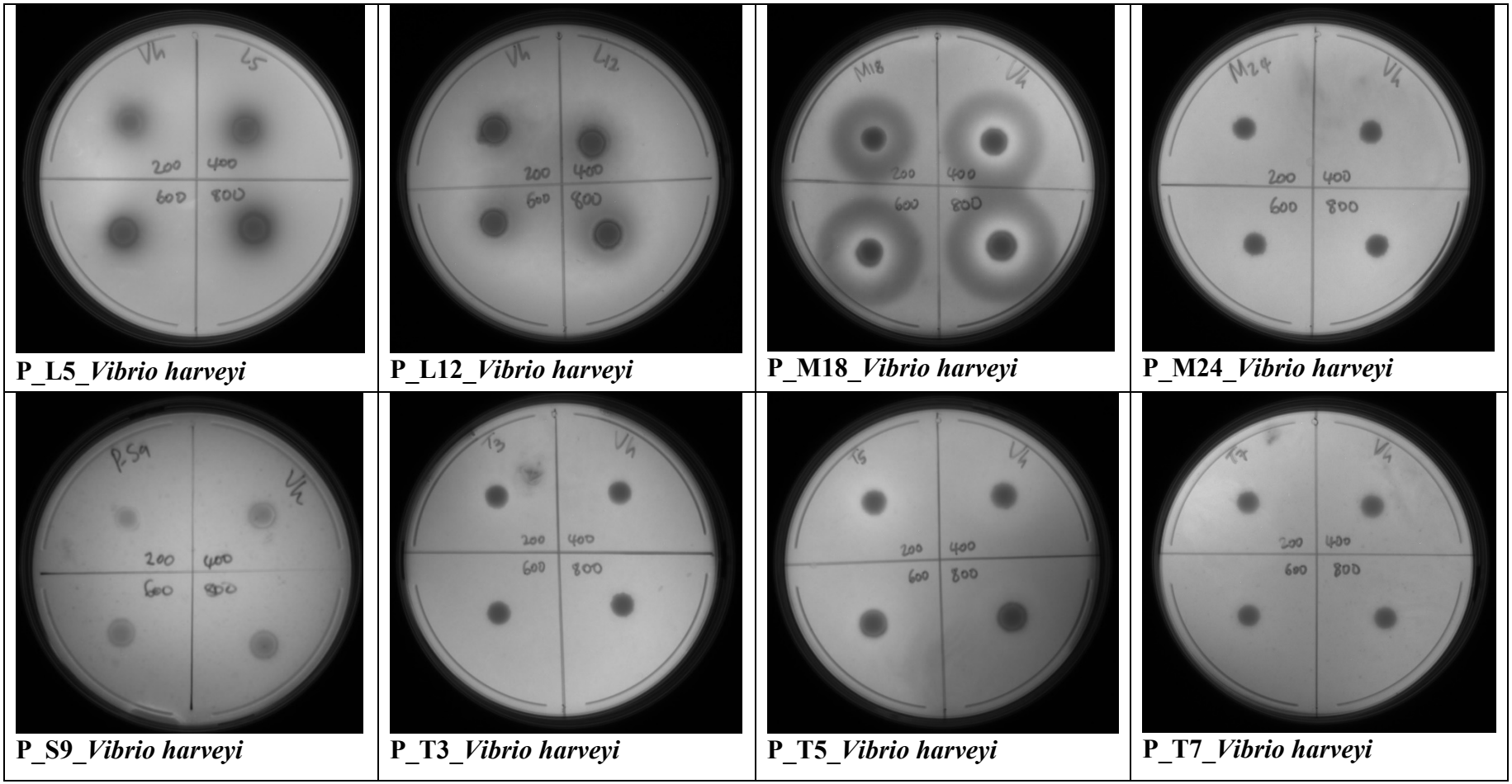
P E20 *Vibrio harveyi*



P G1 *Vibrio harveyi*



P L4 *Vibrio harveyi*



P_L5_*Vibrio harveyi* | **P_L12_*Vibrio harveyi*** | **P_M18_*Vibrio harveyi*** | **P_M24_*Vibrio harveyi***

P_S9_*Vibrio harveyi* | **P_T3_*Vibrio harveyi*** | **P_T5_*Vibrio harveyi*** | **P_T7_*Vibrio harveyi***

Figure S3.5: Autoinducer-2 inhibition of 16 *Pocillopora* CAB extracts against bioluminescent marine strain *Vibrio harveyi* BB120. AI-2 inhibition was identified by the appearance of dark zones, lacking bioluminescence around the discs. Clear zones around the discs were indicative of killing activity (P_A9 - P_T7).

Table S3.1: Quantitative analysis of percentage biofilm reducing potential (%BFR) and percentage growth inhibition (%GI) of *Pocillopora* CAB extracts against initial adhesion and mature biofilms of *Pseudomonas aeruginosa* ATCC 27853.

Extracts	*Pseudomonas aeruginosa* ATCC 27853															
	Initial								Mature							
	200 µg/ml		400 µg/ml		600 µg/ml		800 µg/ml		200 µg/ml		400 µg/ml		600 µg/ml		800 µg/ml	
	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR
A9_*Bacillus altitudinis*	7,63	39,91	11,35	40,44	12,02	40,98	14,22	42,33	18,08	-14,27	17,31	-12,71	18,18	-10,53	21,71	-5,59
E2_*Phaeobacter gallaeciensis*	1,95	8,85	3,84	8,77	4,52	9,53	5,29	10,07	15,51	8,18	15,50	10,19	11,51	16,24	12,14	39,66
E5_*Bacillus pumilus*	0,89	3,50	1,43	5,24	1,99	5,68	2,41	6,74	8,07	7,65	9,91	12,02	9,61	15,99	20,21	23,98
E12_*Phaeobacter gallaeciensis*	-6,24	8,30	-4,43	8,65	-2,91	11,40	-0,98	10,93	2,44	-4,46	8,54	13,41	34,91	43,07	44,94	45,52
E13_*Halomonas venusta*	8,96	3,45	10,05	4,67	11,25	6,57	11,23	7,22	17,64	5,22	19,17	5,20	21,11	11,45	22,21	20,84
E20_*Halomonas venusta*	9,98	5,44	10,12	5,83	10,95	6,07	12,93	8,64	5,57	6,88	9,72	19,77	13,49	25,41	15,07	34,66
G1_*Bacillus altitudinis*	8,69	2,86	9,19	3,08	10,08	5,51	9,73	10,52	11,11	5,60	11,96	8,06	11,97	15,75	13,51	26,13
L4_*Phaeobacter gallaeciensis*	3,68	3,53	3,75	4,40	6,58	8,43	8,69	17,44	1,99	7,61	1,86	9,92	6,72	18,60	12,32	41,25
L5_*Phaeobacter gallaeciensis*	11,01	5,61	13,53	3,89	15,71	15,78	9,11	25,12	14,20	0,18	13,19	4,87	13,76	11,80	35,40	30,06
L12_*Staphylococcus equorum*	-5,70	4,88	-4,71	5,30	-3,69	5,49	-5,06	7,14	-1,84	-0,95	-2,38	2,20	-2,97	4,08	-9,20	3,73
M18_*Phaeobacter gallaeciensis*	15,81	4,58	16,61	5,55	19,90	5,58	28,09	5,84	28,71	-5,91	29,31	4,65	33,66	7,90	58,52	7,48
M24_*Phaeobacter gallaeciensis*	-10,67	5,57	-10,88	6,70	-9,58	12,97	-10,10	14,98	-0,10	8,85	1,40	10,38	2,15	15,95	3,11	15,38
S9_*Streptomyces griseus*	-5,94	3,80	-5,58	3,71	-4,72	3,33	-4,60	3,31	2,25	7,47	3,54	7,97	5,59	12,97	7,22	14,35
T3_*Bacillus paralicheniformis*	2,22	4,86	2,26	4,35	5,69	4,65	8,41	5,73	1,28	6,22	3,53	7,39	4,84	24,19	20,20	31,06
T5_*Bacillus* sp.	-4,89	5,78	-2,53	6,38	-3,05	6,59	-4,44	5,72	-5,34	-1,01	-6,65	1,59	-7,67	6,45	-7,26	8,83
T7_*Bacillus* sp.	-3,18	7,62	0,12	9,33	48,61	15,85	49,21	18,74	-3,76	5,82	-3,72	37,93	67,73	42,10	70,30	48,06
Total extracts displaying ≥ 50% BFR and <40% GI	0		0		0		0		0		0		0		0	
Total extracts displaying ≥ 50% BFR and ≥40% GI	0		0		0		0		0		0		0		0	

Table S3.2: Quantitative analysis of percentage biofilm reducing potential (%BFR) and percentage growth inhibition (%GI) of *Pocillopora* CAB extracts against initial adhesion and mature biofilms of *Staphylococcus aureus* ATCC 43300.

Extracts	*Staphylococcus aureus* ATCC 43300															
	Initial								Mature							
	200 µg/ml		400 µg/ml		600 µg/ml		800 µg/ml		200 µg/ml		400 µg/ml		600 µg/ml		800 µg/ml	
	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR
P_A9_*Bacillus altitudinis*	10,44	1,29	10,45	1,88	10,60	2,84	10,73	3,14	25,85	-11,57	26,74	0,14	34,73	2,32	40,00	12,67
P_E2_*Phaeobacter gallaeciensis*	-0,70	0,32	0,82	-0,33	0,80	-0,60	1,48	-3,22	23,23	17,39	24,49	22,53	29,00	25,88	37,61	28,33
P_E5_*Bacillus pumilus*	0,92	0,65	1,03	0,85	2,31	1,57	3,39	2,46	12,39	16,92	11,20	19,87	12,32	41,79	14,92	69,72
P_E12_*Phaeobacter gallaeciensis*	-4,35	0,29	-4,16	0,77	-4,48	1,70	-4,88	1,64	14,35	48,11	40,54	52,01	44,52	58,87	43,97	115,17
P_E13_*Halomonas venusta*	8,17	0,51	8,91	0,92	9,51	2,39	9,67	4,76	16,21	8,77	21,66	24,18	21,82	41,92	21,82	44,37
P_E20_*Halomonas venusta*	4,01	2,76	11,09	1,64	12,29	-1,73	12,60	-3,02	10,99	12,80	11,61	12,95	18,74	38,62	21,03	39,66
P_G1_*Bacillus altitudinis*	1,51	2,18	1,48	1,81	3,06	1,79	4,79	0,75	11,03	4,46	9,96	32,42	12,04	37,97	10,20	46,66
P_L4_*Phaeobacter gallaeciensis*	-0,29	2,37	1,91	2,07	5,82	1,75	6,19	-1,86	1,74	3,07	6,76	8,42	7,97	32,10	8,10	78,48
P_L5_*Phaeobacter gallaeciensis*	11,90	-5,72	13,71	3,49	14,30	6,07	17,87	27,96	23,37	11,80	20,92	14,27	25,50	15,38	46,40	29,78
P_L12_*Staphylococcus equorum*	7,79	-4,76	7,86	0,26	10,21	1,29	10,50	1,31	0,54	5,60	6,13	7,88	0,69	8,88	2,68	9,82
P_M18_*Phaeobacter gallaeciensis*	16,21	0,84	17,70	1,40	19,56	2,64	24,57	6,63	36,19	0,09	38,62	7,76	40,88	9,92	47,73	20,55
P_M24_*Phaeobacter gallaeciensis*	5,25	-3,49	3,77	-1,76	2,23	-1,03	1,02	0,35	0,41	-6,68	3,72	6,59	8,55	8,64	11,60	9,23
P_S9_*Streptomyces griseus*	5,64	4,79	5,28	4,75	4,53	5,69	3,54	5,61	12,30	1,01	13,77	6,60	18,01	18,17	19,16	22,27
P_T3_*Bacillus paralicheniformis*	2,95	4,51	4,56	4,83	6,41	4,99	7,00	7,54	15,66	11,42	25,01	12,92	29,47	14,84	34,94	22,46
P_T5_*Bacillus* sp.	-0,32	2,07	0,16	3,06	-0,25	4,73	0,37	8,40	1,83	8,05	1,58	18,41	6,70	21,32	12,48	40,10
P_T7_*Bacillus* sp.	3,81	18,10	54,97	26,60	55,94	30,32	65,84	35,31	3,54	19,40	12,63	13,67	19,36	51,42	27,20	62,62
Total extracts displaying ≥ 50% BFR and <40% GI	0		0		0		0		0		0		1		3	
Total extracts displaying ≥ 50% BFR and ≥40% GI	0		0		0		0		0		1		1		1	

Table S3.3: Quantitative analysis of percentage biofilm reducing potential (%BFR) and percentage growth inhibition (%GI) of *Pocillopora* CAB extracts against initial adhesion and mature biofilms of *Shewanella putrefaciens* ATCC 8071.

Extracts	*Shewanella putrefaciens* ATCC 8071															
	Initial								Mature							
	200 µg/ml		400 µg/ml		600 µg/ml		800 µg/ml		200 µg/ml		400 µg/ml		600 µg/ml		800 µg/ml	
	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR
P_A9_*Bacillus altitudinis*	11,03	16,38	14,16	16,81	18,28	18,07	20,76	24,12	25,38	36,38	26,87	38,05	26,94	40,03	29,60	47,25
P_E2_*Phaeobacter gallaeciensis*	4,62	32,20	4,65	41,94	8,95	41,96	12,50	51,65	24,61	41,25	24,88	44,09	25,83	45,82	25,38	48,51
P_E5_*Bacillus pumilus*	0,35	20,69	2,07	37,08	2,18	41,69	2,43	51,65	15,58	43,79	17,32	44,95	20,61	47,83	20,91	50,42
P_E12_*Phaeobacter gallaeciensis*	3,45	32,47	2,81	78,39	2,33	90,65	1,33	120,44	8,75	40,73	17,34	42,94	17,47	43,66	17,68	43,91
P_E13_*Halomonas venusta*	11,15	24,55	12,07	30,66	15,29	34,92	15,32	37,89	22,71	13,44	23,62	18,73	17,49	22,20	20,91	22,10
P_E20_*Halomonas venusta*	11,85	37,05	14,13	28,50	17,74	11,81	23,99	5,51	19,56	13,91	19,61	13,93	19,88	18,24	20,62	20,30
P_G1_*Bacillus altitudinis*	13,53	35,34	13,23	37,28	17,13	41,28	18,41	41,85	23,26	13,33	23,35	13,88	23,75	20,52	24,75	22,39
P_L4_*Phaeobacter gallaeciensis*	5,29	14,40	7,32	14,83	10,67	38,97	13,92	42,50	14,11	10,25	14,69	12,84	14,72	14,03	15,32	14,26
P_L5_*Phaeobacter gallaeciensis*	8,05	3,94	13,60	11,62	16,03	13,36	17,79	15,09	23,40	5,85	25,31	5,22	28,66	8,87	24,43	9,66
P_L12_*Staphylococcus equorum*	12,60	6,99	6,49	7,41	6,60	7,62	8,19	13,11	8,42	1,28	9,66	6,27	11,21	10,24	17,15	10,95
P_M18_*Phaeobacter gallaeciensis*	20,08	18,27	29,48	27,66	31,22	53,77	43,85	69,86	22,18	3,03	28,32	3,37	30,90	4,44	34,68	9,33
P_M24_*Phaeobacter gallaeciensis*	-1,22	31,74	-0,30	31,34	-1,64	34,58	-3,74	57,65	6,12	0,40	6,46	1,60	7,28	3,98	7,25	7,16
P_S9_*Streptomyces griseus*	10,06	17,48	9,74	17,95	10,30	18,18	14,43	30,16	24,91	14,93	31,54	13,29	38,94	11,41	38,58	14,40
P_T3_*Bacillus paralicheniformis*	16,69	26,90	21,78	47,87	22,13	70,87	30,62	84,53	17,49	8,93	18,22	16,56	20,36	16,71	21,48	28,67
P_T5_*Bacillus* sp.	18,94	12,36	28,77	19,07	20,69	27,25	31,28	63,11	20,04	11,22	20,83	13,73	20,75	13,83	14,91	13,23
P_T7_*Bacillus* sp.	35,74	11,87	58,35	12,08	58,48	12,24	62,42	12,71	20,05	10,86	16,12	14,44	6,37	16,63	4,91	16,55
Total extracts displaying ≥ 50% BFR and <40% GI	0		1		3		6		0		0		0		1	
Total extracts displaying ≥ 50% BFR and ≥40% GI	0		0		0		1		0		0		0		0	

Table S3.4: Quantitative analysis of percentage biofilm reducing potential (%BFR) and percentage growth inhibition (%GI) of *Pocillopora* CAB extracts against initial adhesion and mature biofilms of *Vibrio coralliilyticus* ATCC BAA 450.

Extracts	*Vibrio corallyliticus* ATCC_BAA 450															
	Initial								Mature							
	200 µg/ml		400 µg/ml		600 µg/ml		800 µg/ml		200 µg/ml		400 µg/ml		600 µg/ml		800 µg/ml	
	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR
P_A9_*Bacillus altitudinis*	25,59	43,82	26,44	43,73	28,41	42,95	29,28	38,52	16,97	45,40	18,83	47,31	19,28	47,84	23,60	48,53
P_E2_*Phaeobacter gallaeciensis*	6,99	28,93	7,62	32,07	10,27	32,20	14,59	32,91	4,11	45,32	6,29	48,06	7,03	48,78	9,20	49,06
P_E5_*Bacillus pumilus*	27,09	31,38	27,34	33,18	27,49	34,49	32,35	34,76	13,00	48,11	14,22	48,68	14,88	48,60	16,20	51,06
P_E12_*Phaeobacter gallaeciensis*	41,34	37,10	43,36	37,92	43,67	39,80	43,89	42,59	30,84	47,43	34,31	48,35	42,20	48,14	43,79	49,50
P_E13_*Halomonas venusta*	33,92	40,28	36,40	43,00	36,48	43,93	38,02	44,52	15,40	49,39	16,98	51,75	22,52	51,81	27,78	54,53
P_E20_*Halomonas venusta*	41,47	37,92	41,34	38,60	41,71	42,54	43,01	46,21	22,99	51,47	24,02	52,02	31,19	53,10	36,90	56,37
P_G1_*Bacillus altitudinis*	33,69	36,36	34,83	38,91	35,95	40,20	36,57	40,84	13,21	51,26	14,13	52,68	15,81	52,83	16,40	53,28
P_L4_*Phaeobacter gallaeciensis*	22,14	34,15	26,68	36,63	29,52	41,25	32,24	42,86	39,43	50,97	38,98	50,95	38,96	51,73	40,47	52,44
P_L5_*Phaeobacter gallaeciensis*	27,05	38,18	32,18	39,04	34,65	40,86	35,16	41,43	17,13	46,13	17,41	46,22	19,71	48,07	20,07	48,56
P_L12_*Staphylococcus equorum*	17,20	40,83	38,29	40,74	38,67	41,25	42,12	49,02	9,88	47,51	12,09	50,47	25,67	50,89	36,78	53,08
P_M18_*Phaeobacter gallaeciensis*	33,21	38,34	33,94	38,55	33,88	39,23	35,72	41,99	17,45	47,43	18,48	49,20	19,37	50,08	21,84	50,53
P_M24_*Phaeobacter gallaeciensis*	27,51	45,41	30,26	47,92	30,45	52,09	30,78	54,37	33,33	49,12	33,50	51,39	37,03	54,72	41,43	55,77
P_S9_*Streptomyces griseus*	28,92	32,23	29,95	35,00	29,98	36,47	32,66	36,91	17,08	49,37	17,24	49,98	18,39	51,78	22,71	54,01
P_T3_*Bacillus paralicheniformis*	30,72	34,74	34,64	35,30	35,38	35,94	35,94	40,00	15,37	49,33	15,95	49,82	16,33	52,07	17,30	54,50
P_T5_Bacillus sp.	26,83	31,54	38,34	35,20	38,58	35,27	42,45	45,55	22,28	49,68	34,18	51,30	35,10	51,92	38,48	52,43
P_T7_*Bacillus* sp.	35,44	37,21	37,10	45,52	37,60	49,38	41,19	49,40	27,42	48,11	30,06	49,35	36,07	51,47	40,46	51,52
Total extracts displaying ≥ 50% BFR and <40% GI	0		0		1		1		3		9		11		9	
Total extracts displaying ≥ 50% BFR and ≥40% GI	0		0		0		0		0		0		0		3	

Table S3.5: Quantitative analysis of percentage biofilm reducing potential (%BFR) and percentage growth inhibition (%GI) of *Pocillopora* CAB extracts against initial adhesion and mature biofilms of *Vibrio parahaemolyticus* ATCC 17802.

Extracts	*Vibrio parahaemolyticus* ATCC 17802															
	Initial								Mature							
	200 µg/ml		400 µg/ml		600 µg/ml		800 µg/ml		200 µg/ml		400 µg/ml		600 µg/ml		800 µg/ml	
	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR
P_A9_*Bacillus altitudinis*	23,45	24,01	24,13	38,17	29,72	56,48	51,36	57,36	27,29	15,09	27,30	16,38	27,40	17,38	33,80	20,46
P_E2_*Phaeobacter gallaeciensis*	7,78	7,99	14,40	16,56	15,36	23,41	20,73	50,78	30,08	18,32	34,44	18,73	35,27	25,13	36,43	28,92
P_E5_*Bacillus pumilus*	6,78	10,33	7,78	27,40	8,03	31,31	8,50	66,33	17,89	15,00	27,05	17,46	27,61	21,24	27,86	24,04
P_E12_*Phaeobacter gallaeciensis*	14,86	34,19	46,36	45,85	38,34	55,00	20,01	69,26	25,70	22,39	25,84	22,52	25,84	22,13	28,99	27,79
P_E13_*Halomonas venusta*	10,71	0,44	11,20	-0,06	12,07	2,23	19,16	8,99	23,83	5,49	21,73	14,36	22,23	17,03	20,96	17,35
P_E20_*Halomonas venusta*	19,23	0,50	16,98	6,26	21,07	6,13	47,88	17,87	18,00	7,51	22,20	8,61	36,95	20,71	37,51	22,21
P_G1_*Bacillus altitudinis*	11,86	0,90	12,36	0,62	14,80	3,24	19,50	9,92	22,55	9,80	27,48	10,09	28,55	20,68	31,84	26,00
P_L4_*Phaeobacter gallaeciensis*	4,58	1,08	7,33	3,17	10,49	3,14	17,46	7,10	14,98	4,41	17,35	13,40	19,35	20,78	26,58	23,36
P_L5_*Phaeobacter gallaeciensis*	9,52	8,68	11,85	12,36	17,40	16,43	48,84	18,55	20,98	4,72	23,88	8,16	29,14	13,71	30,66	14,63
P_L12_*Staphylococcus equorum*	1,55	3,77	3,70	15,88	22,77	22,02	20,31	42,01	11,47	4,47	12,68	6,63	12,68	12,68	6,63	6,63
P_M18_*Phaeobacter gallaeciensis*	19,01	8,56	34,26	48,15	31,05	53,07	46,54	66,60	28,48	6,58	29,16	8,75	31,14	11,62	34,39	17,59
P_M24_*Phaeobacter gallaeciensis*	33,93	12,56	34,82	29,04	39,62	46,26	39,14	46,12	9,99	0,29	13,19	7,31	14,60	11,30	13,27	27,29
P_S9_*Streptomyces griseus*	10,17	9,27	10,28	9,59	10,61	11,03	8,12	11,13	13,65	7,65	24,57	8,95	25,95	19,56	26,41	19,62
P_T3_*Bacillus paralicheniformis*	9,27	3,23	42,75	39,07	45,81	40,59	48,02	53,61	25,19	6,13	26,92	19,75	33,84	24,70	35,97	40,52
P_T5_*Bacillus* sp.	8,18	3,20	22,66	13,45	48,96	26,58	49,49	31,73	17,06	7,72	17,96	8,31	19,24	12,54	19,73	16,84
P_T7_*Bacillus* sp.	39,05	21,05	40,80	28,87	56,43	42,20	57,59	46,91	28,16	-0,68	20,44	2,67	18,27	5,19	33,65	15,56
Total extracts displaying ≥ 50% BFR and <40% GI	0		0		3		3		0		0		0		0	
Total extracts displaying ≥ 50% BFR and ≥40% GI	0		0		0		3		0		0		0		0	

Table S3.6: Quantitative analysis of percentage biofilm reducing potential (%BFR) and percentage growth inhibition (%GI) of *Pocillopora* CAB extracts against initial adhesion and mature biofilms of *Vibrio shilonii* ATCC_BAA 91.

Extracts	*Vibrio shilonii* ATCC_BAA 91															
	Initial								Mature							
	200 µg/ml		400 µg/ml		600 µg/ml		800 µg/ml		200 µg/ml		400 µg/ml		600 µg/ml		800 µg/ml	
	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR
P_A9_*Bacillus altitudinis*	27,46	42,61	25,85	40,05	27,84	37,31	24,99	36,98	17,06	6,50	19,51	7,28	20,53	8,82	21,88	9,94
P_E2_*Phaeobacter gallaeciensis*	8,57	34,87	6,21	35,18	7,89	36,03	12,12	36,51	2,14	6,44	2,53	6,35	7,06	8,21	7,30	9,58
P_E5_*Bacillus pumilus*	26,76	34,44	26,50	35,61	31,80	35,89	25,68	36,43	6,93	7,64	7,72	8,73	10,56	10,75	12,82	11,49
P_E12_*Phaeobacter gallaeciensis*	40,87	39,39	42,90	46,08	43,22	49,01	43,04	57,89	12,30	4,91	10,66	7,29	64,72	8,68	65,24	12,38
P_E13_*Halomonas venusta*	33,39	36,78	35,89	37,44	35,97	38,91	37,52	40,61	12,72	7,24	14,89	7,90	14,81	9,99	19,20	15,24
P_E20_*Halomonas venusta*	41,00	43,66	41,24	47,38	41,24	48,87	41,98	50,05	11,22	9,87	12,08	11,19	12,65	12,58	14,61	15,80
P_G1_*Bacillus altitudinis*	31,72	38,24	34,43	48,55	35,60	50,28	36,56	51,89	10,64	10,11	20,94	11,37	35,86	11,97	36,30	14,80
P_L4_*Phaeobacter gallaeciensis*	21,51	51,42	26,09	52,95	28,20	56,57	31,69	57,19	37,09	11,17	37,21	16,35	38,18	20,60	39,77	26,69
P_L5_*Phaeobacter gallaeciensis*	26,47	34,71	33,38	34,79	34,64	34,65	37,32	37,04	11,86	3,41	12,58	4,35	13,33	4,64	13,60	6,32
P_L12_*Staphylococcus equorum*	16,53	39,98	38,18	41,31	37,79	77,25	40,90	79,95	5,37	4,47	73,06	6,58	73,24	7,90	73,44	9,32
P_M18_*Phaeobacter gallaeciensis*	32,91	46,85	33,35	46,82	33,18	51,14	35,20	52,13	13,11	3,91	13,67	4,04	17,23	4,61	21,15	9,01
P_M24_*Phaeobacter gallaeciensis*	26,93	36,61	29,89	37,56	30,22	38,77	29,70	38,80	4,42	0,71	4,81	2,92	6,09	4,55	11,18	5,86
P_S9_*Streptomyces griseus*	28,50	38,81	29,24	39,60	29,42	41,09	30,30	41,99	8,68	5,67	10,61	12,02	11,34	13,86	12,55	14,21
P_T3_*Bacillus paralicheniformis*	32,70	65,45	33,83	62,25	35,56	52,81	36,62	49,22	7,57	9,78	12,28	11,04	15,82	15,48	16,86	19,05
P_T5_*Bacillus* sp.	26,24	79,35	37,85	79,40	39,71	79,82	41,99	88,03	9,55	12,04	10,36	13,85	12,41	18,35	12,84	20,48
P_T7_*Bacillus* sp.	36,48	76,30	36,57	81,90	37,73	82,55	40,72	85,45	9,76	11,23	10,43	11,17	10,86	14,85	10,89	17,70
Total extracts displaying ≥ 50% BFR and <40% GI	4		4		6		3		0		0		0		0	
Total extracts displaying ≥ 50% BFR and ≥40% GI	0		0		1		5		0		0		0		0	

CHAPTER 4

ANTIMICROBIAL, ANTI-QUORUM SENSING AND ANTIBIOFILM POTENTIAL OF *Acropora* SPECIES-ASSOCIATED BACTERIAL EXTRACTS AGAINST CLINICAL INDICATORS, MARINE BACTERIA AND MARINE PATHOGENS

Abstract

The increase of bacterial resistance to antibiotics has led to a search for new natural compounds, and coral-associated bacteria are emerging as an untapped source for bioactive compounds. The antimicrobial, anti-quorum sensing and anti-biofilm potential of selected *Acropora* CAB extracts (n=24) was assessed. Shake-flask fermentations and ethyl acetate extractions of the selected bacterial isolates were carried out. Bacterial isolates were identified by 16S rRNA gene amplification and sequencing. Extracts were assessed for antimicrobial activity using antimicrobial susceptibility testing (AST) against selected indicator organisms, *Pseudomonas aeruginosa* ATCC 27853*, Staphylococcus aureus* ATCC 43300*, Shewanella putrefaciens* ATCC 8071*, Vibrio alginolyticus* ATCC 17749*, V. coralliilyticus* ATCC_BAA 450*, V. parahaemolyticus* ATCC 17802 *,* and *V. shilonii* ATCC_BAA 91. Quorum sensing (QS) inhibition was assessed using qualitative and quantitative violacein inhibition assays against biosensors *Chromobacterium violaceum* ATCC 12472 and *Chromobacterium. subtsugae* CV017, while autoinducer-2 (AI-2) inhibition was detected using *Vibrio harveyi*. Anti-biofilm potential was assessed using crystal violet microtiter plate assay against indicators. Antimicrobial activity was demonstrated by 12.5% (3/24) of extracts against clinical pathogens (two against *S. aureus* and one against *P. aeruginosa*) and 20.83% (5/24) against marine indicators. Qualitatively, QS inhibition was demonstrated by 25% (6/24) of extracts against *C. violaceum* and 29.16% (7/24) against *C. subtsugae*. Quantitatively, QS inhibition was demonstrated by 62.50% of extracts against *C. violaceum* and *C. subtsugae*, respectively. Autoinducer-2 inhibition was exhibited by 25% of extracts. For initial adhesion inhibition, 87.50% (21/24) of extracts demonstrated ≥50% activity, with three extracts demonstrating activity against three and six against two indicators. Mature biofilm reduction (≥50%) was exhibited by 70.83% (17/24) of extracts, with one extract showing activity against four indicators, three against three indicators and five showing activity against two indicators. Extracts demonstrated better activity against mature biofilms of *S. aureus, S. putrefaciens,* and *V. shilonii,* while better initial adhesion reduction was observed against, *P. aeruginosa, V. coralliilyticus* and *V. parahaemolyticus*. Members of the genus *Bacillus* showed broad spectrum activity across all bioassays. This may be due to *Bacillus* species producing a variety

of biomolecules with desired bioactivity. This study reports *Acropora*-associated bacteria as a promising source of novel bioactive compounds for use in environmental and pharmaceutical industries.

4.1 Introduction

Coral-associated bacteria (CAB) are considered to exhibit a great biotechnological potential through their production of biologically active secondary metabolites (Rajasabapathy *et al*., 2020). Corals are an untapped source of bacterial diversity with bioactive compounds where natural drugs can be obtained (Ahila *et al*., 2017; ElAhwany *et al.,* 2015). Bioactive compounds used in environmental and clinical studies have been obtained from CAB (El Samak *et al*., 2018; Garrido *et al.,* 2020). Corals and their associated bacteria are essential to maintain host homeostasis and health as defensive agents against pathogens due to the production of antimicrobial compounds, quorum sensing (QS) inhibitors and biofilm inhibitors to respond to environmental disturbances (Garrido *et al.,* 2020; Nofiani *et al.*, 2020; Thompson *et al*., 2015). This is due to the increase in antibiotic-resistant pathogens that threaten both coral and human health (Rajasabapathy *et al*., 2020). Coral-associated bacteria are suggested to produce broad spectrum antibiotics to fight against resistant pathogens (Vanwonterghem and Webster, 2020), hence the exploration of these microorganisms for antibiotics and drugs important in the treatment of diseases in both corals and humans (Arachchige *et al.,* 2021).

The exploration of new antimicrobial compounds from CAB is essential not only for commercial purposes, but also to contribute towards different approaches in the maintenance of coral health due to the increase in antibiotic resistance in both coral and human pathogens (Sarmiento-Vizcaíno *et al*., 2017). Pereira *et al.* (2017) explored the antimicrobial activity of CAB isolated from a Brazilian coral and reported that 83% of the isolated bacteria demonstrated antimicrobial activity against the opportunistic coral pathogen, *Serratia marcescens*, and most isolates that exhibited activity belonged to the genus *Bacillus*. Li *et al*. (2014) screened the antimicrobial activity of actinomycetes isolated from the coral *Acropora millepora*. Six strains of actinomycetes demonstrated greater antimicrobial activity against the marine pathogen *Vibrio alginolyticus*. Kvennefors *et al*. (2012) reported that a CAB *Pseudoalteromonas* strain exhibited antagonistic characteristics against the marine pathogen *Vibrio coralliilyticus*. Their study further observed other species that produced antimicrobial activities including *Bacillus, Erythrobacter, Labrenzia, Micrococcus, Roseobacter* and *Streptomyces* species *Bacillus amyloliquefaciens* was also observed to have demonstrated activity against *Bacillus subtilis, Escherichia coli* and *Staphylococcus aureus.* Garrido *et al*. (2020) reported the antimicrobial activity demonstrated by *Bacillus* species against the human

pathogens; methicillin-resistant *S. aureus* and multi-drug resistant *Pseudomonas aeruginosa*. This bacterial strain inhibited the growth of *P. aeruginosa* and signs of growth reduction were observed for *S. aureus*.

Quorum sensing signalling molecules of bacteria from different genera have been reported and are known to regulate motility genes and biofilm formation in opportunistic bacteria from different genera such as *S. marcescens* and *P. aeruginosa* and *Vibrio* species (Papenfort and Bassler, 2016). Several studies have screened CAB for anti-QS compounds to minimize the infection of the host by pathogens (Pham *et al*., 2016; Singh *et al*., 2017; Zhao *et al*., 2019). These studies have demonstrated that CAB produce novel anti-QS compounds. El-Kurdi *et al*. (2021) reported that the anti-QS activity of CAB strains was higher than the anti-QS activity of bacteria from other marine organisms. A total of 66% of quorum quenching CAB were recorded and 35% of the tested isolates demonstrated degradation of the purple pigment of the *Chromobacterium* biosensor. Goldberg *et al*. (2013) screened 120 CAB isolated from a healthy coral species against three biosensors, *E. coli, Chromobacterium violaceum* ATCC 12472 and *Agrobacterium tumefaciens*. Anti-QS activity against these biosensors was observed with 12, 11 and 24% of CAB, respectively. Bakkiyaraj *et al*. (2013) reported CAB23 and CAB41 isolated from coral *Acropora digitifera* to exhibit anti-QS activity against the opportunistic human pathogen *P. aeruginosa*. The detected bioactive compounds from CAB23 and CAB41 extracts inhibited the expression of virulence genes of *P. aeruginosa* responsible for the production of enzymes or biofilm formation. About 21.95% (9/41) of the CAB demonstrated anti-QS activity against the reporter strain *C. violaceum* ATCC 12472.

Coral-associated bacteria are a rich source of bioactive compounds with promising anti-biofilm properties (Thenmozhi *et al*., 2009; Qian *et al*., 2009; Wang *et al.,* 2018). Wang *et al*. (2018) isolated a compound hygrocin C from a coral-associated *Streptomyces* species which was reported to inhibit biofilm activity at the early stage against *S. aureus* and *Bacillus. amyloliquefaciens.* Several CAB isolated from coral *A. digitifera* were reported to inhibit biofilm activity of *Streptococcus pyogenes* (Nithyanand *et al*., 2010). The *Streptococcus* sp. strains clearly demonstrated efficient anti-biofilm activity against all biofilm-forming M serotypes. Moreover, their study suggested actinomycetes produced pharmaceutical compounds able to inhibit biofilm formation of marine pathogens such *Vibrio* species Gowrishankar *et al*. (2012) evaluated two CAB extracts from *Bacillus firmus* and *Vibrio parahaemolyticus* for anti-biofilm activity against *S. aureus*. Their results demonstrated excellent anti-biofilm activity against *S. aureus* and these CAB appeared to be a promising source of anti-biofilm agents particularly against this clinical pathogen. The present study aims

to demonstrate the importance and ability of bacteria isolated from the coral *Acropora* species to produce natural bioactive compounds with antimicrobial, anti-QS and anti-biofilm properties.

4.2 Materials and Methods

4.2.1 Fermentation and ethyl acetate extraction

Twenty-four bacterial isolates cultured from a *Acropora* species which demonstrated antimicrobial activity in the colony drop assay (Chapter 2) were selected for fermentation and further screening (Suppl. Fig. S4.1). These isolates were pre-cultured in 5 ml ISP2 broth per litre (4g yeast extract powder, 10g malt exrtact powder, 4g dextrose, 1L distilled water) in duplicate for 2 d and 3 ml of each pre-culture was inoculated into 250 ml of medium Mannitol. Isolates were incubated with 150 rpm shaking at 30 °C for 7 d (Varela *et al*., 2021). Bacterial cells were centrifuged at 9 500 rpm for 10 min to collect cell-free supernatant (CFS). The organic layer was collected using a separatory funnel, where the solvent and the liquid separate into two layers, then majority of the bottom layer is drained into a labelled flask for extraction An equal volume (1:1 of ethyl acetate) was added to each cell-free supernatant followed by an agitation of 1 h at 30 °C. The organic layer was collected was subjected to a second (1:1) ethyl acetate extraction and agitation for 4 h to collect the organic layer. Both layers were combined, and the ethyl acetate was evaporated in a rotary evaporator (Ilmvac, ROdist digital 230V 50/60Hz). Each extract was solubilized in 3 ml methanol and was added into a pre-weighed vial. Methanol was allowed to evaporate for 5-7 d and each extract obtained was weighed and dissolved in 10% (v/v) dimethyl sulfoxide (DMSO) or 100% methanol for oily extracts to a final standardized concentration of 20 mg/ml.

4.2.2 Molecular identification

4.2.2.1 Genomic DNA isolation purification

Twenty-four selected bacterial strains from *Acropora* spp. were subjected to phylogenetic analysis. Genomic DNA isolation was carried out using the GeneJet Genomic DNA purification (Thermo Scientific) or ZR Fungal/Bacterial DNA Miniprep (Zymo Research) kits according to manufacturers' instructions. Plates were flooded with 1 ml of sterile distilled water and bacterial cells were scraped off using a glass Pasteur pipette into 1.5 ml microcentrifuge tubes. Thereafter the contents in the microfuge tube were centrifuged for 10 min at 12 000 rpm. The DNA was eluted with 75 µl of DNA elution buffer and stored at -20 °C until required.

4.2.2.2 16S rRNA gene amplification and sequencing

The 16S ribosomal RNA genes (1.5 kb) from selected isolates were amplified using the universal primer sets F1 5'- AGAGTTTGATCITGGCTCAG-3-3'; R5 5'- ACGGITACCTTGTTACGACTT -3' (Coram and Rawlings *et al*., 2002) and 27F-DEG 5'- AGAGTTTGATCMTGGCTCAG -3'; 1492R-deg 5′-GGYTACCTTGTTACGACTT-3' (Weisburg *et al*., 1991). PCR reaction mixtures included 2 µl of template DNA, 18 µl ddH_2O, 2.5 µl buffer, 1.2 µl dNTPs, 0.2 µl of each primer set, 1.2 µl $MgCl_2$ and 0.1 µl DNA polymerase (SuperTherm). Amplification was performed in a PCR machine (MJ MINI^{TM} personal Thermal cycler; Bio-Rad) using the following conditions: DNA denaturation at 94 °C for 1 min, followed by 35 cycles of denaturation at 94 °C for 30 s, primer annealing at 52 °C for 1 min and extension at 72 °C for 1 min. A total of 5 µl of PCR mixture and 3 µl gel loading buffer were loaded into agarose gels together with the molecular weight marker, GeneRuler 100 bp plus DNA ladder (Thermo Scienfic). Amplified PCR products were subjected to gel electrophoresis in 1% (w/v) agarose gels at 80 V for 90 min in 1% TAE buffer. PCR products were visualized by UV transillumination (Syngene, UK) after staining in 0.1 mg/ml ethidium bromide for 15 min. DNA was sequenced, sequences were processed using BioEdit (version 7.0) and subjected to identification using the NCBI-Blast nucleotide database.

4.2.3 Antimicrobial susceptibility testing of the extracts against clinical and marine pathogens by disc diffusion method

Antimicrobial activity of the bacterial extracts from *Pocillopora* spp. (n=24) was assessed using the disc diffusion assay against clinical pathogens (methicillin-resistant *S. aureus* ATCC 43300, multidrug-resistant *P. aeruginosa* ATCC 27853) and marine indicators *Shewanella putrefaciens* ATCC 8071, *V. alginolyticus* ATCC 17749, *V. coralliilyticus* ATCC-BAA 450, *V. parahaemolyticus* ATCC 17802 and *V. shilonii* ATCC-BAA 91. *Staphylococcus aureus* ATCC 43300, *P. aeruginosa* ATCC 27853*, S. putrefaciens* ATCC 8071, *V. alginolyticus* ATCC 17749 and *V. parahaemolyticus* ATCC 17802 were grown on Mueller-Hinton (MH) agar plates and incubated at 30 °C for 24 h, while *V. coralliilyticus* ATCC-BAA 450, and *V. shilonii* ATCC-BAA 91 were grown on Marine Agar (MA) (Difco^{TM}) plates and incubated at 30 °C for 24 h. Following incubation, turbid cell suspensions equivalent to a 0.5 McFarland standard were prepared (Chenia, 2013) and cultures were uniformly swabbed over the entire surface of MH and MA plates. Extracts (200, 400, 600 and 800 µg) were loaded into 6 mm blank discs. Ampicillin (AP10 µg), ceftriaxone (CRO30 µg), ciprofloxacin (CIP5 µg), erythromycin (E15 µg), gentamicin (GN10 µg), streptomycin (S10 µg) and tetracycline (T30

µg) discs were used as antibiotic controls (Chenia, 2013) and 10% DMSO served as the solvent control. *Pseudomonas aeruginosa* and *S. aureus* plates were incubated at 37 °C (optimal temperature) for 24 h, while *S. putrefaciens*, *V. alginolyticus*, *V. coralliilyticus*, *V. parahaemolyticus* and *V. shilonii* were incubated at 30 °C for 24 h. Following incubation, the diameter of zones of inhibition was measured and considered to be indicative of antimicrobial activity (Nithya *et al*., 2011). The following criteria of zone diameter were used to indicate activity of tested extracts: Strong activity ≥16 mm, Intermediate activity = 11 – 15 mm, and weak activity ≤11 mm (Chenia, 2013).

4.2.4 Anti-quorum sensing ability screening

4.2.4.1 Qualitative anti-quorum sensing ability

The quorum quenching (QQ) potential of the 24 selected bacterial extracts was assessed using the agar-overlay assay. Wild-type pigmented biosensor strains *C. violaceum* ATCC 12472 and *C. subtsugae* CV017 were grown overnight in Luria Bertani (LB) broth at 150 rpm at 30 °C using a rotary shaker (Chenia, 2013). Molten soft LB agar (0.5% w/v) was prepared and 5 ml was inoculated with 150 µl of the respective biosensor strains equivalent to 0.5 McFarland standard. The soft agar-culture solution was immediately poured over the LB agar plates and left to solidify. Thereafter, 6 mm blank discs were loaded with extracts (200, 400, 600, and 800 µg) and incubated in an upright position overnight at 30 °C. The inhibition of the violacein pigment production appearing as opaque zones indicated quorum sensing inhibition and clear zones around the discs were indicative of killing (Chenia, 2013). (*Z*-)-4-bromo-5-bromoethylene)-2-(5*H*)-furanone (Sigma) (20 µg/ml) was used as a positive control and 10% (v/v) DMSO and 100% methanol were used as solvent controls (Chenia, 2013).

4.2.4.2 Quantitative quorum sensing inhibition using violacein assay

The QS inhibitory potential CAB extracts was quantified using the violacein inhibition assay (Chenia, 2013). The biosensor strains *C. violaceum* ATCC 12472 and *C. subtsugae* CV017 were grown overnight and 150 µl was inoculated in 3 ml of LB broth in 36 ml test tubes and varying concentrations of the extracts (200, 400, 600, 800 and 1000 µg/ml) were added and incubated at 30 °C for 24 h with shaking. Broth tubes containing *C. violaceum* ATCC 12472 and *C. subtugae* CV017 with no extracts were used as growth controls. Following incubation, 200 µl of the overnight culture was added to microtiter plate wells, and growth absorbance readings were taken at $OD_{600\ nm}$ using a microtitre plate reader (Glomax Multi+ Detection System, Promega) to determine the percentage growth inhibition (GI). If the growth inhibition

at $OD_{600\ nm}$ was ≥40% of the positive, untreated control, the extract was considered to have growth inhibitory activity and not considered to be good quorum sensing inhibition (QSI) indicator.

One ml of the culture supernatant was centrifuged at 12 000 rpm for 10 min to remove the cells and the pellet was evenly resuspended in 1 ml of DMSO and centrifuged at 12 000 rpm for 10 min and the violacein supernatant was collected. Violacein was quantified using a microtitre plate reader at $OD_{560\ nm}$ using Glomax Multi+ Detection System (Promega). Cinnamaldehyde and vanillin (20 µg/ml) were used as a QS inhibition positive control and LB broth as a purity control (Chenia, 2013). The percentage of violacein inhibition (VI) was calculated as follows (Packiavathy *et al*., 2012): %VI = (control $OD_{560\ nm}$ – test $OD_{560\ nm}$ /control $OD_{560\ nm}$) × 100 (Chenia, 2013). Violacein inhibition was assessed in triplicate on two separate occasions.

4.2.5 Qualitative autoinducer-2 inhibition

An autoinducer-2 inhibition assay was also carried against the indicator *Vibrio harveyi* BB120, a wild-type bioluminescent strain using an agar overlay assay (Teasdale *et al.,* 2011). *Vibrio harveyi* was cultured on a MA plate (Difco™) and incubated at 30 °C overnight. The culture was then inoculated into Marine broth and incubated with agitation at 30 °C for 24 h. Following incubation, 150 µl of the *V. harveyi* broth equivalent to 0.5 McFarland standard was inoculated in Marine soft agar (0.5% w/v) and poured over the LB-seawater agar plates and left to solidify. Once the overlay solidified, 6 mm blank discs were loaded with extracts (200, 400, 600 and 800 µg) and incubated in an upright position overnight at 30 °C. Zones of growth inhibition were observed visually, while bioluminescence inhibition was observed by using GeneSys image analyzer (Syngene). Autoinducer-2 inhibition was identified by the appearance of dark zones and lack of bioluminescence around the bacterial colonies. Cinnamaldehyde (10 µg/ml) was used as positive AI-2 inhibitor control (Brackman *et al*., 2008).

4.2.6 Detection of anti-biofilm activity of seaweed-associated bacteria extracts

Prior to the anti-biofilm assay, the 24 selected *Pocillopora* spp. bacterial extracts were tested for antibacterial activity with sub-inhibitory and inhibitory concentrations (200, 400, 600 and 800 µg), using the disc diffusion assay. Extracts were tested against clinical pathogens *P. aeruginosa* ATTC 27853 and *S. aureus* ATCC 43300 and marine indicators *S. putrefaciens* ATCC 27853, *V. coralliilyticus* ATCC_BAA 450, *V. parahaemolyticus* ATCC 17802 and *V. shilonii* ATCC_BAA 91 to assess their effect on initial adhesion and detachment of mature

biofilms. Overnight cultures were used to prepare cell suspensions, which were standardized equivalent to a 0.5 McFarland standard (Basson *et al*., 2008). For initial adhesion studies, extracts were added to 90 µl tryptic soy broth (TSB) and 10 µl of standardized cell suspension (to a final volume of 200 µl) and incubated for 24 h at 37 ˚C with agitation. For pre-formed biofilm detachment assays, 24 h biofilms were established following addition of 90 µl TSB and 10 µl of 0.5 McFarland standardized cell suspension to microtiter plate wells, and incubation at 37 ºC for 24 h. Microtiter plates were washed three times with sterile deionised water and allowed to air-dry. Following air-drying, 90 µl TSB as well as extracts at the relevant, respective concentrations were added to wells (to a final volume of 200 µl) and microtiter plates were incubated for 24 h with agitation at 37 ˚C. The negative control contained only broth, while positive controls contained respective cell suspensions with no extracts added. After incubation, growth $OD_{600\ nm}$ values were determined using the Glomax multi + detection system (Promega) and wells with ≥40% reduction in growth were considered growth inhibitory. Thereafter, planktonic cells were removed by discarding the liquid media. Plates were processed for biofilm inhibition as described by Basson *et al*. (2008). Microtiter plates were washed three times with sterile dH_2O. Cells were fixed with 200 µl of methanol for 15 min, then air-dried. Wells were stained with 150 µl of 2% Hucker's crystal violet for 5 min. Wells were rinsed gently under running tap water then plates were allowed to dry. Glacial acetic acid (150 µl; 33% (v/v)) was used to re-solubilise cells (Basson *et al*., 2008). The OD was read at 600 nm using the Glomax multi + detection system (Promega). Tests were conducted in triplicate on two separate occasions. A measure of efficacy called percentage reduction was calculated from the blank, control, and treated absorbance values (Pitts *et al*., 2003): %reduction =[(C−B) − (T−B) C−B]/C-B ×100, where B denotes the average absorbance per well for blank wells (no biofilm, no treatment), C denotes the average absorbance per well for control wells (biofilm, no treatment), and T denotes the average absorbance per well for treated wells (biofilm and treatment).

4.2.7 Statistical analysis

Data was obtained after assays had been performed in either duplicate or triplicate to ensure validity of obtained results. One-way ANOVA analysis was employed to determine the significance of the CAB extract's bioactivity. For all analyses, a p-value of ≤ 0.05 was accepted as significant, such that the null hypothesis may be rejected. The differences in violacein inhibition with and without the addition of varying concentrations of extracts was determined using pair-wise testing based on Student's t-tests using IBM SPSS (Statistical Package for the

Social Sciences) (IBM SPSS Statistics for Windows, 2017. Version 27.0. Armonk, NY: IBM Corp.), with $p \leq 0.05$ being considered significant. The difference in violacein inhibition mean values between extracts was determined using One-way repeated measures ANOVA with $p \leq 0.05$ being considered significant (Chenia, 2013).

4.3 Results

4.3.1 Preparation of ethyl acetate bacterial extracts

Twenty-four *Acropora* CAB isolates were selected from primary screening for fermentation and secondary metabolite extraction using ethyl acetate and rotary evaporation (Suppl. Fig. S4.1). Extracts were dissolved in 10% (v/v) DMSO or methanol for further screening.

4.3.2 Molecular identification of selected bacterial isolates

Genomic DNA was isolated from the 24 selected bacterial isolates and amplified to get 16S rRNA gene fragments of ~ 1500 bp. These were compared with 16S rRNA gene sequences in the GenBank database. Both Gram-positive and Gram-negative bacterial isolates were dominant, particularly *Bacillus* (n=11), *Erythrobacter* (n=2), *Labrenzia* (n=2), *Phaeobacter* (n=3) and *Streptomyces* (n=3) species (Table 4.1).

4.3.3 Secondary screening of coral-associated bacterial isolates using antimicrobial susceptibility testing (AST)

All extracts demonstrated no-to-weak activity (≤10 mm) against multi-drug resistant *P. aeruginosa* ATCC 27853, i.e., 200 - 800 µg, with only a single *Bacillus altitudinis* (Ac_A20) extract demonstrating intermediate activity (11- 15 mm) at 200 µg and strong activity (18 – 24 mm) at 400 - 800 µg (Table 4.2; Suppl. Fig. S4.2).

Against methicillin-resistant *S. aureus* ATCC 43300, only two extracts, both from genus *Bacillus* demonstrated activity, i.e., *B. altitudinis* (Ac_A20) and *Bacillus aerophilus* (Ac_L15). Both extracts demonstrated intermediate activity at 200 µg and strong activity at 400 - 800 µg (Table 4.2; Suppl. Fig. S4.2).

All extracts demonstrated no activity against *S. putrefaciens* (Table 4.3), i.e., 200 - 800 µg. Against *V. alginolyticus*, only four extracts demonstrated activity, i.e., Ac_G37, Ac_G56, Ac_L15 and Ac_L27. The *Bacillus velezensis* (Ac_G37) extract demonstrated strong activity (16 -19 mm) from 200 - 800 µg compared to *Erythrobacter nanhaisediminis* (Ac_G56) which demonstrated intermediate activity (14 - 15 mm) across 200 - 800 µg. The *B. aerophilus*

(Ac_L15) and *B. altitudinis* (Ac_L27) extracts demonstrated intermediate activity (15 mm) at 200 µg and strong activity (16 - 18 mm) at 400 – 800 µg (Table 4.3; Suppl. Fig. S4.2).

Table 4.1: 16S rRNA identities of selected *Acropora* -associated bacterial isolates with colony description and Gram-stain characteristics.

Isolate Code	16S rRNA confirmation	Sequence ID	Sequence %	Colony description	Gram stain result
Ac_A18	*Streptomyces globisporus*	MH819503.1	100.00%	Yellow actinomycetes	Gram-positive rods
Ac_A20	*Bacillus altitudinis*	MT598007.1	99.86%	Pink	Gram-positive rods
Ac_E5	*Bacillus altitudinis*	MN421109.1	99.86%	Yellow	Gram-positive rods
Ac_E13	*Labrenzia marina*	MH283853.1	97.90%	Yellow	Gram-negative rods
Ac_E14	*Labrenzia alexandrii*	MH283852.1	99.55%	Pink	Gram-negative rods
Ac_E17	*Bacillus aerophilus*	MT598007.1	100.00%	Cream white	Gram-positive rods
Ac_E19	*Phaeobacter gallaeciensis*	CP010784.1	100.00%	Brown	Gram-negative rods
Ac_G37	*Bacillus velezensis*	CP054714.1	99.44%	Yellow	Gram-positive rods
Ac_G56	*Erythrobacter nanhaisediminis*	KJ009560.1	99.48%	Orange	Gram-negative rods
Ac_G57	E*rythrobacter nanhaisediminis*	KJ009560.1	99.48%	Orange	Gram-negative rods
Ac_L5	*Phaeobacter piscinae*	CP010715.1	100.00%	brown	Gram-negative rods
Ac_L8	*Bacillus altitudinis*	KY357421.1	100.00%	Cream white	Gram-positive rods
Ac_L12	*Phaeobacter gallaeciensis*	KY357427.1	95.58%	Brown	Gram-negative rods
Ac_L15	*Bacillus aerophilus*	MT598007.1	100.00%	Light yellow	Gram-positive rods
Ac_L27	*Bacillus altitudinis*	KP236332.1	93.03%	Cream white	Gram-positive rods
Ac_L38	*Ruegeria atlantica*	MW828512.1	99.85%	Light yellow	Gram-negative rods
Ac_L39	*Sporosarcina pasteurii*	KF214757.1	99.86%	Cream white	Gram-positive rods
Ac_L41	*Streptomyces pratensis*	KU973961.1	99.49%	Yellow actinomycetes	Gram-positive rods
Ac_L43	*Microbulbifer variabilis*	MH283822.1	100.00%	Light yellow	Gram-negative rods
Ac_M40	*Metabacillus indicus*	FM992830.1	100.00%	Yellow	Gram-positive rods
Ac_S14	*Streptomyces violascens*	EU906929.1	99.93%	Yellow actinomycetes	Gram-positive rods
Ac_T15	*Bacillus pumilus*	KR085935.1	99.79%	Cream white	Gram-positive rods
Ac_T16	*Bacillus pumilus*	KR085935.1	99.93%	Cream white	Gram-positive rods
Ac_T20	*Bacillus pumilus*	KR085935.1	99.93%	Cream white	Gram-positive rods

Two extracts demonstrated activity against *V. coralliilyticus*, i.e., Ac_A20 and Ac_L15. The *B. altitudinis* Ac_A20 extract demonstrated intermediate activity (11 - 13 mm) across 200 - 800 µg, while the *B. aerophilus* Ac_L15 extract demonstrated intermediate (11 mm) activity at 600 - 800 µg (Table 4.4; Suppl. Fig. S4.2). Against *V. shilonii*, the *B. altitudinis* Ac_A20 extract demonstrated intermediate activity (11 - 13 mm) across 200 - 800 µg, while the *B. aerophilus* Ac_L15 extract demonstrated intermediate (11 - 12 mm) activity at 600 - 800 µg (Table 4.4; Suppl. Fig. S4.2). None of the extracts demonstrated activity against *V. parahaeomolyticus* (Table 4.3; Suppl. Fig. S4.2).

Overall, extracts Ac_A20 and Ac_L15 showed a broad spectrum of activity. The *B. altitudinis* Ac_A20 extract demonstrated intermediate to strong activity against *P. aeruginosa, S. aureus, V. coralliilyticus* and *V. shilonii* at varying exposures of 200 - 800 µg (Tables 4.2 - 4.4; Suppl. Fig. S4.2). The *B. aerophilus* Ac_L15 extract demonstrated intermediate activity against *S. aureus, V. alginolyticus, V. coralliilyticus* and *V. shilonii* at varying exposures of 200 - 800 µg. A trend of intermediate activity (11 - 15 mm) by these extracts was observed at 200 µg, with strong activity (16 - 24 mm) at 400 - 800 µg (Tables 4.2 - 4.4; Suppl. Fig. S4.2).

Out of the nine genera which were identified, *Bacillus, Phaeobacter* and *Streptomyces* isolates demonstrated better activity. *Bacillus* extracts were, however, more effective as antimicrobials as they demonstrated activity against multiple indicators, especially Ac_A20 and Ac_L15 which demonstrated activity against four indicators, respectively (Tables 4.2 – 4.4; Suppl. Fig. S4.2). Three extracts, Ac_G37, Ac_G56, Ac_L27 demonstrated activity against a single indicator (Table 4.3; Suppl. Fig. S4.2). A single *Erythrobacter* sp. strain Ac_G56 extract demonstrated activity against *V. alginolyticus* (Table 4.3). The rest of the extracts demonstrated weak-to-no activity against the tested indicators (Tables 4.2 – 4.4; Suppl. Fig. S4.2).

Table 4.2: Antimicrobial activity of *Acropora* CAB extracts against clinical indicators *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 43300 using the disc diffusion assay.

Zone diameter								
Extract	***Pseudomonas aeruginosa***				***Staphylococcus aureus***			
	200 µg	**400 µg**	**600 µg**	**800 µg**	**200 µg**	**400 µg**	**600 µg**	**800 µg**
Ac_A18	0	0	0	0	0	0	8	9
Ac_A20	14	18	20	24	15	17	17	18
Ac_E5	0	0	0	0	0	0	0	0
Ac_E13	0	0	0	0	0	0	0	0
Ac_E14	0	0	0	0	0	0	0	0
Ac_E17	0	0	0	0	0	0	0	0
Ac_E19	0	0	0	0	0	0	0	0
Ac_G37	0	0	0	0	0	0	0	0
Ac_G56	0	0	0	0	0	0	0	0
Ac_G57	0	0	0	0	0	0	0	0
Ac_L5	0	0	0	0	0	0	0	0
Ac_L8	0	0	0	0	0	8	9	9
Ac_L12	0	0	0	0	0	0	0	0
Ac_L15	0	0	0	0	15	16	20	20
Ac_L27	0	0	0	0	0	0	0	0
Ac_L38	0	0	0	0	0	0	0	0
Ac_L39	0	0	0	0	0	0	0	0
Ac_L41	0	0	0	0	0	0	0	0
Ac_L43	0	0	0	0	0	0	0	0
Ac_M40	0	0	0	0	0	0	0	0
Ac_S14	0	0	0	0	0	8	10	10
Ac_T15	0	0	0	0	0	0	0	0
Ac_T16	0	0	0	0	0	0	0	0
Ac_T20	0	0	0	0	0	0	0	0
Antibiotic controls								
Ampicillin (AMP10)	0				0			
Ceftriaxone (CTX30)	17				11			
Ciprofloxacin (CIP5)	28				24			
Erythromycin (E15)	15				0			
Gentamicin (GN10)	18				0			
Streptomycin (S10)	12				13			
Tetracycline (TE30)	9				22			
Solvent								
10% (v/v) DMSO	0				0			

*Color codes:

*Blue – Weak activity ≤ 10 mm

*Green – Intermediate 11 - 15 mm

*Yellow – Strong ≥ 16 mm.

Table 4.3: Antimicrobial activity of *Acropora* CAB extracts against marine bacteria *Shewanella putrefaciens* ATCC 27853, *Vibrio alginolyticus* ATCC 17749 and *Vibrio parahaemolyticus* ATCC 17802 using the disc diffusion assay.

Zone diameter												
Extract	***Shewanella putrefaciens***				***Vibrio alginolyticus***				***Vibrio parahaemolyticus***			
	200 µg	**400 µg**	**600 µg**	**800 µg**	**200 µg**	**400 µg**	**600 µg**	**800 µg**	**200 µg**	**400 µg**	**600 µg**	**800 µg**
Ac A18	0	0	0	0	0	0	0	0	0	0	0	0
Ac A20	0	0	0	0	0	0	0	0	0	0	0	0
Ac E5	0	0	0	0	0	0	0	0	0	0	0	0
Ac E13	0	0	0	0	0	0	0	0	0	0	0	0
Ac E14	0	0	0	0	0	0	0	0	0	0	0	0
Ac E17	0	0	0	0	0	0	0	0	0	0	0	0
Ac E19	0	0	0	0	0	0	0	0	0	0	0	0
Ac G37	0	0	0	0	16	17	18	19	0	0	0	0
Ac G56	0	0	0	0	15	14	15	14	0	0	0	0
Ac G57	0	0	8	8	0	0	0	0	0	0	0	0
Ac L5	0	0	0	0	0	0	0	0	0	0	0	0
Ac L8	0	0	0	0	0	0	0	0	0	0	0	0
Ac L12	0	0	0	0	0	0	0	0	0	0	0	0
Ac L15	0	0	0	0	15	16	17	18	0	0	0	0
Ac L27	0	0	0	0	15	16	16	16	0	0	0	0
Ac L38	0	0	0	0	0	0	0	0	0	0	0	0
Ac L39	0	0	0	0	0	0	0	0	0	0	0	0
Ac L41	0	0	0	0	0	0	0	0	0	0	0	0
Ac L43	0	0	0	0	0	0	0	0	0	0	0	0
Ac M40	0	0	0	0	0	0	0	0	0	0	0	0
Ac S14	0	0	0	0	0	0	0	0	0	0	0	0
Ac T15	0	0	0	0	0	0	0	0	0	0	0	0
Ac T16	0	0	0	0	0	0	0	0	0	0	0	0
Ac T20	0	0	0	0	0	0	0	0	0	0	0	0
Antibiotic controls												
Ampicillin (AMP10)	9				9				0			
Ceftriaxone (CTX30)	14				15				28			
Ciprofloxacin (CIP5)	31				30				25			
Erythromycin (E15)	10				21				22			
Gentamicin (GN10)	10				10				19			
Streptomycin (S10)	16				19				20			
Tetracycline (TE30)	29				20				21			
Solvent												
10% (v/v) DMSO	0				0				0			

*Color codes:

*Blue – Weak activity ≤ 10 mm

*Green – Intermediate 11 - 15 mm

*Yellow – Strong ≥ 16 mm.

Table 4.4: Antimicrobial activity of *Acropora* CAB extracts against marine bacteria *Vibrio corallilyticus* ATCC_BAA_450 and *Vibrio shilonii* ATCC_BAA_91 using the disc diffusion assay.

Zone diameter								
Extract	***Vibrio corallilyticus***				***Vibrio shilonii***			
	200 µg	**400 µg**	**600 µg**	**800 µg**	**200 µg**	**400 µg**	**600 µg**	**800 µg**
Ac_A18	0	0	0	0	0	0	0	0
Ac_A20	12	13	13	13	11	12	12	13
Ac_E5	0	0	0	0	0	0	0	0
Ac_E13	0	0	0	0	0	0	0	0
Ac_E14	0	0	0	0	0	0	0	0
Ac_E17	0	0	0	0	0	0	9	9
Ac_E19	0	0	0	0	0	0	0	0
Ac_G37	0	0	0	0	0	0	0	0
Ac_G56	0	0	0	0	0	0	0	0
Ac_G57	0	0	0	0	0	0	0	0
Ac_L5	0	0	0	0	0	0	0	0
Ac_L8	0	0	0	0	0	0	0	0
Ac_L12	0	0	0	0	0	0	0	0
Ac_L15	9	10	11	11	10	10	12	11
Ac_L27	0	0	0	0	0	0	0	0
Ac_L38	0	0	0	0	0	0	0	0
Ac_L39	0	0	0	0	0	0	0	0
Ac_L41	0	0	0	0	0	0	0	0
Ac_L43	0	0	0	0	0	0	0	0
Ac_M40	0	0	0	0	0	0	0	0
Ac_S14	0	0	0	0	0	0	0	0
Ac_T15	0	0	0	0	0	0	0	0
Ac_T16	0	0	0	0	0	0	0	0
Ac_T20	0	0	0	0	0	0	0	0
Antibiotic controls								
Ampicillin (AMP10)	0				25			
Ceftriaxone (CTX30)	41				30			
Ciprofloxacin (CIP5)	34				33			
Erythromycin (E15)	24				31			
Gentamicin (GN10)	30				30			
Streptomycin (S10)	20				22			
Tetracycline (TE30)	30				28			
Solvent								
10% (v/v) DMSO	0				0			

*Color codes:

*Blue – Weak activity ≤ 10 mm

*Green – Intermediate 11 - 15 mm

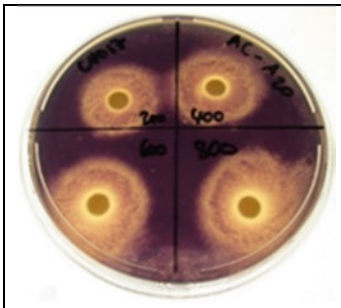
*Yellow – Strong ≥ 16 mm.

4.3.4 Gram-negative anti-quorum sensing ability screening

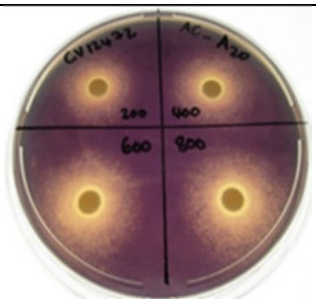
4.3.4.1 Qualitative agar-overlay assay

The anti-quorum sensing ability of *Acropora* CAB extracts was assessed against both *C. subtsugae* CV017 and *C. violaceum* ATCC 12472. Quorum sensing inhibition against *C. subtsugae* CV017 was demonstrated by 29.17% (7/24) of the extracts at 200 - 800 µg, with the *S. violascens* Ac_S14 extract demonstrating QSI activity at 400 - 800 µg (Fig. 4.1; Table 4.5; Suppl. Fig. S4.3).

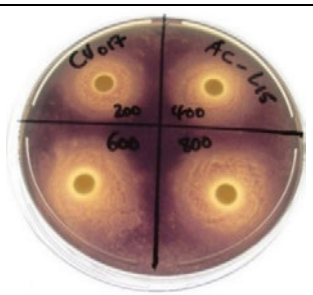
Inhibition activity against *C. violaceum* ATCC 12472 was demonstrated by 25% (6/24) of the extracts, with three extracts, i.e., *B. altitudinis* Ac_A20, *E. nanhaisediminis* Ac_G57 and *B. altitudinis* Ac_L8 demonstrating activity across 200 - 800 µg, and three extracts i.e., *S. globisporus* Ac_A18, *B. aerophilus* Ac_L15 and *S. violascens* Ac_S14 demonstrating activity at 400 - 800 µg (Fig. 4.1; Table 4.6; Suppl. Fig. S4.3). No killing activity was observed with any of the extracts. The best activity was observed with *B. altitudinis* Ac_A20, *B. altitudinis* Ac_L8 and *B. aerophilus* Ac_L15 extracts (Fig. 4.1; Table 4.5; Suppl. Fig. S4.3). Five extracts demonstrated activity against both *C. subtsugae* CV017 and *C. violaceum* ATCC 12472 (Tables 4.5 - 4.6; Suppl. Fig. S4.3).



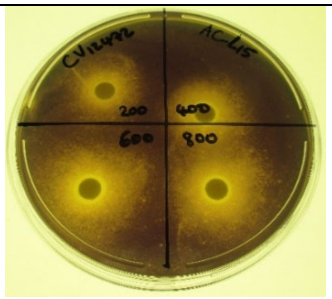
(A) Ac_A20_CV017



(A) Ac_A20_ATCC 12472



(B) Ac_L15_CV017



(C) Ac_L15_ATCC 12472

Figure 4.1: Anti-quorum sensing activity of selected *Acropora* CAB extracts against *Chromobacterium subtsugae* CV017 and *Chromobacterium violaceum* ATCC 12472 (A-B) *Bacillus altitudinis* Ac_A20 and (C-D) *Bacillus aerophilus* Ac_L15. The inhibition of the violacein pigment production appearing as opaque zones was indicative of QS inhibition and clear zones around the discs were indicative of killing.

Table 4.5: Quorum sensing inhibitory potential (opaque zones) demonstrated by the CAB bacterial extracts using the *Chromobacterium subtsugae* CV017 agar-overlay assays.

Extract	*Chromobacterium subtsugae* CV017											
	Zone diameter (mm)				Clear zone (mm)				QSI zone (mm)			
	200 µg	400 µg	600 µg	800 µg	200 µg	400 µg	600 µg	800 µg	200 µg	400 µg	600 µg	800 µg
Ac_A18	8	10	9	10	0	0	0	0	8	10	9	10
Ac_A20	9	10	10	11	0	0	0	0	9	10	10	11
Ac_E5	0	0	0	0	0	0	0	0	0	0	0	0
Ac_E13	0	0	0	0	0	0	0	0	0	0	0	0
Ac_E14	0	0	0	0	0	0	0	0	0	0	0	0
Ac_E17	0	0	0	0	0	0	0	0	0	0	0	0
Ac_E19	0	0	0	0	0	0	0	0	0	0	0	0
Ac_G37	0	0	0	0	0	0	0	0	0	0	0	0
Ac_G56	8	9	9	9	0	0	0	0	8	9	9	9
Ac_G57	0	0	0	00	0	0	0	0	0	0	0	0
Ac_L5	8	8	8	8	0	0	0	0	8	8	8	8
Ac_L8	8	9	10	10	0	0	0	0	8	9	10	10
Ac_L12	0	0	0	0	0	0	0	0				0
Ac_L15	9	10	11	11	0	0	0	0	9	10	11	11
Ac_L27	0	0	0	0	0	0	0	0	0	0	0	0
Ac_L38	0	0	0	0	0	0	0	0	0	0	0	0
Ac_L39	0	0	0	0	0	0	0	0	0	0	0	0
Ac_L41	0	0	0	0	0	0	0	0	0	0	0	0
Ac_L43	0	0	0	0	0	0	0	0	0	0	0	0
Ac_M40	0	0	0	0	0	0	0	0	0	0	0	0
Ac_S14	0	9	9	9	0	0	0	0	0	9	9	9
Ac_T15	0	0	0	0	0	0	0	0	0	0	0	0
Ac_T16	0	0	0	0	0	0	0	0	0	0	0	0
Ac_T20	0	0	0	0	0	0	0	0	0	0	0	0
Furanone (10 µg/ml)	10				0				10			

*Color codes:

*Green – selected extracts showing QSI activity

*Orange – extracts demonstrating no activity

*Yellow - extracts demonstrating QSI activity

*If an opaque halo was present, the QSI zone was calculated as follows: Zone diameter – Clear zone.

Table 4.6: Quorum sensing inhibitory potential (opaque zones) demonstrated by the *Acropora* CAB extracts using the *Chromobacterium violaceum* ATCC 12472 agar-overlay assays.

Extract	*Chromobacterium violaceum* ATCC12472											
	Zone diameter (mm)				Clear zone (mm)				QSI zone (mm)			
	200 µg	400 µg	600 µg	800 µg	200 µg	400 µg	600 µg	800 µg	200 µg	400 µg	600 µg	800 µg
Ac_A18	0	9	10	10	0	0	0	0	0	9	10	10
Ac_A20	9	9	10	11	0	0	0	0	9	9	10	11
Ac_E5	0	0	0	0	0	0	0	0	0	0	0	0
Ac_E13	0	0	0	0	0	0	0	0	0	0	0	0
Ac_E14	0	0	0	0	0	0	0	0	0	0	0	0
Ac_E17	0	0	0	0	0	0	0	0	0	0	0	0
Ac_E19	0	0	0	0	0	0	0	0	0	0	0	0
Ac_G37	0	0	0	0	0	0	0	0	0	0	0	0
Ac_G56	0	0	0	0	0	0	0	0	0	0	0	0
Ac_G57	8	10	10	10	0	0	0	0	8	10	10	10
Ac_L5	0	0	0	0	0	0	0	0	0	0	0	0
Ac_L8	9	11	11	12	0	0	0	0	9	11	11	12
Ac_L12	0	0	0	0	0	0	0	0	0	0	0	0
Ac_L15	0	9	10	10	0	0	0	0	0	9	10	10
Ac_L27	0	0	0	0	0	0	0	0	0	0	0	0
Ac_L38	0	0	0	0	0	0	0	0	0	0	0	0
Ac_L39	0	0	0	0	0	0	0	0	0	0	0	0
Ac_L41	0	0	0	0	0	0	0	0	0	0	0	0
Ac_L43	0	0	0	0	0	0	0	0	0	0	0	0
Ac_M40	0	0	0	0	0	0	0	0	0	0	0	0
Ac_S14	0	10	10	11	0	0	0	0	0	10	10	11
Ac_T15	0	0	0	0	0	0	0	0	0	0	0	0
Ac_T16	0	0	0	0	0	0	0	0	0	0	0	0
Ac_T20	0	0	0	0	0	0	0	0	0	0	0	0
Furanone (10 µg/ml)	13				0				13			

*Color codes:

*Green – selected extracts showing QSI activity

*Orange – extracts demonstrating no activity

*Yellow - extracts demonstrating QSI activity

*If an opaque halo was present, the QSI zone was calculated as follows: Zone diameter – Clear zone.

4.3.4.2 Quantitative quorum sensing inhibition using violacein assay

This assay was carried out to quantify violacein inhibition and ascertain whether the inhibition was due to cell death or QSI (Fig. 4.2; Suppl. Fig. S4.4). Extracts that demonstrated a percentage growth inhibition (%GI) ≥40% were considered bactericidal rather than QS inhibition. Optimal QSI of extracts was indicated by a percentage violacein inhibition (%VI) ≥50% with <40% (%GI).

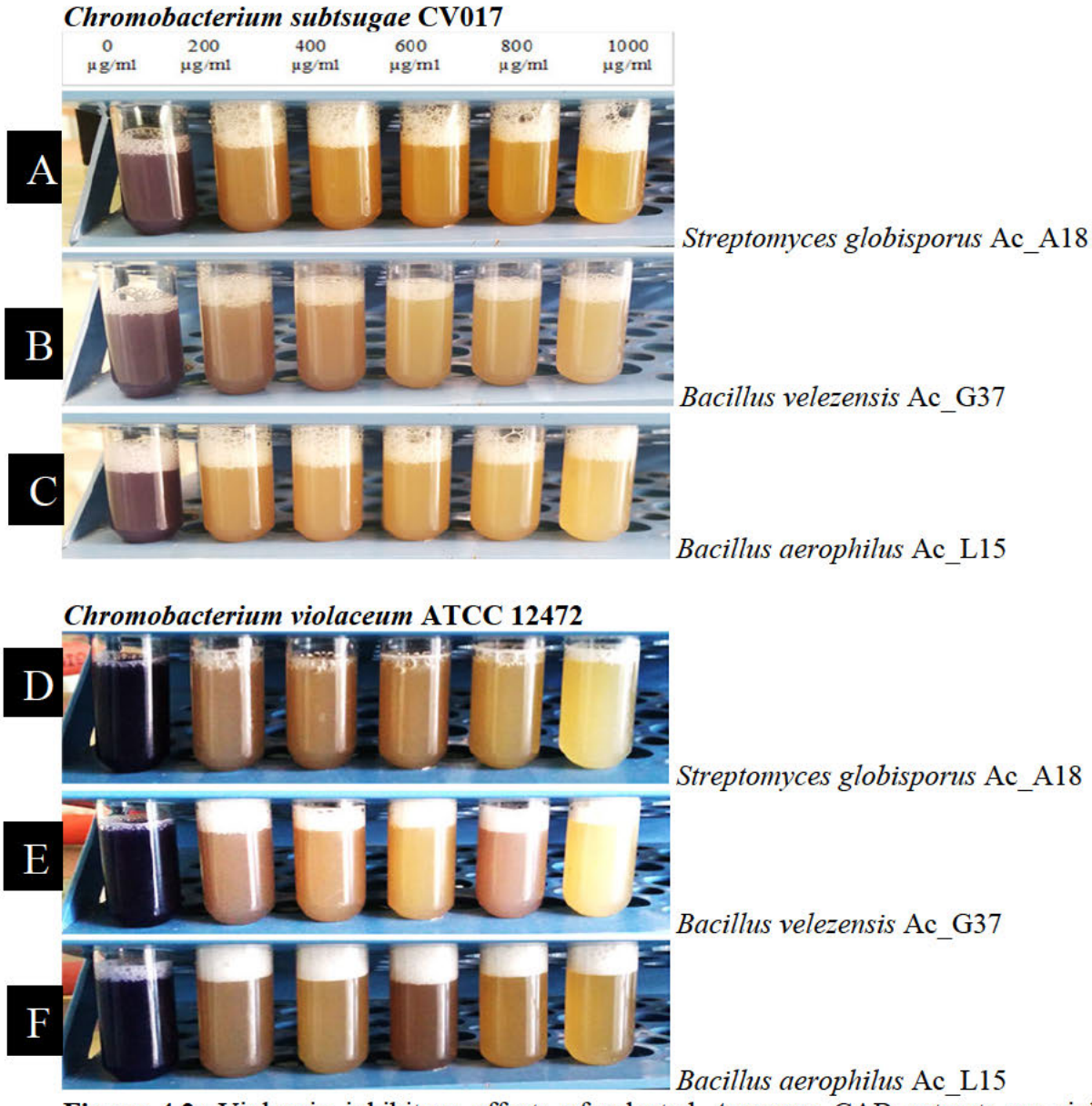


Figure 4.2: Violacein inhibitory effects of selected *Acropora* CAB extracts on violacein production by *Chromobacterium subtsugae* CV017 and *Chromobacterium violaceum* ATCC 12472 at 200 - 1000 µg/ml. Strong purple violacein pigment indicated less or no QS inhibition, while loss of it indicated QS inhibition or killing. A18, G37 and L15 against *C. subtsugae* CV017 (A - C) and *C. violaceum* ATCC 12472 (D - F) at various concentrations (200 µg/ml – 1000 µg/ml).

Fifteen extracts (62.50%) demonstrated QS inhibition activity against short-chain AHL producing *C. subtsugae* CV017 at varying concentrations tested, i.e., 200 - 1000 µg/ml (Figs. 4.3 - 4.4; Suppl. Fig. S4.5). Six extracts (29.16%) demonstrated the desired activity of ≥50% VI with < 40% GI at 200 - 1000 µg/ml, Ac_A18, Ac_ E19, Ac_ G37, Ac_ L5, Ac_ L15 and Ac_L39. These extracts demonstrated dose-dependent activity and treatments were statistically significant ($p \leq 0.05$). Four extracts, Ac_A20, Ac_G56, Ac_ L27, Ac_L43 demonstrated inhibition activity at 400 - 1000 µg/ml. Three extracts, Ac_G57, Ac_M40, Ac_ S14 demonstrated activity at 600 - 1000 µg/ml with nine extracts (Ac_A18, Ac_E5, Ac_E13, Ac_E14, Ac_E17, Ac_L12, Ac_ L38, Ac_M40) and Ac_T16 demonstrating ≥50% VI with >40% GI (bactericidal activity) at 1000 µg/ml (Figs. 4.3 - 4.4; Suppl. Fig. S4.4).

Fifteen extracts (62.50%) demonstrated QS inhibition activity against long-chain AHL-producing *C. violaceum* ATCC 12472 at varying concentrations tested, i.e., 200 - 1000 µg/ml (Figs. 4.3 - 4.4; Suppl. Fig. S4.4). The seven best performing extracts were Ac_A18, Ac_A20, Ac_G37, Ac_G56, Ac_G57, Ac_L5, and Ac_L15, which demonstrated dose-dependent activity at 200 - 1000 µg/ml and treatments were statistically significant ($p \leq 0.05$). Two extracts, Ac_L8 and Ac_L27, demonstrated inhibition activity at 400 - 1000 µg/ml. One extract demonstrated activity at 600 - 1000 µg/ml, three extracts (Ac_L39, Ac_S14 and Ac_T15) demonstrated activity at 800 - 1000 µg/ml, while two extracts, Ac_L43 and Ac_T20, demonstrated activity at 1000 µg/ml. Only a single extract (Ac_M40) demonstrated bactericidal activity at 1000 µg/ml (Figs. 4.3 - 4.4; Suppl. Fig. S4.4).

Thirteen extracts (54.16%; Ac_A18, Ac_A20, Ac_G37, Ac_G56, Ac_G57, Ac_L5, Ac_L8, Ac_L15, Ac_L27, Ac_L39, Ac_L43, Ac_S14 and Ac_ T15) showed broad spectrum QS inhibition activity against both *C. subtsugae* CV017 and *C. violaceum* ATCC 12472. These extracts were effective at varying concentrations for both biosensors, i.e., 200 -1000 µg/ml (Figs. 4.3 - 4.4; Suppl. Fig. S4.4). Four extracts, i.e., *S. globisporus* Ac_A18, *B. velezensis* Ac_G37, *P. piscinae* Ac_L5 and *B. aerophilus* Ac_L15 were effective at all concentrations tested, i.e., 200 - 1000 µg/ml for both biosensors and treatments were statistically significant ($p \leq 0.05$). *Bacillus* extracts demonstrated better activity compared to *Phaeobacter* and *Streptomyces* extracts, as 60% (6/10) of the *Bacillus* extracts demonstrated activity against both biosensors (Figs. 4.3 - 4.4; Suppl. Fig. S4.4). *Phaeobacter* extracts were more effective against short chain AHLs produced by *C. subtsugae* CV017 compared to long chain AHLs produced by *C. violaceum* ATCC 12472. *Streptomyces* extracts, however, were more effective against long chain AHLs than short chain AHLs (Figs. 4.2 - 4.4; Suppl. Fig. S4.4).

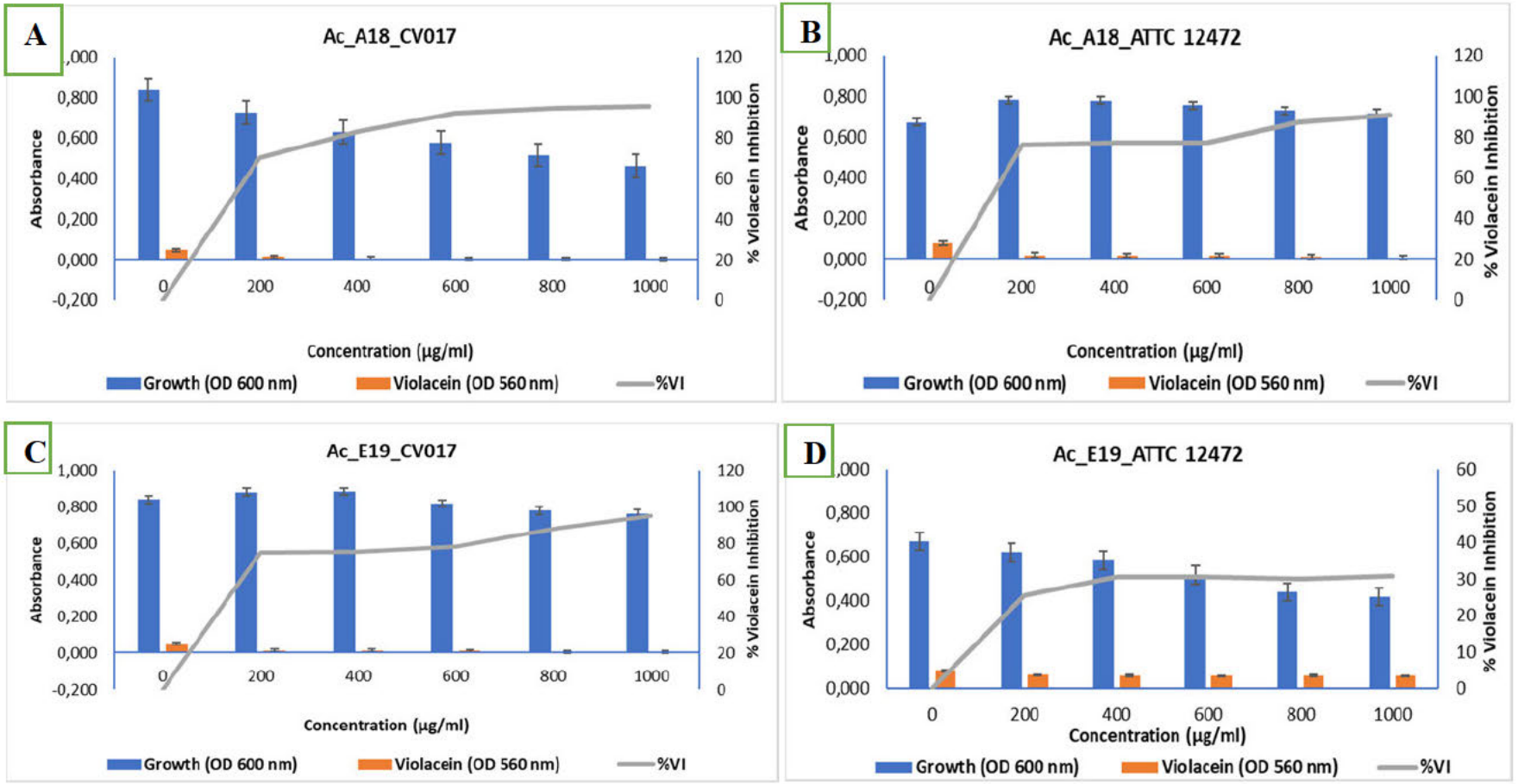


Figure 4.3: Quantitative assessment of violacein production inhibitory effects of strain Ac_A18 extract against *Chromobacterium subtsugae* CV017 (A), *Chromobacterium violaceum* 12472 (B), and strain Ac_E19 extract against *C. subtsugae* CV017 (C) and *C. violaceum* ATCC 12472 (D). Bacterial growth measured at $OD_{600\ nm}$ and violacein production at OD_{560} nm are indicated as bar graphs, while the percentage violacein inhibition (%VI) is indicated by the line graph. Data represent the mean of two independent experiments performed in triplicate.

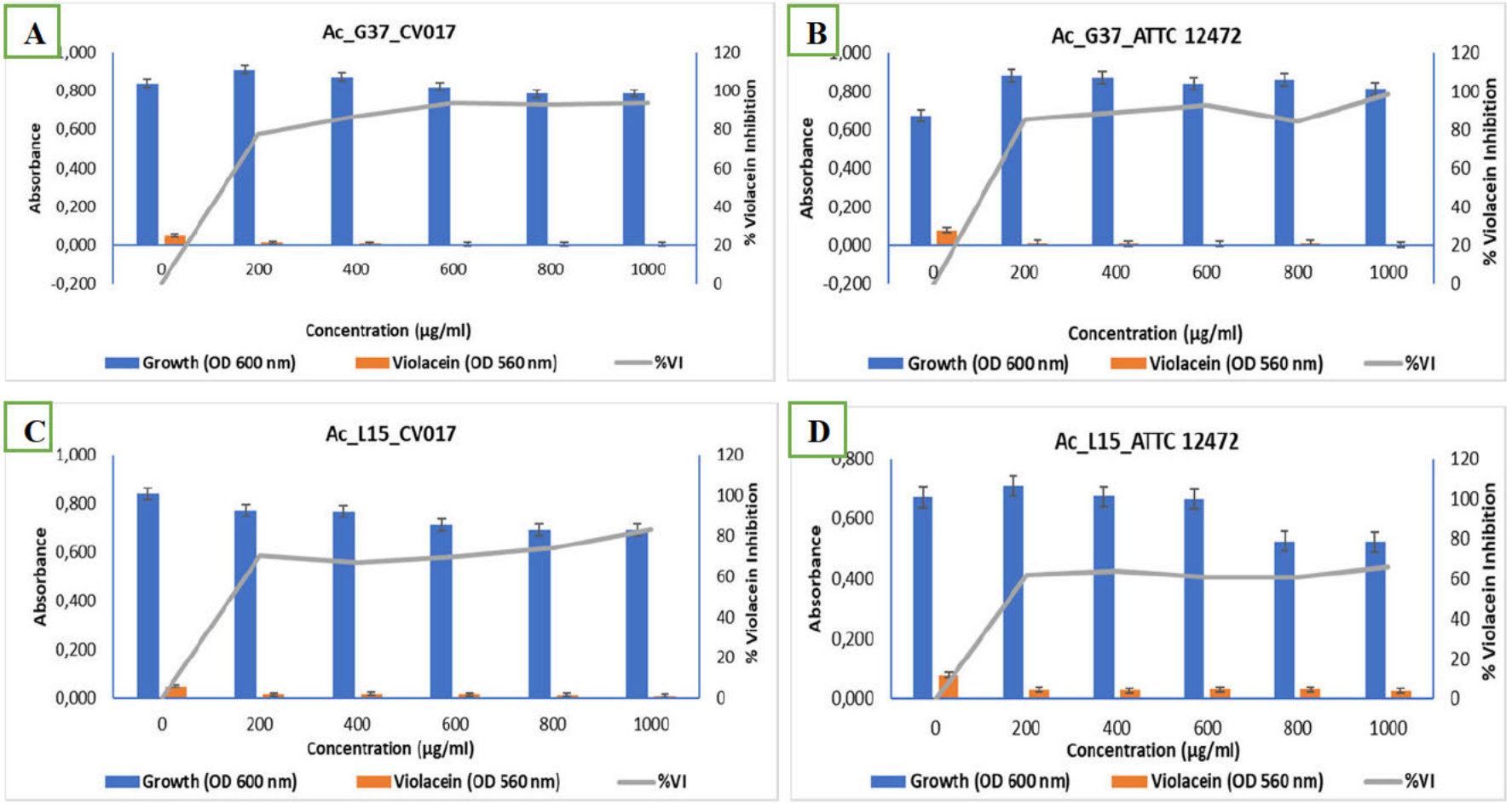


Figure 4.4: Quantitative assessment of violacein production inhibitory effects of strain Ac_G37 extract against *Chromobacterium subtsugae* 017 (A) and *Chromobacterium violaceum* 12472 (B), and strain Ac_L15 extract against *C. subtsugae* CV017 (C) and *C. violaceum* ATCC 12472 (D). Bacterial growth measured at $OD_{600\ nm}$ and violacein production at OD_{560} nm are indicated as bar graphs, while the percentage violacein inhibition (%VI) is indicated by the line graph. Data represent the mean of two independent experiments performed in triplicate.

4.3.5 Qualitative autoinducer-2 inhibition

Acropora CAB extracts were assessed for autoinducer-2 inhibition using the bioluminescent marine strain *V. harveyi* BB120. Growth inhibition was denoted by the presence of dark zones, lacking bioluminescence and growth around the discs (Fig. 4.5; Table 4.7; Suppl. Fig. S4.5). Inhibitory activity was demonstrated by 25% (6/24; Ac_A18, Ac_E19, Ac_L12, Ac_L15, Ac_L38 and Ac_S14) of the extracts against *V. harveyi* BB120. These extracts exhibited weak activity (≤10 mm) at 200 - 800 µg, with a single extract demonstrating intermediate activity at 800 µg. No killing activity was observed with any of the extracts (Fig. 4.5; Table 4.7; Suppl. Fig. S4.5).

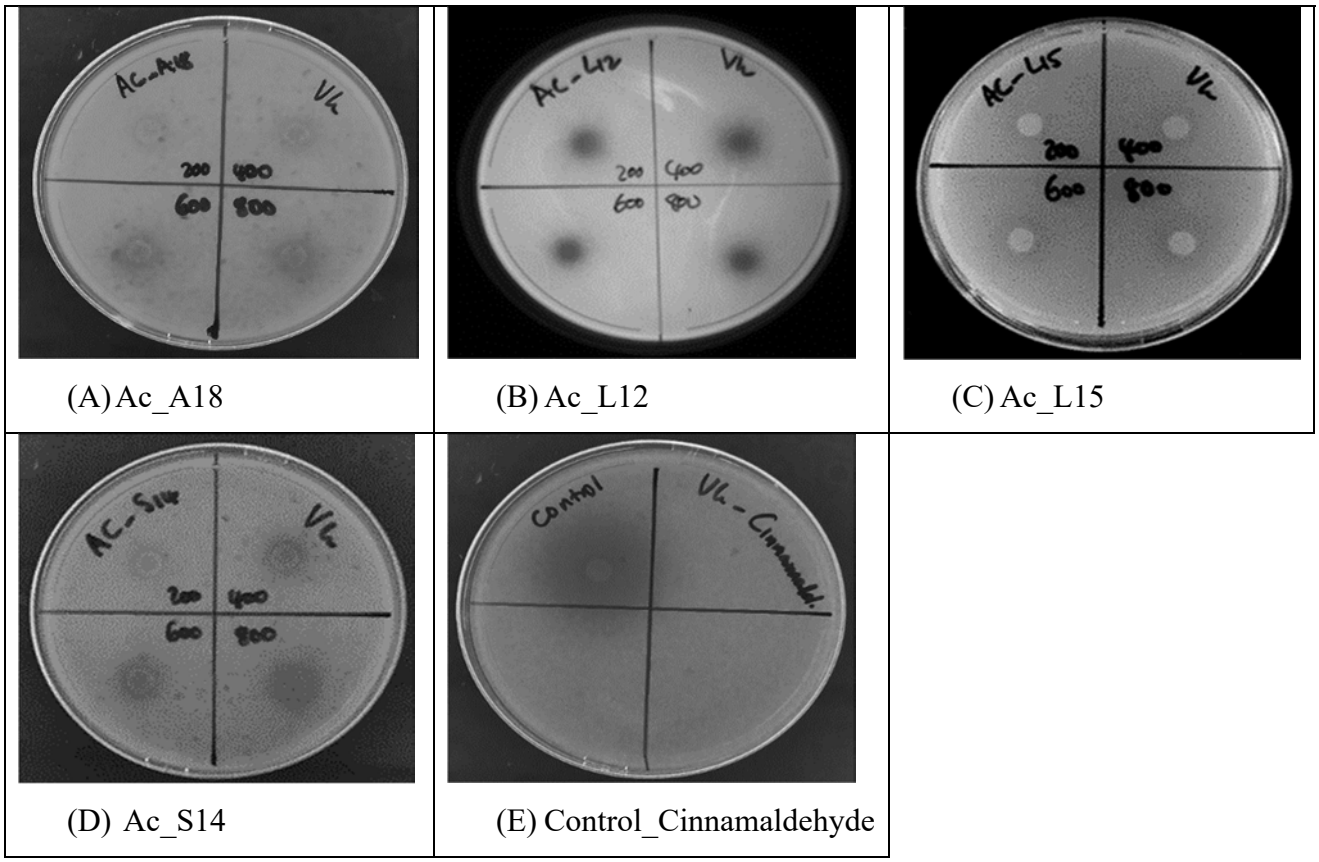


Figure 4.5: Autoinducer-2 inhibition of selected *Acropora* CAB extracts against marine bacterium *Vibrio harveyi* BB120. AI-2 inhibition was indicated by the appearance of dark zones and lack of bioluminescence around the discs (A) Ac_A18 (B) Ac_L12 (C) Ac_L15 (D) Ac_S14 and (E) Control - Cinnamaldehyde.

Table 4.7: Qualitative autoinducer-2 inhibitory potential (opaque zones) demonstrated by the *Acropora* CAB extracts against marine pathogen *Vibrio harveyi* BB120 using agar-overlay assays.

Extract	*Vibrio harveyi* BB120											
	Zone diameter (mm)				Clear zone (mm)				QSI zone (mm)			
	200 µg	400 µg	600 µg	800 µg	200 µg	400 µg	600 µg	800 µg	200 µg	400 µg	600 µg	800 µg
Ac_A18	0	0	9	10	0	0	0	0	0	0	9	10
Ac_A20	0	0	0	0	0	0	0	0	0	0	0	0
Ac_E5	0	0	0	0	0	0	0	0	0	0	0	0
Ac_E13	0	0	0	0	0	0	0	0	0	0	0	0
Ac_E14	0	0	0	0	0	0	0	0	0	0	0	0
Ac_E17	0	0	0	0	0	0	0	0	0	0	0	0
Ac_E19	8	9	10	10	0	0	0	0	8	9	10	10
Ac_G37	0	0	0	0	0	0	0	0	0	0	0	0
Ac_G56	0	0	0	0	0	0	0	0	0	0	0	0
Ac_G57	0	0	0	0	0	0	0	0	0	0	0	0
Ac_L5	0	0	0	0	0	0	0	0	0	0	0	0
Ac_L8	0	0	0	0	0	0	0	0	0	0	0	0
Ac_L12	0	8	8	8	0	0	0	0	0	8	8	8
Ac_L15	0	8	8	8	0	0	0	0	0	8	8	8
Ac_L27	0	0	0	0	0	0	0	0	0	0	0	0
Ac_L38	0	8	8	8	0	0	0	0	0	8	8	8
Ac_L39	0	0	0	0	0	0	0	0	0	0	0	0
Ac_L41	0	0	0	0	0	0	0	0	0	0	0	0
Ac_L43	0	0	0	0	0	0	0	0	0	0	0	0
Ac_M40	0	0	0	0	0	0	0	0	0	0	0	0
Ac_S14	8	9	9	12	0	0	0	0	8	9	9	12
Ac_T15	0	0	0	0	0	0	0	0	0	0	0	0
Ac_T16	0	0	0	0	0	0	0	0	0	0	0	0
Ac_T20	0	0	0	0	0	0	0	0	0	0	0	0
Cinnamaldehyde (10 µg/ml)	25				0				25			

* **Color codes**:
*Yellow – selected extracts showing AI-2 activity
*Blue – extracts showing no AI-2 activity
*AI-2 inhibition zone = Total zone diameter – Clear zone

4.3.6 Detection of anti-biofilm activity of coral-associated bacterial extracts

Selected *Acropora* CAB extracts (n=24) were assessed for their potential to disrupt initial adhesion and mature biofilm using the crystal violet microtitre plate assay. Extracts were screened against clinical indicators *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 43300 as well as marine indicators *S. putrefaciens* ATCC 8071, *V. coralliilyticus* ATCC_BAA 450, *V. parahaemolyticus* ATCC 17802 and *V. shilonii* ATCC_BAA 91 (Figs. 4.6 - 4.9; Suppl. Tables S4.1 - S4.6). Biofilm reduction (BFR) was defined as extracts with ≥50% BFR with <40% growth inhibition, while antimicrobial activity was defined as extracts with ≥40% GI.

When initial adhesion inhibition was observed against *P. aeruginosa*, 29.16% (7/24) of the extracts demonstrated the desired BFR activity (Fig. 4.6A; Suppl. Table S4.1). Two extracts, i.e., *B. altitudinis* Ac_E5 and *L. marina* Ac_E13 demonstrated BFR activity at 200 - 800 µg/ml. A single *P. gallaeciensis* Ac_L12 extract demonstrated activity at 200 - 400 µg/ml and two extracts, *B. altitudinis* Ac_L8 and *B. altitudinis* Ac_L27 demonstrated initial adhesion at 600 - 800 µg/ml. The *S. violascens* Ac_S14 extract demonstrated activity at 800 µg/ml. Four extracts (16.66%; *B. altitudinis* Ac_A20, *P. gallaeciensis* Ac_E19, *P. piscinae* Ac_L5, and *B. aerophilus* Ac_L15 demonstrated bactericidal activity at varying exposures, i.e., 200 - 800 µg/ml. Two of these extracts (*B. altitudinis* Ac_A20 and *P. gallaeciensis* Ac_E19) demonstrated bactericidal activity across all concentrations tested, with the *B. aerophilus* Ac_L15) extract demonstrating bactericidal activity at 400 - 800 µg/ml and the *P. piscinae* Ac_L5 extract demonstrating activity at 800 µg/ml.

Against *P. aeruginosa* mature biofilms, only 16.66% (4/24) of the extracts demonstrated BFR activity (Fig. 4.6B; Suppl. Table S4.1). The *B. altitudinis* extracts Ac_L8 and Ac_L27 demonstrated activity at 200 - 800 µg/ml. The *S. violascens* extract Ac_S14 demonstrated activity at 400 - 800 µg/ml while the *P. gallaeciensis* Ac_L12 extract reduced mature biofilm at the highest concentration, 800 µg/ml. Only a single *B. aerophilus* Ac_L15 extract demonstrated bactericidal activity across all concentrations, i.e., 200 - 800 µg/ml (Fig. 4.6B; Suppl. Table S4.1). Extracts demonstrating initial adhesion inhibition and/or mature biofilm reduction belonged to the genera *Bacillus, Labrenzia, Phaeobacter* and *Streptomyces.*

Initial adhesion inhibition by extracts against *S. aureus* was demonstrated by 12.5% (3/24; Ac_E5; Ac_E13, Ac_L15) of extracts (Fig. 4.6C; Suppl. Table S4.2). The *L. marina* E13 extract demonstrated activity at 600 - 800 µg/ml and *B. altitudinis* Ac_E5 and *B. aerophilus* Ac_L15 extracts demonstrated activity at the highest concentration, 800 µg/ml. Only two extracts demonstrated bactericidal activity, i.e., *B. altitudinis* Ac_A20 demonstrating activity

across all concentrations i.e., 200 - 800 µg/ml and *P. gallaeciensis* Ac_E19 extract demonstrated activity at 600 - 800 µg/ml (Fig. 4.6; Suppl. Table S4.2).

For *S. aureus* mature biofilms, 58.33% (14/24; Ac_E5, Ac_L5, Ac_L8, Ac_L12, Ac_L27, Ac_L38, Ac_L39, Ac_L41, Ac_L43, Ac_M40, Ac_S14, Ac_T15, Ac_T16 and Ac_T20) of the extracts demonstrated good BFR activity across varying concentrations, i.e., 200 - 800 µg/ml (Fig. 4.6D; Suppl. Table S4.2). Ten (41.66%) of these extracts demonstrated BFR at 200 - 800 µg/ml. *B. altitudinis* Ac_E5 and *R. atlantica* Ac_L38 extracts demonstrated activity at 400 - 800 µg/ml, while the *P. piscinae* Ac_L5 extract demonstrated biofilm reduction at 600 - 800 µg/ml. The *B. pumilus* Ac_T20 extract demonstrated activity at the highest concentration, 800 µg/ml. The *B. aerophilus* Ac_L15 extract demonstrated activity across all concentrations, tested, i.e., 200 - 800 µg/ml, and these treatments were statistically significant ($p \leq 0.05$) (Fig. 4.6D; Suppl. Table S4.2). Extracts demonstrated better inhibition activity against *S. aureus* mature biofilms compared to *P. aeruginosa*, where better activity initial adhesion inhibition was observed.

Against *S. putrefaciens*, the initial adhesion activity was predominantly bactericidal with no %BFR ≥50% and <40% GI exhibited by any of the extracts (Fig. 4.7A; Suppl. Table S4.3). Nine extracts (37.5%) demonstrated antimicrobial activity at varying concentrations, i.e., 200 - 800 µg/ml. Against mature *S. putrefaciens* biofilms, 20.83% (5/24; Ac_L8, Ac_L15, Ac_S14, Ac_T15 and Ac_T20) of the extracts demonstrated BFR activity at varying concentrations. A single *S. violascens* Ac_S14 extract demonstrated activity across all concentrations, i.e., 200 - 800 µg/ml and was statistically significant ($p \leq 0.05$). Two extracts, *B. aerophilus* Ac_L15 and *B. pumilus* Ac_T20 demonstrated activity at 400 - 800 µg/ml, with *B. aerophilus* Ac_L15 demonstrating bactericidal activity at 800 µg/ml. Two extracts, *B. altitudinis* Ac_L8 and *B. pumilus* Ac_T15 demonstrated biofilm inhibition at the highest concentration, 800 µg/ml (Fig. 4.7B; Suppl. Table S4.3).

Against marine pathogen *V. coralliilyticus*, 41.66% (10/24; Ac_L15, Ac_L27, Ac_L38, Ac_39, Ac_L41, Ac_L43, Ac_M40, Ac_T15, Ac_T16, and Ac_T20) of the extracts demonstrated initial adhesion inhibition at various concentrations, with 50% (12/24) of the extracts predominantly demonstrating antimicrobial activity at 200 - 800 µg/ml. The *B. aerophilus* Ac_L15 extract demonstrated activity at 200 µg/ml, while the *S. pasteurii* Ac_L39 and *S. pratensis* Ac_L41 extracts demonstrated activity at higher concentration, 800 µg/ml. Two extracts, *M. variabilis* Ac_L43 and *B. pumilus* Ac_T16 demonstrated activity at 600 µg/ml (Fig. 4.7C; Suppl. Table S4.4). For mature biofilms, 8.33% (2/24; Ac_E5, Ac_L27) of the extracts demonstrated BFR activity at 400 µg/ml. Twenty-two (91.66%) of the extracts

demonstrated bactericidal activity (Fig. 4.7D; Suppl. Table S4.4). None of the extracts demonstrated activity against both initial adhesion and mature biofilm inhibition (Fig. 4.7D; Suppl. Table S4.4).

Initial adhesion inhibition was demonstrated by 41.66% (10/24; Ac_E5, Ac_E13, Ac_E17, Ac_E19, Ac_G37, Ac_G56, Ac_G57, Ac_L5, Ac_L8, and Ac_S14) of the extracts against *V. parahaemolyticus* at varying concentrations. A single *P. gallaeciensis* Ac_E19 extract demonstrated good BFR activity at 200 µg/ml (Fig. 4.8A; Suppl. Table S4.5). Two extracts, (8.33%; *E. nanhaisediminis* Ac_G57 and *P. piscinae* Ac_L5), demonstrated activity at 400 - 800 µg/ml, with Ac_L5 demonstrating bactericidal activity at the highest concentration. Four extracts (16.66%; Ac_E13, Ac_G37, Ac_G56 and Ac_L8) demonstrated activity at 600 - 800 µg/ml, while three extracts (12.5%; Ac_E5, Ac_E17 and Ac_S14) demonstrated activity at 800 µg/ml. Only two extracts, (8.33%; Ac_A20 and Ac_T15) demonstrated bactericidal activity only. Against *V. parahaemolyticus* mature biofilms, no BFR activity was demonstrated by any of the extracts, with a single extract (Ac_T20) demonstrating bactericidal activity at 800 µg/ml (Fig. 4.8B; Suppl. Table S4.5).

Against *V. shilonii*, 12.5% of the extracts (3/24; Ac_G37, Ac_L27 and Ac_T20) demonstrated initial adhesion activity (Fig. 4.8C; Suppl. Table S4.6). Two extracts, Ac_G37 and Ac_L27, demonstrated good BFR activity at 200 µg/ml and a single extract, Ac_T20 demonstrated activity at 400 µg/ml. Bactericidal activity was demonstrated by 87.5% (21/24) of the extracts (Fig. 4.8C; Suppl. Table S4.6). For mature *V. shilonii* biofilms, 29.16% (7/24; Ac_E17, Ac_E19, Ac_L12, Ac_L27, Ac_L41, Ac_L43, and Ac_T20) of the extracts demonstrated good BFR activity at 200 - 400 µg/ml. Seventeen (70.83%) of the extracts demonstrated bactericidal activity at varying concentrations, i.e., 200 - 800 µg/ml (Fig. 4.8D; Suppl. Table S4.6).

At initial adhesion, extracts demonstrated inhibition activity against five indicators, *P. aeruginosa, S. aureus, V. coralliilyticus, V. parahaemolyticus* and *V. shilonii* (Fig. 4.6 - 4.8; Suppl. Tables S4.1 - S4.6). Three extracts, Ac_E5, Ac_E13 and Ac_L27 demonstrated initial adhesion inhibition against three indicators. Six extracts, Ac_E17, Ac_G37, Ac_L8, Ac_L15, Ac_S14 and Ac_T20, demonstrated inhibitory activity against two indicators. For mature biofilm inhibition, extracts demonstrated inhibition activity against five indicators, *P. aeruginosa, S. aureus, S. putrefaciens, V. coralliilyticus,* and *V. shilonii* (Fig. 4.6 - 4.8; Suppl. Tables S4.1 - S4.6). A single extract, Ac_L27 demonstrated inhibition activity against four indicators. Three extracts, Ac_L8, Ac_L12 and Ac_T20 demonstrated inhibition against three indicators. Five extracts, Ac_E5, Ac_L41, Ac_L43, Ac_S14 and Ac_T15, demonstrated

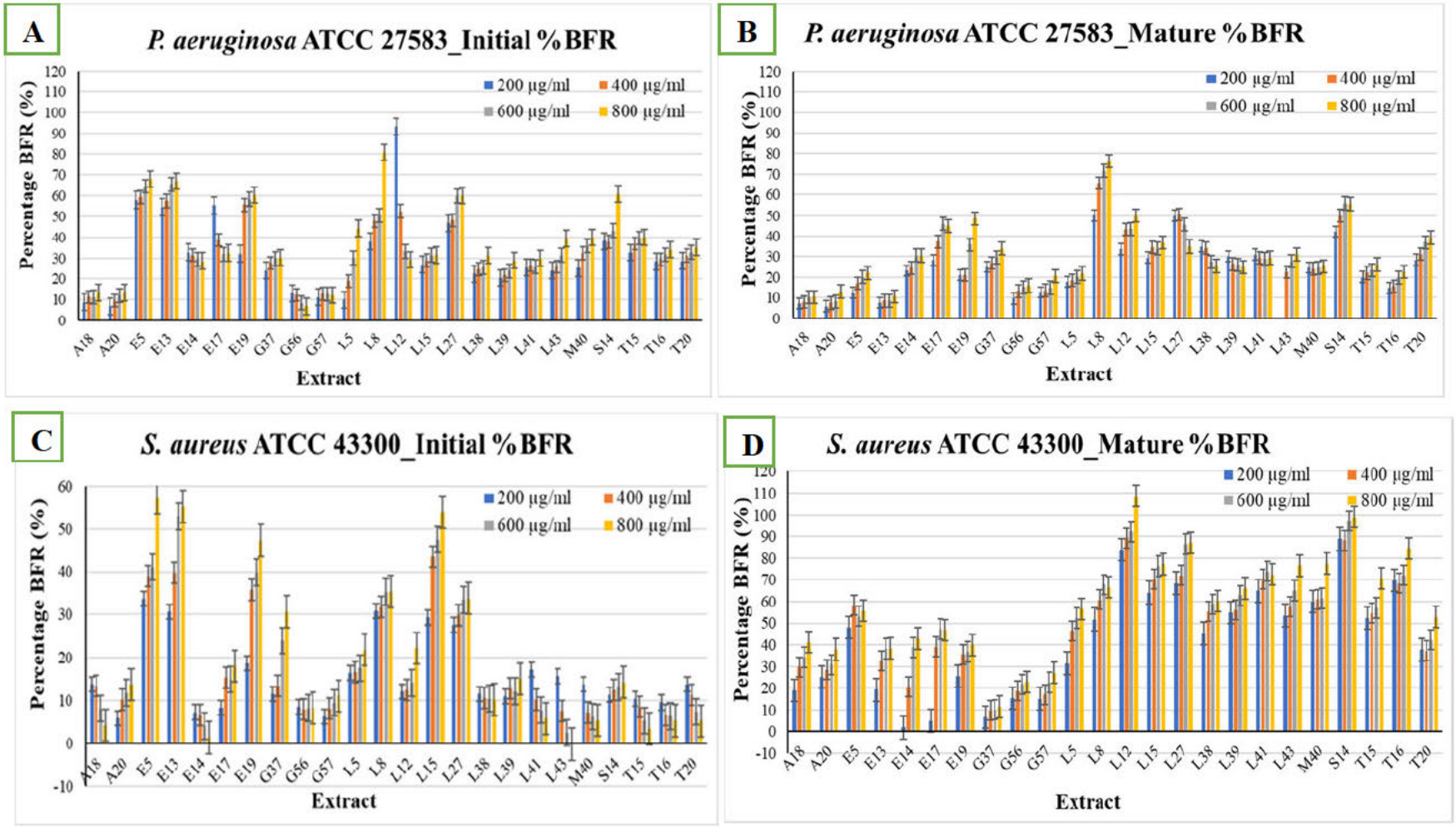


Figure 4.6: Percent biofilm reduction of *Acropora* CAB extracts on initial adhesion and mature biofilm of clinical indicators *Pseudomonas aeruginosa* ATCC 27853 (A-B) and *Staphylococcus aureus* (C-D), as quantified by the crystal violet staining in microtitre plate assay. Mean values of two replicates are shown. Differences in mean values of 200 - 800 µg of extracts were statistically significant ($p \leq 0.05$) based on indifferences in values.

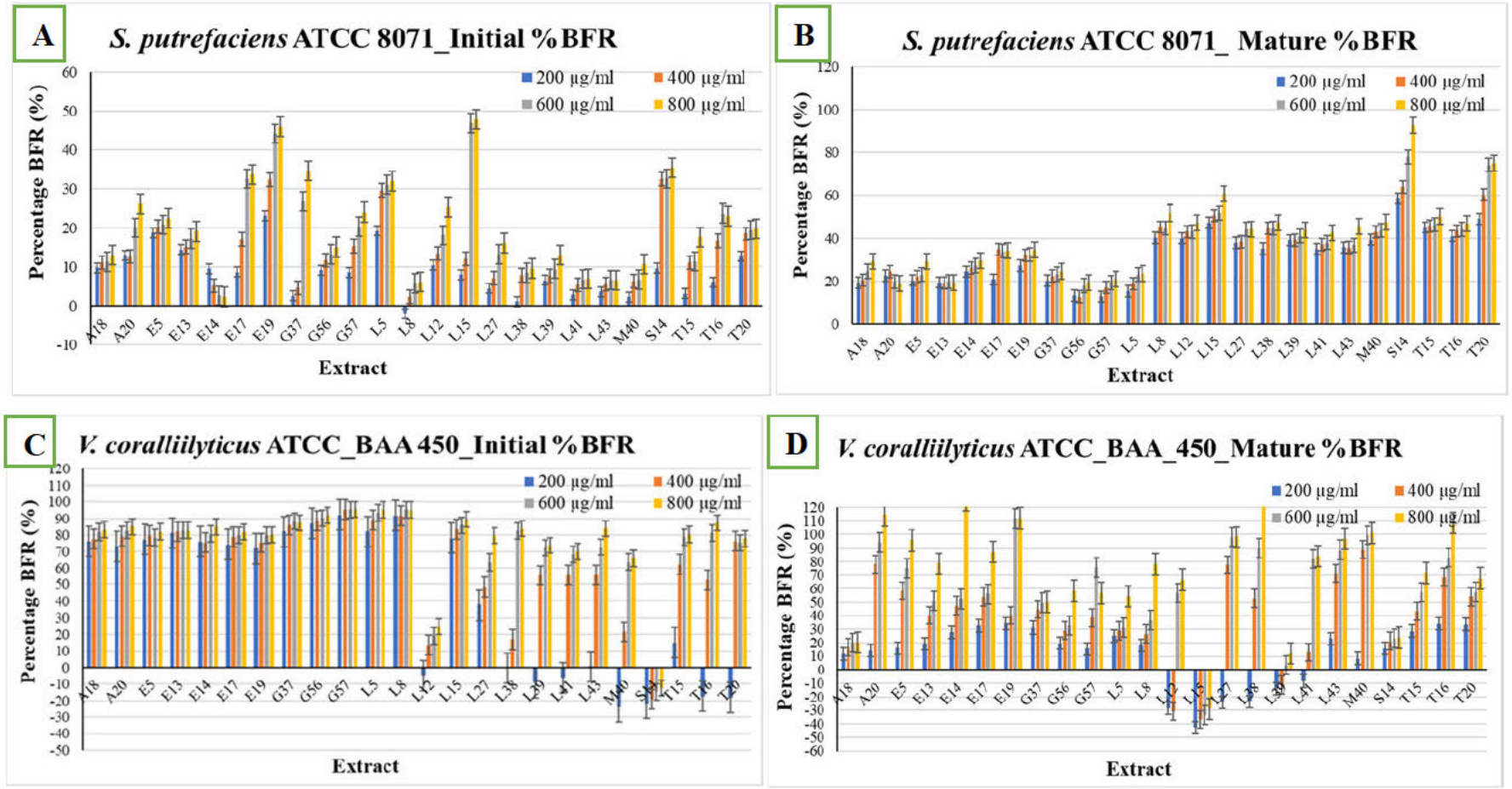


Figure 4.7: Percent biofilm reduction of *Acropora* CAB on initial adhesion and mature biofilm of clinical indicators *Shewanella putrefaciens* ATCC 8071 (A-B) and *Vibrio coralliilyticus* ATCC_BAA 450 (C-D), as quantified by the crystal violet staining in microtitre plate assay. Mean values of two replicates are shown. Differences in mean values of 200 - 800 µg of extracts were statistically significant ($p \leq 0.05$) based on indifferenced in values.

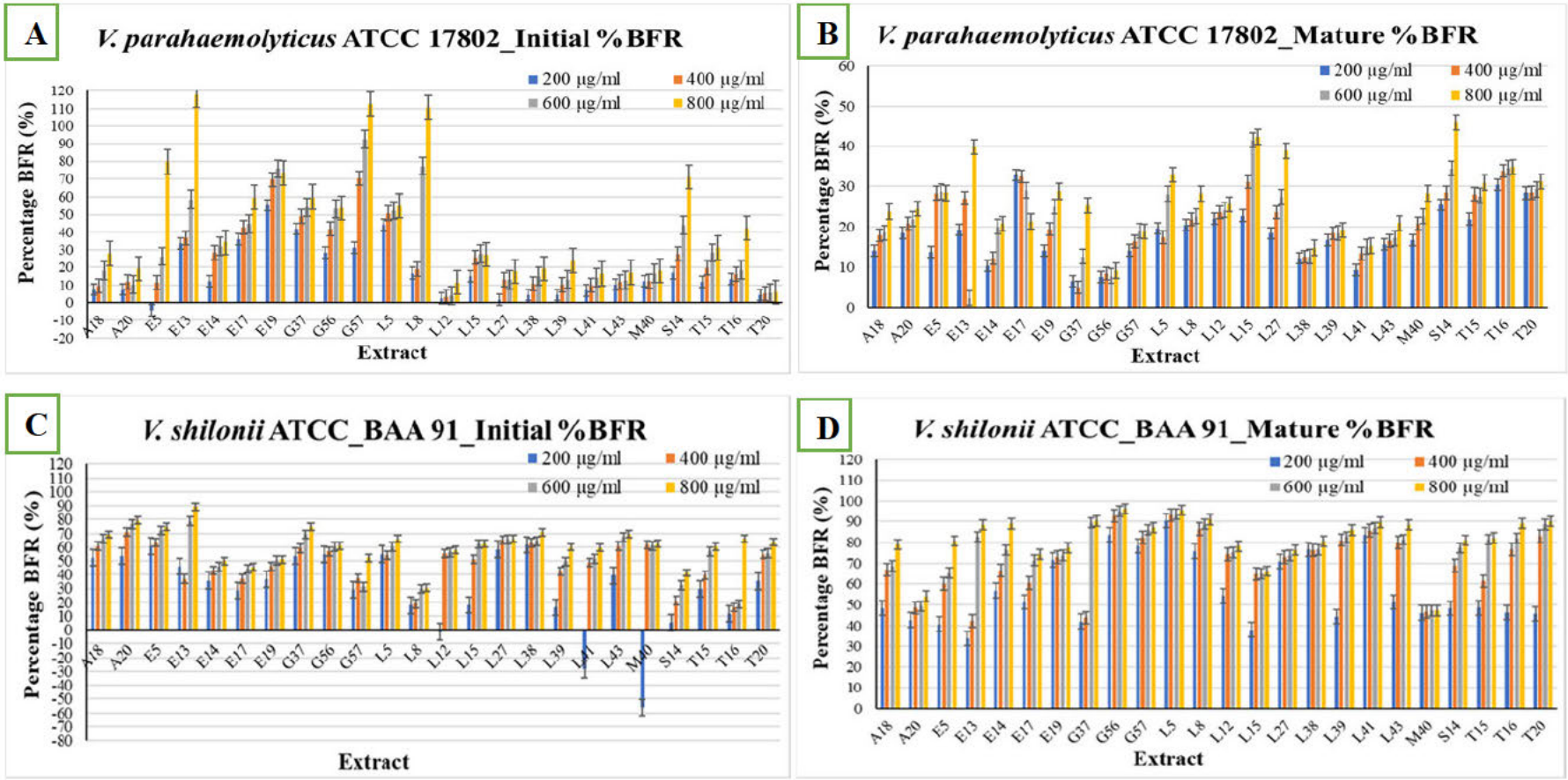


Figure 4.8: Percent biofilm reduction of *Acropora* CAB extracts on initial adhesion and mature biofilm of clinical indicators *Vibrio parahaemolyticus* ATCC 17802 (A-B) and *Vibrio shilonii* ATCC_BAA 91 (C-D), as quantified by the crystal violet staining in microtitre plate assay. Mean values of two replicates are shown. Differences in mean values of 200 - 800 µg of extracts were statistically significant ($p \leq 0.05$) based on indifferences in values.

inhibitory activity against two indicators. For initial adhesion inhibition and mature biofilm inhibition, the *Bacillus* extracts demonstrated significant ($p \leq 0.05$) activity compared to *Erythrobacter, Labrenzia, Phaeobacter, Ruegeria, Sporosarcina, Streptomyces, Microbulbifer* and *Metabacillus* extracts, although inhibitory activity was also observed by these genera. The *Bacillus* extracts demonstrated inhibition activity against multiple indicators and inhibited both clinical and marine indicators.

4.4 Discussion

The search for new natural compounds in the marine environment especially from marine invertebrates has emerged as a potential solution to the increased resistance of bacterial communities to the existing antibiotics (Garrido *et al*., 2020; Gignoux-Wolfsohn *et al*., 2017; Glasl *et al*., 2019; Marston *et al*., 2016). Bacteria associated with marine invertebrates such corals are suggested to produce natural products that exhibit a variety of bioactivities used naturally by the host as a chemical defence mechanism for protection against pathogens (Kemp *et al*., 2018; Nofiani *et al*., 2020). These compounds are an extraordinary source of new therapeutics with potential applications in the environmental and pharmaceutical fields (Neave *et al*., 2017; Rajasabapathy *et al*., 2020). Under harsh environmental conditions, CAB are suggested to be capable of producing antimicrobial compounds, cell-to-cell communication inhibitors and other compounds that inhibit biofilm formation (Glasl *et al*., 2019; Sang *et al*., 2019). This study aimed to explore the antimicrobial activity, quorum sensing inhibition and anti-biofilm activity of bacteria isolated from an *Acropora* species coral.

Molecular identification of selected *Acropora* CAB isolates (n=24) through 16S RNA gene sequencing identified nine genera (Table 4.1), i.e., *Bacillus* (n=11), *Erythrobacter* (n=2), *Labrenzia* (n=2), *Metabacillus* (n=1), *Microbulbifer* spp. (n=1), *Phaeobacter* (n=3), *Ruegeria* (n=1), *Sporosarcina* (n=1) and *Streptomyces* (n=3). Both Gram-positive and Gram-negative bacteria were represented, however, *Bacillus* species were more dominant in the initial screening assays which is in line with previous studies due to their production of AiiA lactonase for enzyme degradation and their production of a wide variety of compounds with bioactivity (Ma *et al*., 2018; Raina *et al.,* 2016; Teasdale *et al.,* 2011) and because *Bacillus* species are reported to be the most abundant groups of cultivable bacteria isolated from corals (Pereira *et al*., 2017).

The selected *Acropora* CAB extracts were assessed for antimicrobial activity against clinical pathogens, i.e., multi-resistant drug *P. aeruginosa* ATCC 27853 and methicillin-resistant *S. aureus* ATCC 43300. Extracts demonstrated antimicrobial activity against both

clinical pathogens, ranging from weak (≤10 mm), intermediate (11 - 15 mm) and strong activities (≥15 mm) (Table 4.2; Suppl. Fig. S4.2). All extracts demonstrated no-to-weak activity for both clinical pathogens, except for the *B. altitudinis* Ac_A20 extract demonstrating intermediate and strong activity against both *P. aeruginosa* and *S. aureus*, and the *B. aerophilus* Ac_L15 extract which demonstrated intermediate and strong activity against *S. aureus* only (Table 4.2; Suppl. Fig. S4.2). The dominant antimicrobial activity by *Bacillus* species shows broad spectrum activity against clinical pathogens. This is in line with findings of Abriouel *et al.* (2011) who reported that *Bacillus* strains produce more than a dozen antibiotics that are structurally and functionally different depending on the niche inhabited. *Bacillus* isolates are also suggested to produce a wide range of structurally different compounds that deal with multidrug resistance issues (Ramachandran *et al.,* 2014). Ramachandran *et al.* (2014) further reported the broad-spectrum antimicrobial ability of a *Bacillus* strain against clinical strains such as *E. coli* and *S. aureus*. ElAhwany *et al.* (2015) reported the antimicrobial activity of 20 CAB isolates tested against four pathogens, *S. aureus, K. pneumoniae, P. aeruginosa* and *Vibrio fluvialis*. Bacterial isolates from *Bacillus* and *Pseudomonas* genera demonstrated the highest antimicrobial activity. Pham *et al.* (2016) assessed bacterial diversity of the coral *Alcyonium digitatum* for antimicrobial activity. These were tested against four microorganisms, i.e., *B. subtilis, Staphylococcus epidermidis, E. coli* and *Candida albicans*. *Bacillus* species were the most abundant, with *Bacillus methylotrophicus* and *B. amyloliquefaciens* exhibiting activity against three of the four test isolates.

When extracts were targeted against marine indicators, no antimicrobial activity was observed against *S. putrefaciens* and *V. parahaemolyticus,* but was observed against *V. alginolyticus, V. coralliilyticus*, and *V. shilonii*. *Bacillus* extracts exhibited intermediate and strong activity against the three *Vibrio* species with the *E. nanhaisediminis* (Ac_G56) extract demonstrating intermediate activity against *V. alginolyticus* (Tables 4.3 - 4.4; Suppl. Fig. S4.2). This is line with Rypien *et al*. (2010) who reported *Vibrio* species to exhibit antimicrobial resistance to a wide range of antibiotics and CAB, which is the reason only four (44.44% (4/9)) *Bacillus* spp. and one *Erythrobacter* species demonstrated activity (Table 4.3 - 4.4). Vizcaino *et al.* (2010) screened several *Erythrobacter* isolates and demonstrated that they could effectively inhibit *V. coralliilyticus.* Furthermore, several other bacteria isolated from coral mucus including *Bacillus* strains have been reported to inhibit a variety of pathogens such as *E. coli, S. aureus* and *V. parahaemolyticus* (Vizcaino *et al.*, 2010). Antimicrobial results of the current study agree with Pereira *et al.* (2017) who explored the antimicrobial activity of CAB isolated from most isolates that exhibited activity belonged to the genus *Bacillus.* These studies

support the hypothesis that CAB have a protective role and may be a promising source of antimicrobial compounds.

The interaction between microbial communities is mediated by QS signalling molecules which play an important role in shaping these communities (Zhou *et al.,* 2020). Quorum sensing molecules of bacterial genera commonly found in corals have been reported, including production by *Roseobacter, Vibrio* and *Pseudoalteromonas* specied Quorum sensing in these bacteria is responsible for the regulation of gene expression, motility, antimicrobial compound production and for formation of biofilms (Papenfort and Bassler, 2016; Tang *et al*., 2019). Several studies have reported that QS systems control production of virulence factors in some opportunistic pathogens such as *P. aeruginosa, S. aureus, S. marcescens* and *Vibrio* species (El-Kurdi *et al*., 2021; Lami, 2019; Ma *et al.,* 2018). Therefore, screening for anti-QS compounds is vital to combat pathogenesis (Zhao *et al*., 2015).

The anti-QS ability of selected bacterial isolates was assessed qualitatively against biosensors *C. subtsugae* CV017 and *C. violaceum* ATCC 12472, using an agar-overlay assay while violacein inhibition was quantified using the violacein inhibition assay. Extracts with activity against both *C. subtsugae* CV017 and *C. violaceum* ATCC 12472 extracts were identified, while some demonstrated no inhibition activity against both biosensors (Fig. 4.1; Suppl. Fig. S4.3; Table 4.5 - 4.6). More QS inhibition was observed against *C. subtsugae* CV017 (29.16%) compared to *C. violaceum* ATCC 12472 where 25% of the extracts inhibited QS at 200 - 1000 µg/ml. Three genera were dominant in activity, i.e., *Bacillus* sp. strains Ac_A20, Ac_L8 and Ac_L15, *Streptomyces* sp. strains Ac_A18 and Ac_S14, and *Erythrobacter* sp. strains Ac_G56 and Ac_G57. Thirteen extracts demonstrated broad-spectrum activity, with four extracts Ac_A18, Ac_G37, Ac_L5, and Ac_L15 demonstrating inhibition of both biosensors at 200 µg/ml. These extracts were dose-dependent ($p \leq 0.05$). The best inhibitory activity was observed with the *B*. *velezensis* Ac_G37 extract as it had the highest %VI at the lowest concentration (200 µg/ml) with <40% GI against both biosensors. The *Microbulbifer* and *Sporosarcina* extracts demonstrated QS inhibition against both biosensors at varying concentrations. Studies on QS inhibition by these species are limited, however, Dow (2021) investigated QS interference in macrooalgae and reported QS detoxification by *Microbulbifer* sp. Strain H211 which does not usually carry out quinolone-mediated QS but was able to detoxify alkylquinolones. Ma *et al*. (2018) screened CAB from *Pocillopora damicormis* and among them was the *Staphylococcus hominis* D11 strain which demonstrated significant QS inhibition activity. Song *et al.* (2018) also reported that *V. alginolyticus* H12

isolated from *P. damicormis* demonstrated QS activity against *P. aeruginosa*, however, it did not affect its growth. El-Kurdi *et al.* (2020) screened CAB strains from the genera *Bacillus, Enterobacter* and *Norcodiopsis.* These were tested against three pathogenic bacteria *Aeromonas hydrophila, P. aeruginosa* and *V. alginolyticus.* Their results demonstrated that the CAB isolates inhibited the QS activity and biofilm formation of these three pathogenic organisms, respectively. The results of the current study are in line with these studies as some of the CAB extracts demonstrated QS inhibition against the tested biosensors. *Bacillus* extracts were the most dominant and demonstrated good QS inhibition activity compared to other genera.

Certner and Vollmer (2018) examined the role of QS in bacteria associated with white band disease (WBD) infected *Acropora* species. These bacteria were isolated and exposed to QS inhibitors and results demonstrated that WBD-associated bacteria added with a QS inhibitor could not establish diseases. Bacterial species from the *Vibrionaceae* and *Flavobacteriaceae* were strongly inhibited by the QS inhibitor supplemented to WBD- infected corals. This is also in line with Thenmozhi *et al*. (2009) who screened nine CAB extracts for QS inhibition and observed ≥80% violacein inhibition by *B. horikoshi* extract E6.

Acropora CAB bacterial extracts were assessed for autoinducer-2 inhibition using bioluminescent marine strain *V. harveyi* BB120. Inhibition activity was demonstrated by 25% (6/24) at varying concentrations, i.e., 200 - 800 µg, and weak activity (≤ 10 mm) was observed. Results were in line with Zimmer *et al.* (2014) who reported the presence of AHLs in CAB collected from black band disease-infected coral. About 199 bacterial isolates were screened for the production of AHLs and autoinducer-2, with AHLs being detected in 100% of the isolates and 50% tested positive for AI-2. Tait *et al*. (2010) also screened CAB of diseased coral and detected AHLs and AI-2 with 58.62% (17/29) producing AHLs and 100% AI-2. The production of AHLs is detected by the inhibition of AI-2 molecules through the inhibition of AHL-dependent AI-2 reporters which are unable to induce bioluminescence (Zimmer *et al.,* 2014). The results of this study are in line with these studies as some CAB inhibited autoinducer-2 and may suggest that QS inhibition may be an active process in corals.

Biofilm formation has become a major threat in ecological and biotechnological settings. It is a mechanism developed by bacteria to protect themselves from environmental stress and is regulated by QS signalling molecules (Haque *et al*., 2016; Lami, 2019) and controls the production of virulence factors in biofilms against opportunistic bacteria (Lami, 2019). Formation of biofilms occurs on living and non-living surfaces through attachment and rapid bacterial growth, which is the initial stage (Ma *et al*., 2018; Modolon *et al.,* 2020). At this stage,

adhesion is reversible and, therefore, preventing adhesion at this stage can reduce the risk of further biofilm formation. For example, *V. coralliilyticus* use biofilm formation as a virulence factor for successful infection of corals (Kimes, 2012). Gowrishankar *et al*. (2012) have suggested that CAB metabolites are strong candidates for controlling clinical pathogens. Previous studies have reported strategies on inhibiting biofilm formation of microbes without being bactericidal or bacteriostatic (El Samak *et al.,* 2018; El-Kurdi *et al*., 2021). Alagely *et al.* (2011) reported CAB proteobacterium 44B9 and *Marinobacter* sp. 47A11 isolated from *Acropora palmata* to have inhibited biofilm formation by the pathogen *S. marcescens* PDL100. Nithyanand *et al.* (2009) reported biofilm inhibition activity of a *Streptomyces* extract isolated from *Acropora digitifera* to have significantly inhibited biofilm formation of all M serotypes.

Greater initial adhesion inhibition was obtained for *P. aeruginosa*, *V. coralliilyticus* and *V. parahaemolyticus*, while greater mature biofilm reduction was obtained against *S. aureus*, *S. putrefaciens* and *V. shilonii* (Figs. 4.6 – 4.8; Suppl. Tables S4.1 - 4.6). Against *P. aeruginosa*, extracts from the genera *Bacillus, Streptomyces* and *Phaeobacter* demonstrated better activity for both initial adhesion and mature biofilm inhibition. Four extracts from genera *Bacillus* (strain Ac_L8) and (strain Ac_L27)*, Phaeobacter* (strain Ac_L12) and *Streptomyces* (strain Ac_S14) inhibited both initial adhesion and mature biofilm. Against *S. aureus*, extracts from *Bacillus* and *Labrenzia* genera demonstrated better initial adhesion inhibition. For mature biofilms the genera *Bacillus, Phaeobacter, Metabacillus, Microbulbifer, Reugeria, Sporosarcina* and *Streptomyces* exhibited activity. A single *Bacillus* Ac_E5 extract inhibited both initial and mature biofilms. This extract was dose-dependent ($p \leq 0.05$). For mature biofilm inhibition, fourteen extracts demonstrated better inhibition activity. Against both clinical indicators, two extracts from *Bacillus* sp. strain Ac_E5 and *Labrenzia* sp. strain Ac_E13 extracts demonstrated initial adhesion inhibition, while four extracts Ac_L5, Ac_L8, Ac_L27, and Ac_L41 demonstrated reduction of mature biofilms. The *Labrenzia* extract Ac_E13 inhibited initial adhesion of both clinical indicators at 600 - 800 µg and *V. parahaemolyticus*. Bacterial extracts of *Bacillus* and *Phaeobacter* inhibited both initial and mature biofilms compared to other extracts at varying concentrations, i.e., 200 - 800 µg (Suppl. Table S4.1 - S4.2). Against marine indicators, *Bacillus* extracts demonstrated inhibited both initial and mature biofilms compared to other extracts tested at varying concentrations i.e., 200 - 800 µg (Suppl. Table S4.3 - S4.6). *Bacillus* extracts the best activity compared to other genera against *Vibrio* species as it demonstrated to be more effective at varying concentrations. This is in line with data from a study by Gowrishankar *et al*. (2012) who evaluated CAB extracts for anti-biofilm activity and virulence production inhibition. They observed that 22% (2/9) of

the isolates screened demonstrated excellent anti-biofilm activity against *S. aureus,* i.e., *B. firmus* and *V. parahaemolyticus*. The *B. firmus* extract reduced exopolysaccharide production by 79% and the *V. parahaeomolyticus* extract reduced it by 57%, which is a clear indication that *Bacillus* species are a promising source of anti-biofilm compounds against clinical pathogens. El-Kurdi *et al*. (2021) reported anti-biofilm activity of CAB against clinical and marine pathogens *P. aeruginosa, Aeromonas hydrophila* and *V. alginolyticus*. From the extracts tested, extracts from genera *Bacillus* and *Enterobacter* inhibited biofilm formation of these three pathogens by 67%. It was also reported that 91% of the inhibition by these bacteria was due to lactonase activity. Quorum quenching of *P. aeruginosa* by bacteria isolated from *A. digitifera* was observed as CAB inhibited biofilm formation and production of enzymes such as protease and elastase (Bakkiyaraj *et al.,* 2013), as well as demonstrating 98% QS inhibition of *C. violaceum* ATCC 12472. *Streptomyces* species are also major producers of biomolecules that exhibit a wide range of bioactivities (Ramaligam *et al*., 2019). El-Kurdi *et al*. (2020) screened CAB including *Streptomyces* species which demonstrated to produce QSI molecules with a strong ability to inhibit biofilm formation in *A. hydrophila*. Their study further reported *Streptomyces* species to inhibit biofilm formation of the pathogen *P. aeruginosa*. In this study the *Labrenzia*, *Metabacillus, Microbulbifer, Reugeria, Sporosarcina* extracts demonstrated anti-QS and anti-biofilm activities although few studies have reported their anti-QS and anti-biofilm activities on pathogens (Dow, 2021; Bakkiyaraj *et al.,* 2013**)**. Three *Bacillus* extracts from strains Ac_A20, Ac_L8, and Ac_L15 demonstrated multiple bioactivities. The *Bacillus, Phaeobacter* and *Streptomyces* extracts would be candidate extracts for bioactive compounds as they demonstrated multiple bioactivities against both Gram-negative and Gram-positive pathogens, making them bacteria with broad-spectrum activity. The results of the current study demonstrate that the anti-QS strategy in corals is important, with ecological and biotechnological implications and could serve in the discovery of new biofilm inhibitors for identification of new biofilm compounds.

4.5 Conclusions

This study uncovered the antimicrobial, anti-QS and anti-biofilm activity of CAB isolated from *Acropora* species. Selected bacterial extracts demonstrated bioactivity with potential environmental application. *Bacillus* extracts have demonstrated activity against multiple indicators and bioassays tested and members of this genera continue to be a promising candidates of new drugs as previous studies have reported. These bacterial isolates may help in the development of antimicrobial and QS inhibitors with broad spectrum activity and

facilitate the discovery of novel drugs that can deal with marine bacterial infections (aquaculture, coral disease, etc.) in the current antibiotic resistance era.

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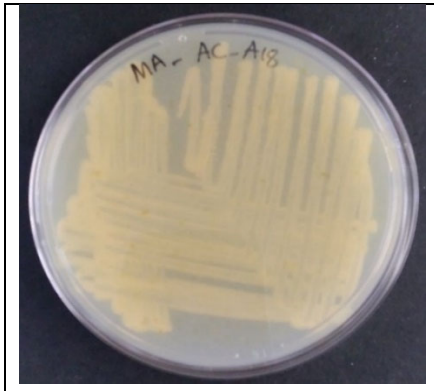
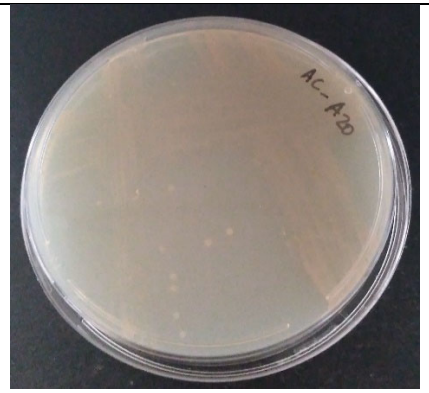
4.6 Supplementary information

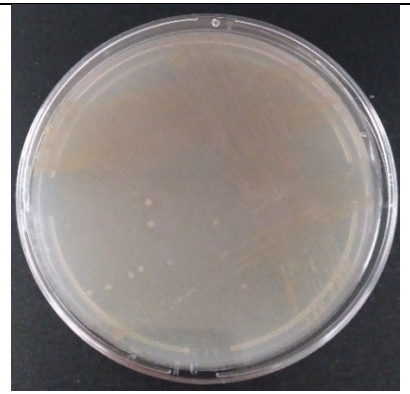
4.6.1 Selected *Acropora* CAB cultures

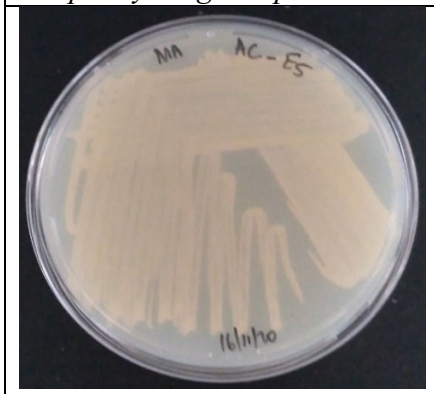


Ac_A18
Streptomyces globisporus

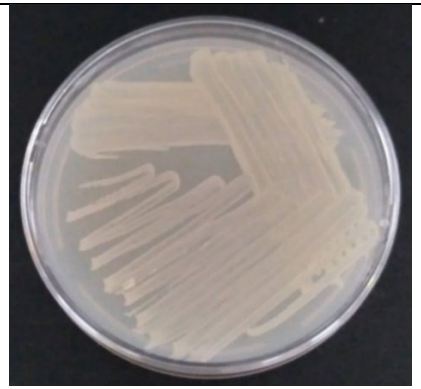


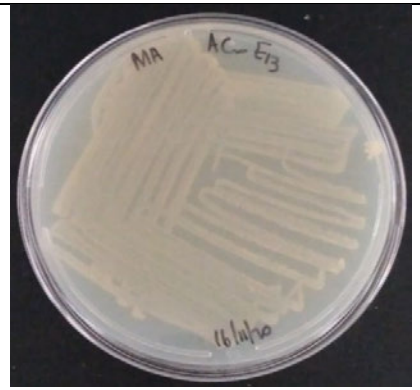
Ac_A20
Bacillus altitudinis



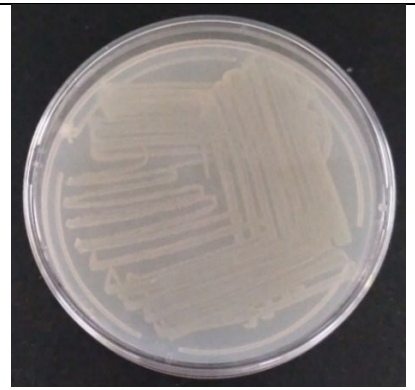


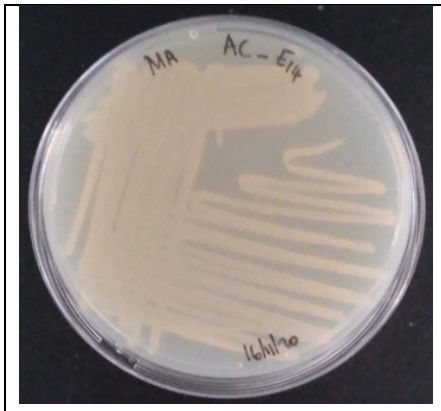
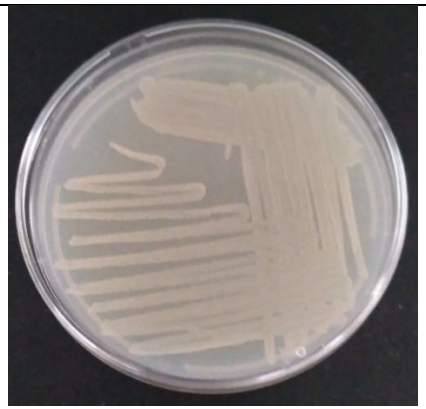
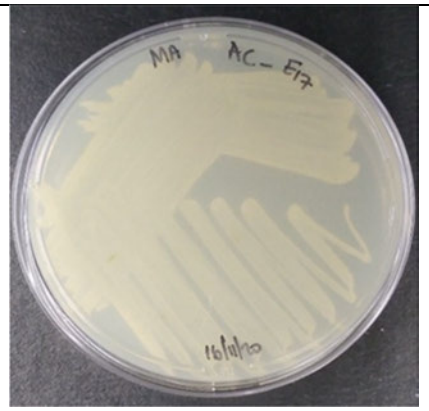
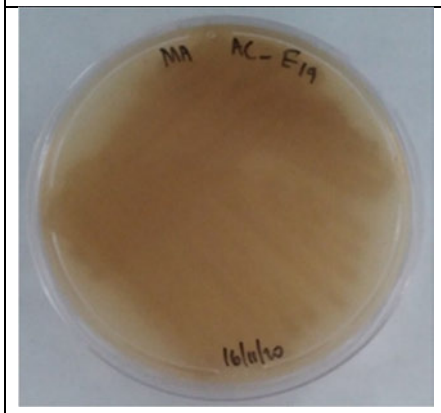
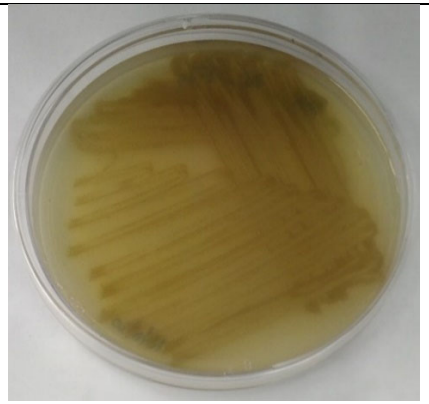
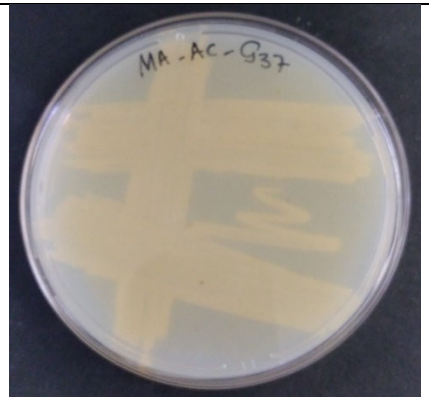
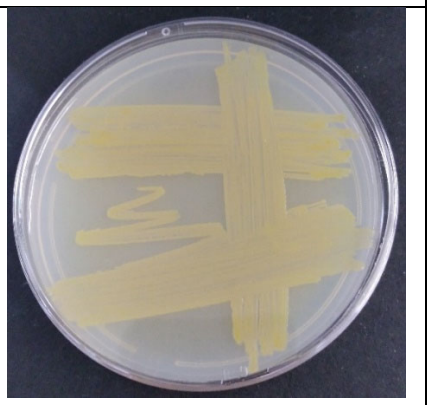
Ac_E5
Bacillus altitudinis

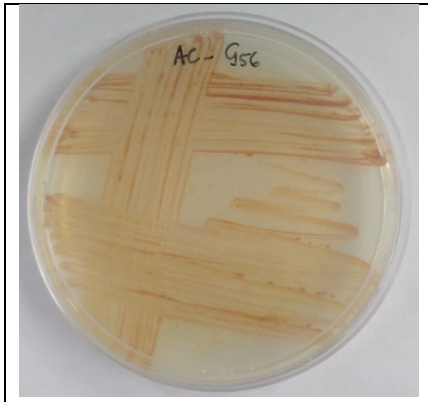
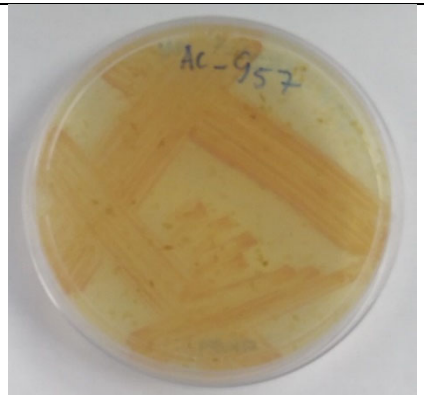
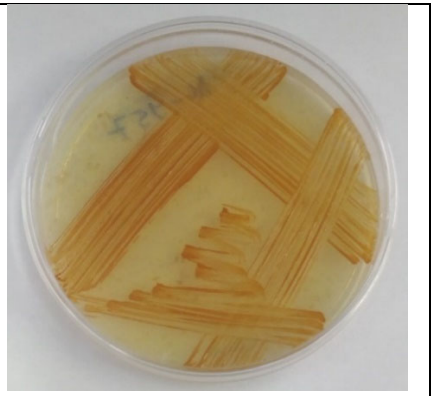
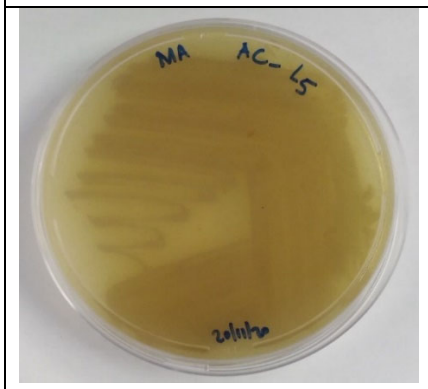
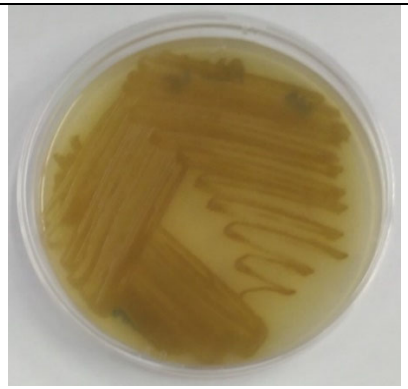
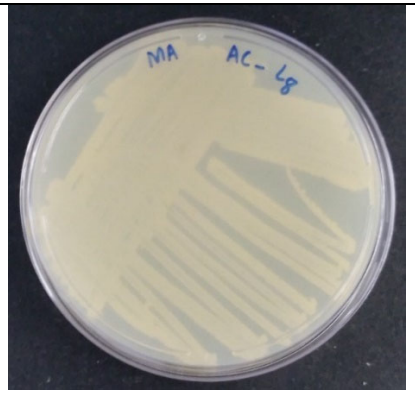


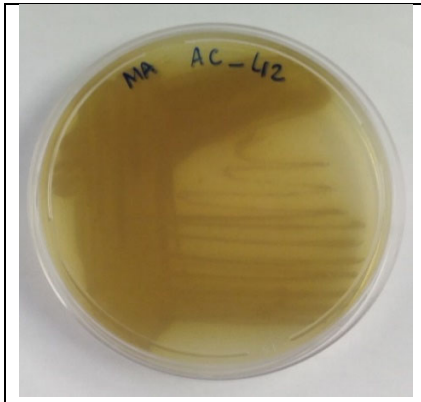
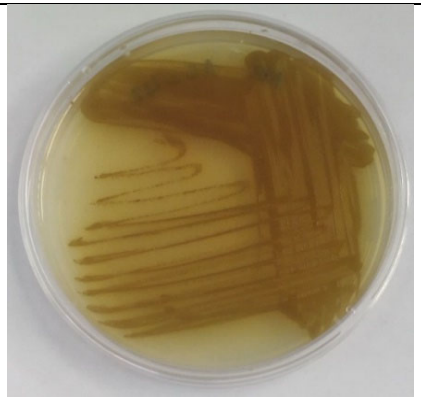
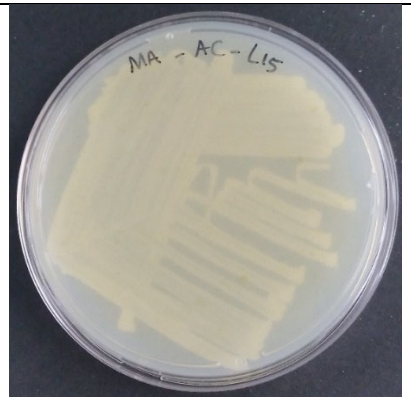
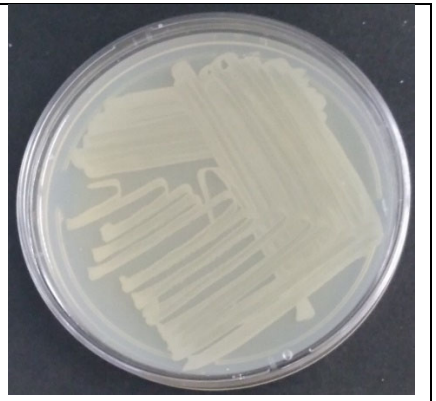
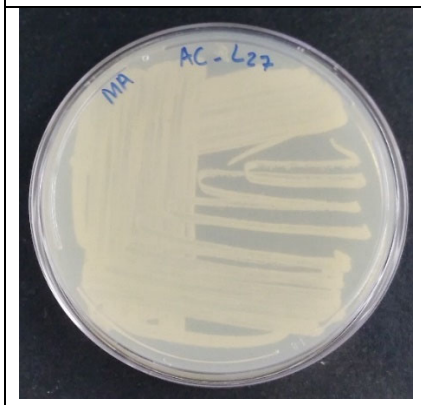
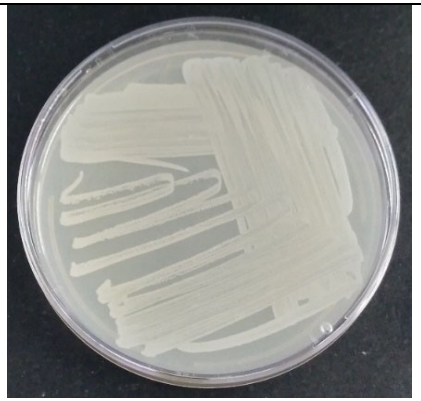
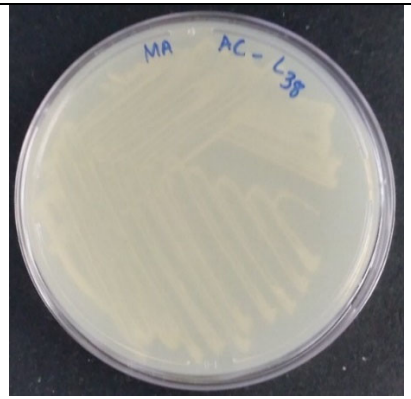
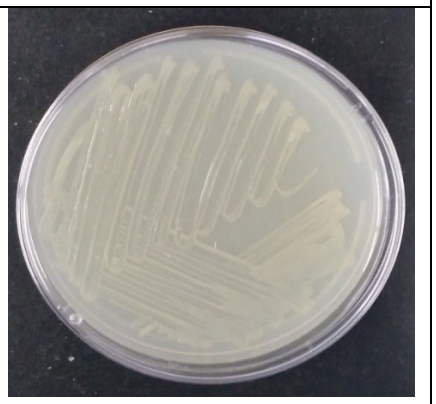


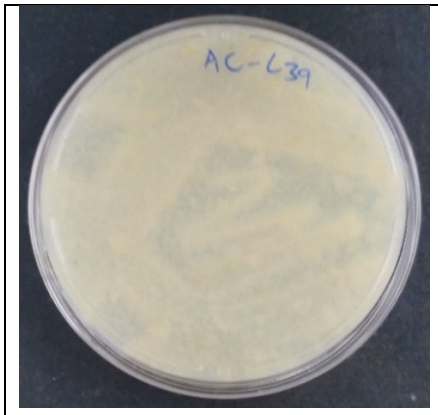
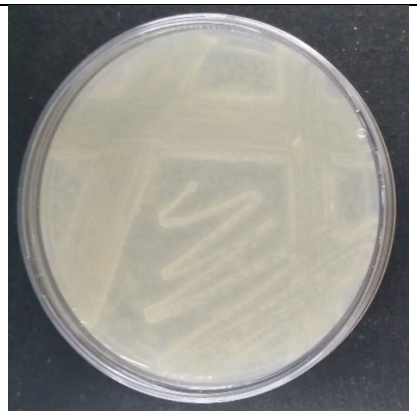
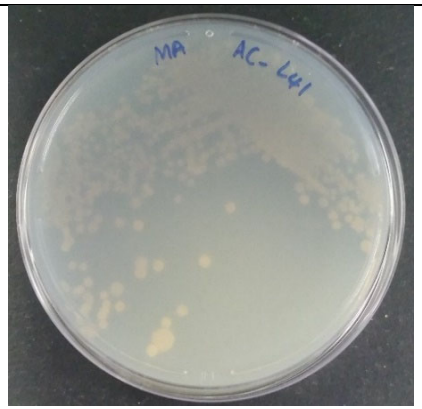
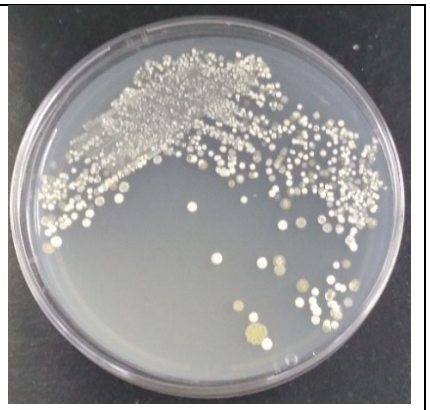
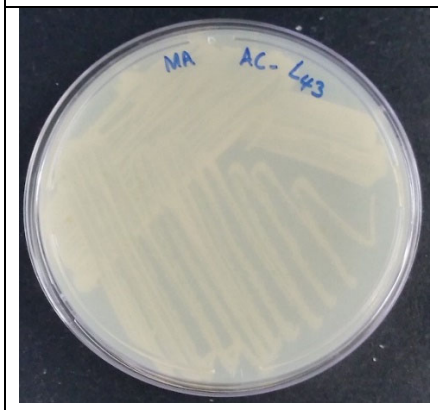
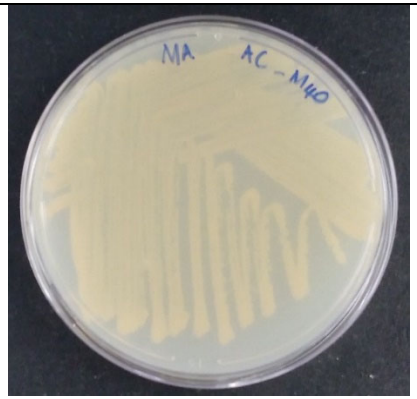
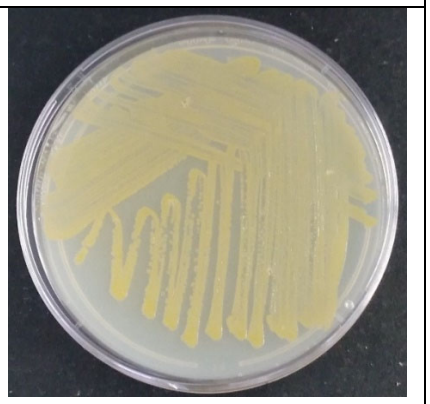
Ac_E13
Labrenzia marina

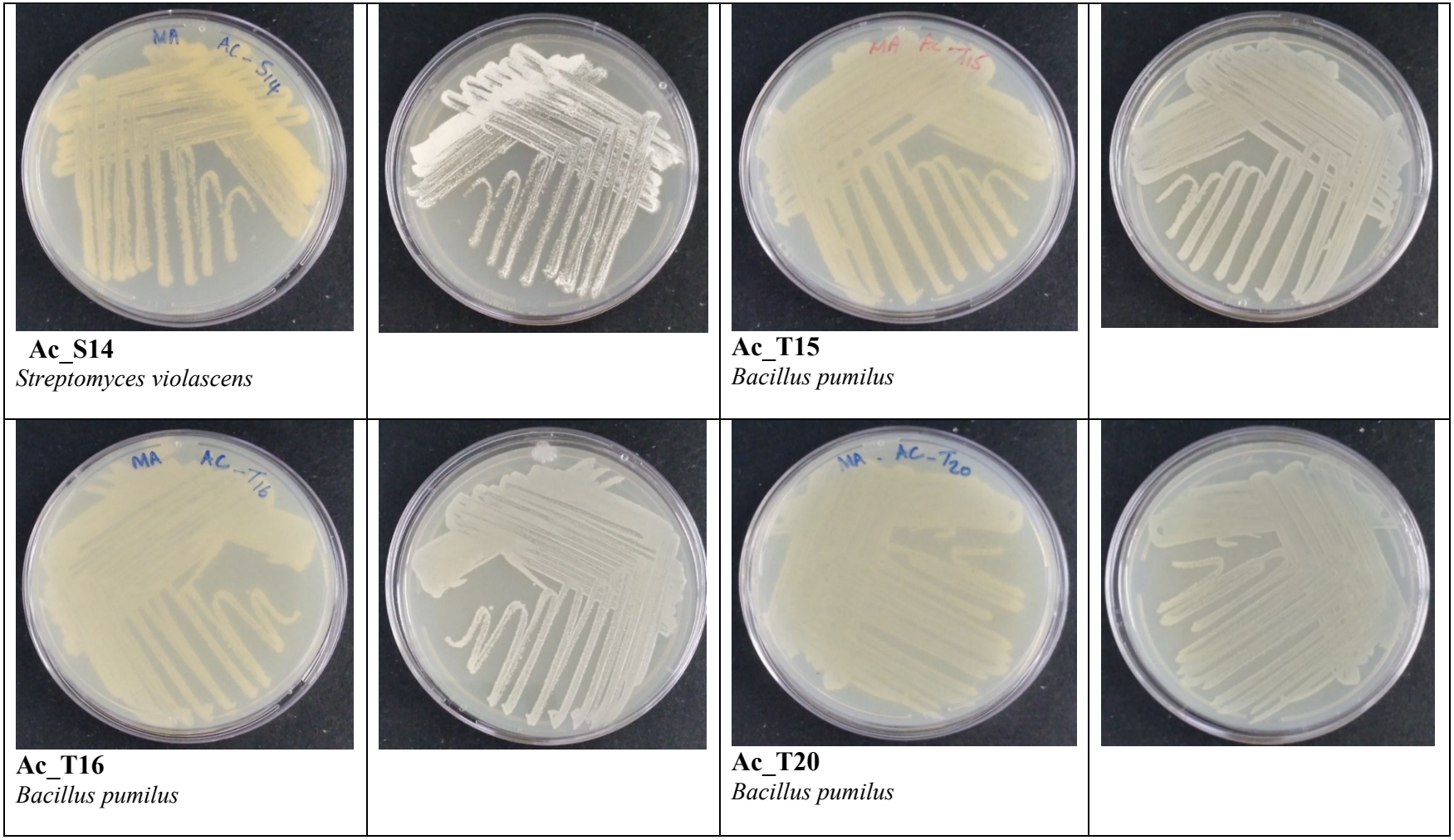


 **Ac_E14** *Labrenzia alexandrii*		 **Ac_E17** *Bacillus aerophilus*	
 **Ac_E19** *Phaeobacter gallaeciensis*		 **Ac_G37** *Bacillus velezensis*	

 **Ac_G56** E*rythrobacter nanhaisediminis*		 **Ac_G57** E*rythrobacter nanhaisediminis*	
 **Ac_L5** *Phaeobacter piscinae*		 **Ac_L8** *Bacillus altitudinis*	

 **Ac_L12** *Phaeobacter gallaeciensis*		 **Ac_L15** *Bacillus aerophilus*	
 **Ac_L27** *Bacillus altitudinis*		 **Ac_L38** *Ruegeria atlantica*	

 **Ac_L39** *Sporosarcina pasteurii*		 **Ac_L41** *Streptomyces pratensis*	
 **Ac_L43** *Microbulbifer variabilis*		 **Ac_M40** *Metabacillus indicus*	



Ac_S14
Streptomyces violascens

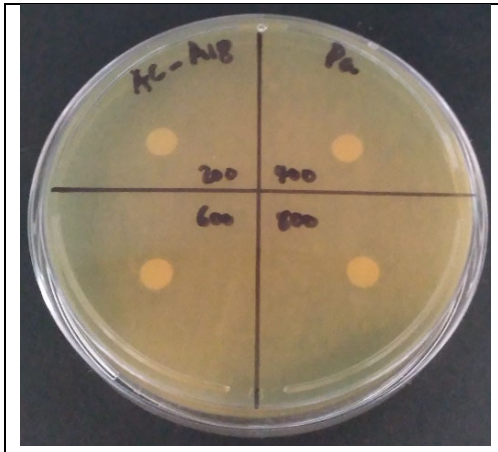
Ac_T15
Bacillus pumilus

Ac_T16
Bacillus pumilus

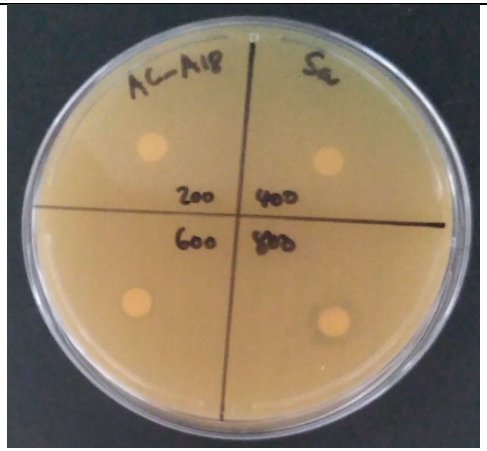
Ac_T20
Bacillus pumilus

Figure S4.1: Twenty-four *Acropora* CAB isolates selected from primary screening for fermentation and secondary metabolite extraction.

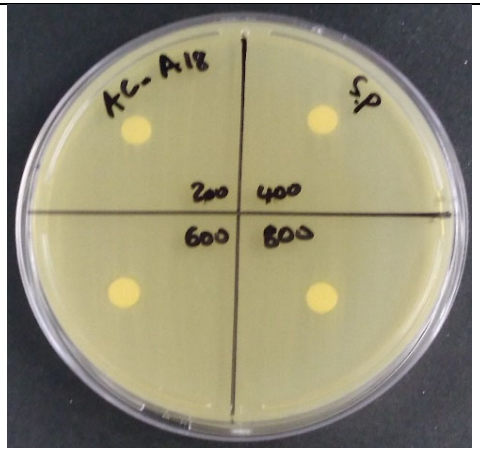
4.6.2 Antimicrobial activity screening of 24 *Acropora* CAB extracts.



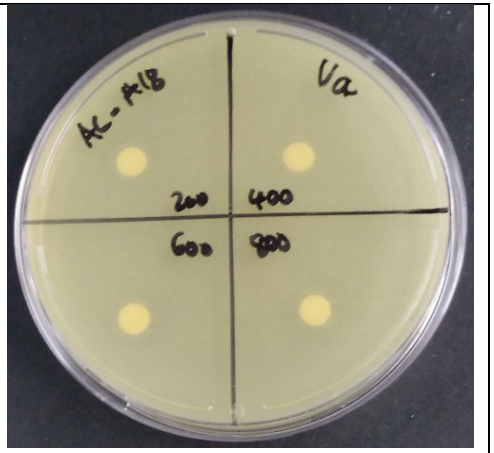
Ac_A18_*P. aeruginosa*



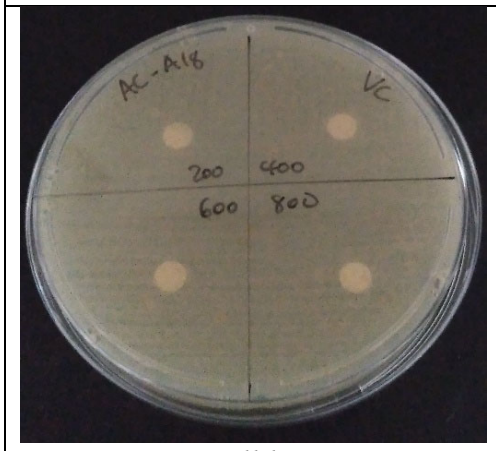
Ac_A18_*S. aureus*



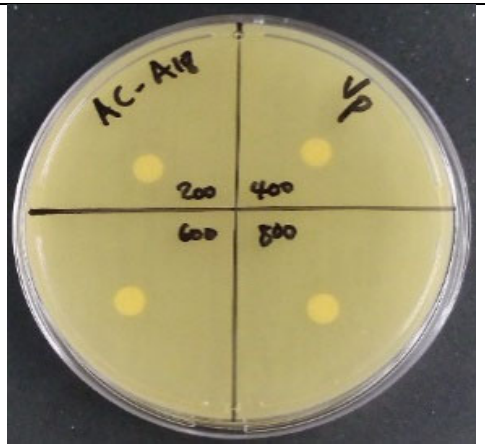
Ac_A18_*S. putrefaciens*



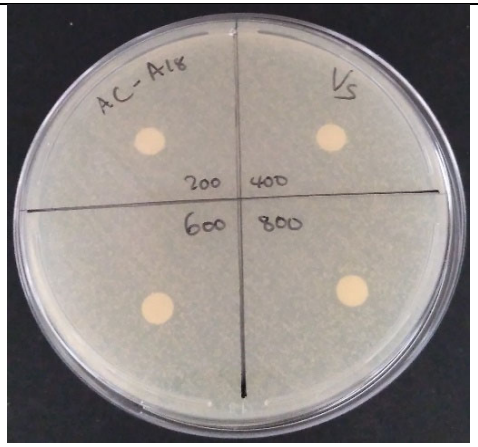
Ac_A18_*V. alginolyticus*



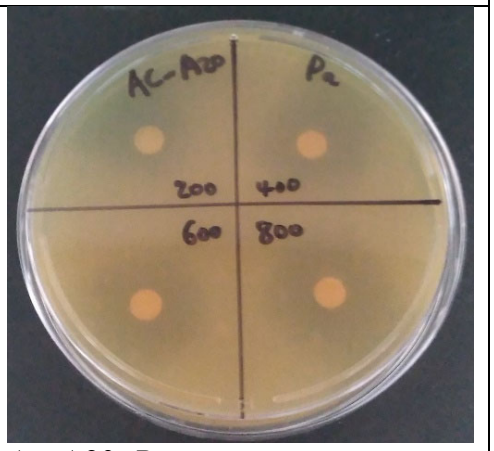
Ac_A18_*V. corallilyticus*



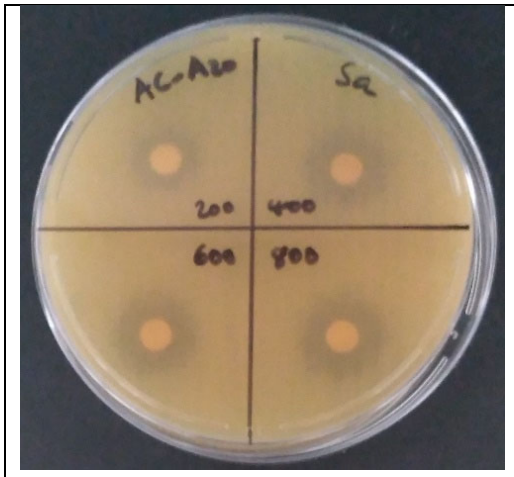
Ac_A18_*V. parahaemolyticus*



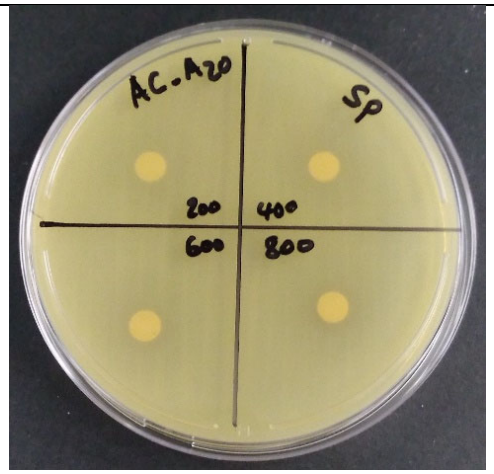
Ac_A18_*V. shilonii*



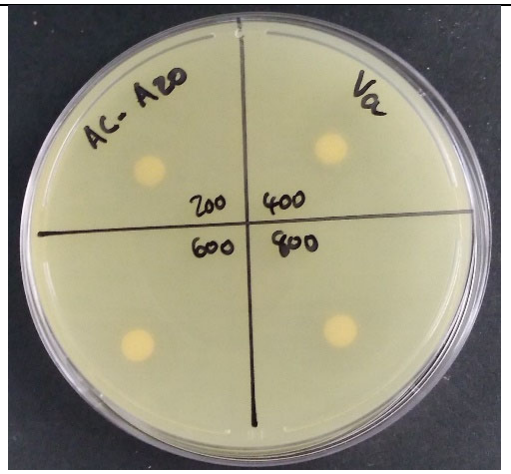
Ac_A20_*P. aeruginosa*



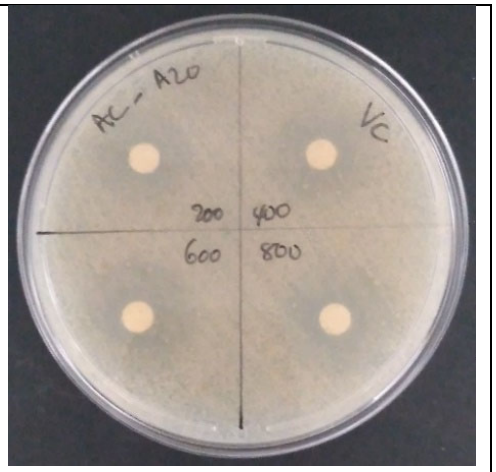
Ac_A20_*S. aureus*



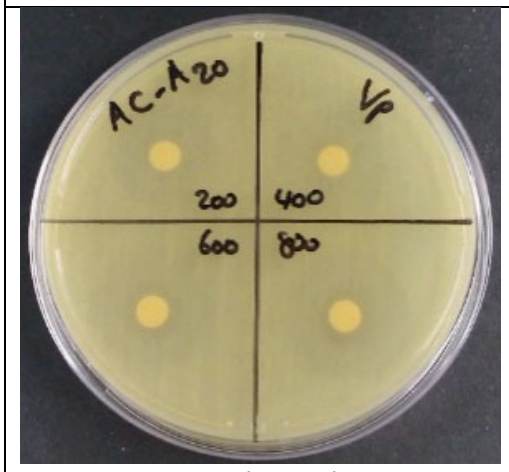
Ac_A20_*S. putrefaciens*



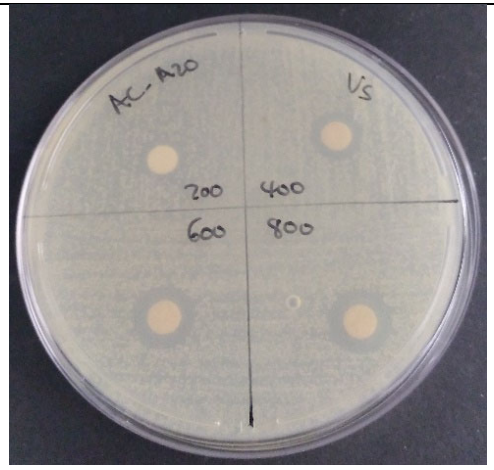
Ac_A20_*V. alginolyticus*



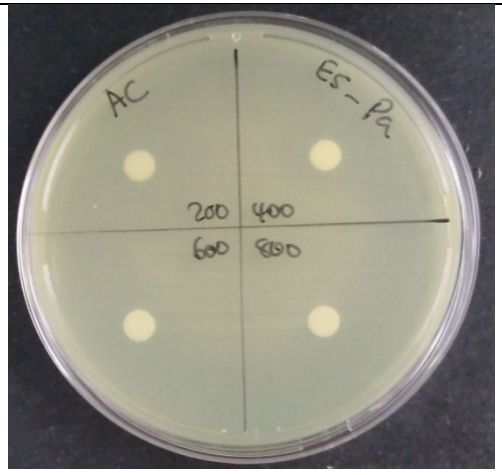
Ac_A20_*V. corallilyticus*



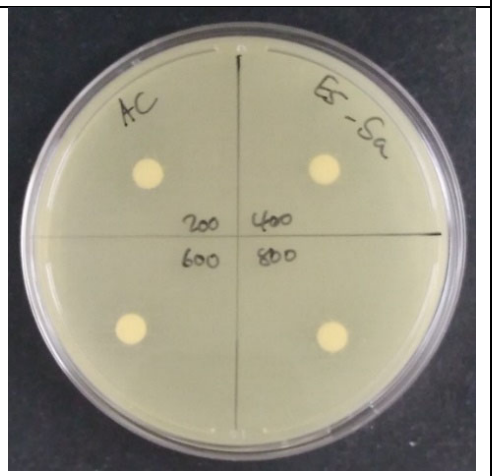
Ac_A20_*V. parahaemolyticus*



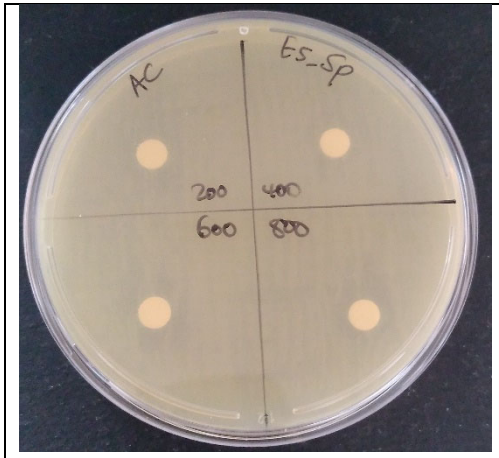
Ac_A20_*V. shilonii*



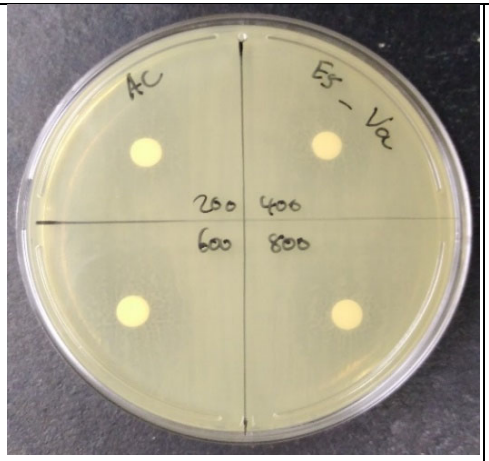
Ac_E5_*P. aeruginosa*



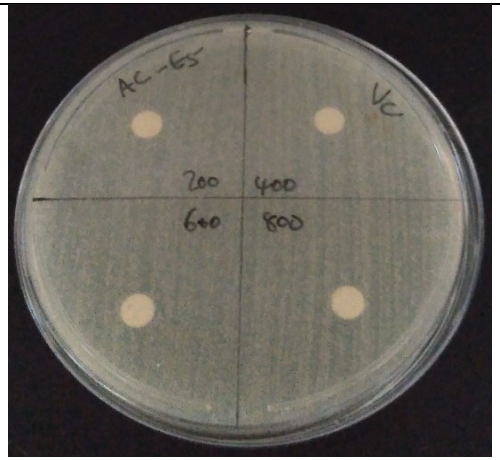
Ac_E5_*S. aureus*



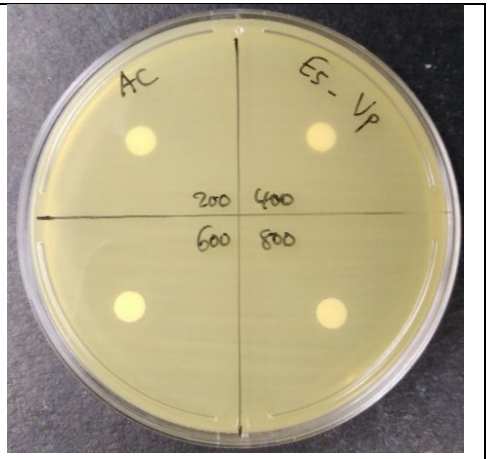
Ac_E5_*S. putrefaciens*



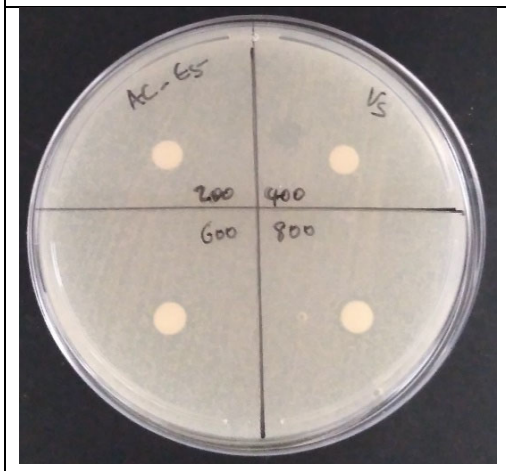
Ac_E5_*V. alginolyticus*



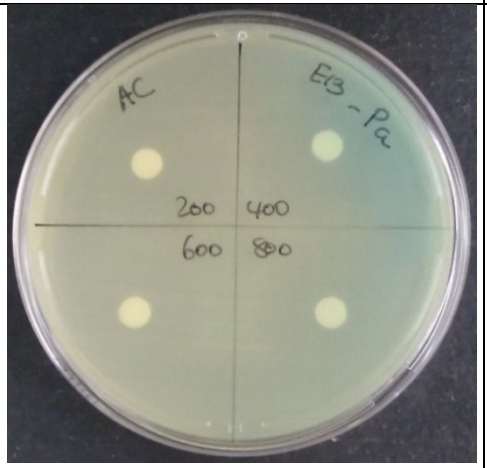
Ac_E5_*V. corallilyticus*



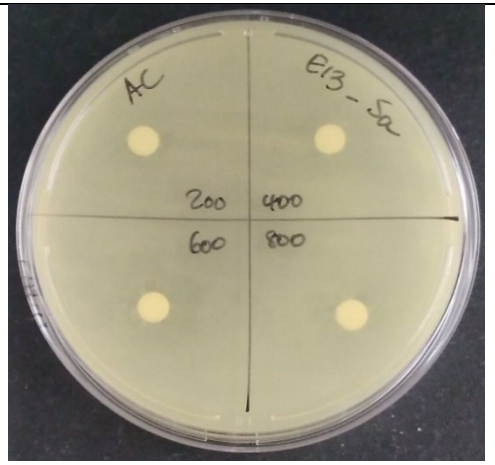
Ac_E5_*V. parahaemolyticus*



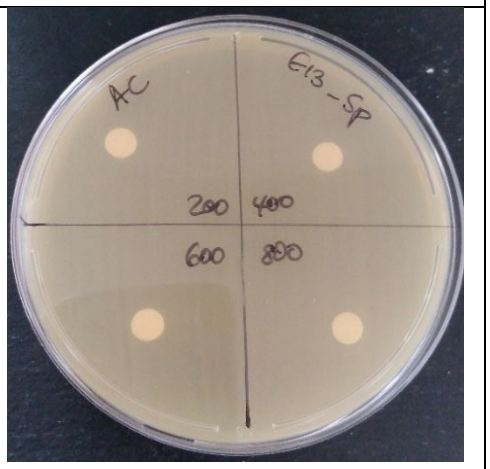
Ac_E5_*V. shilonii*



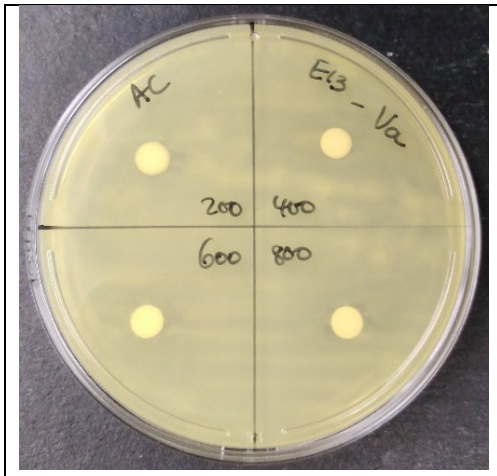
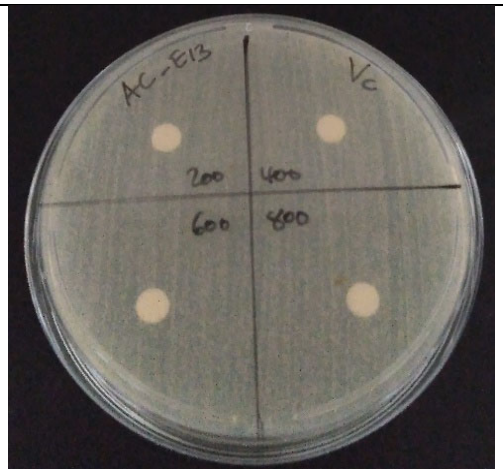
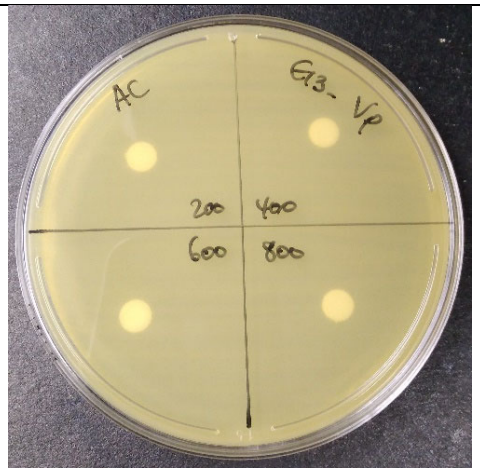
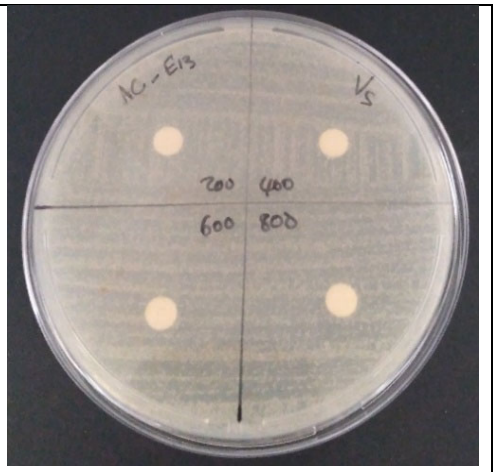
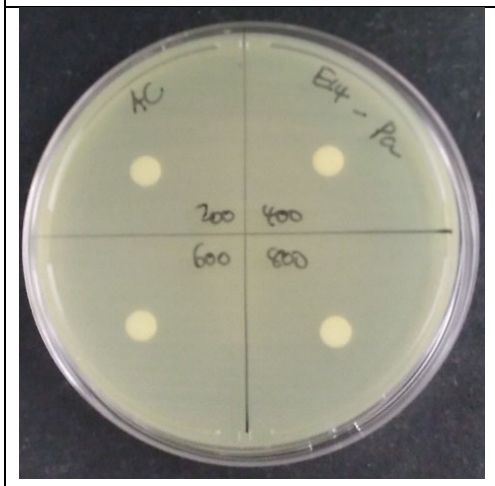
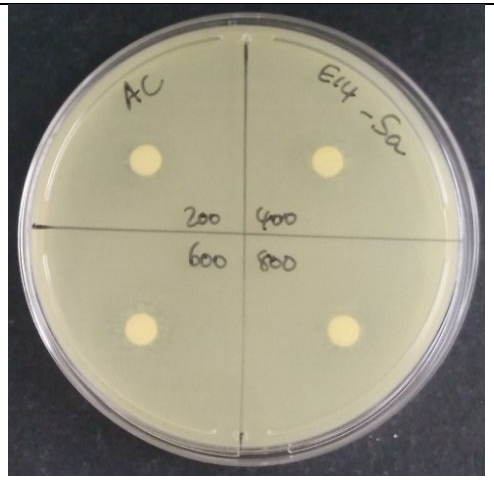
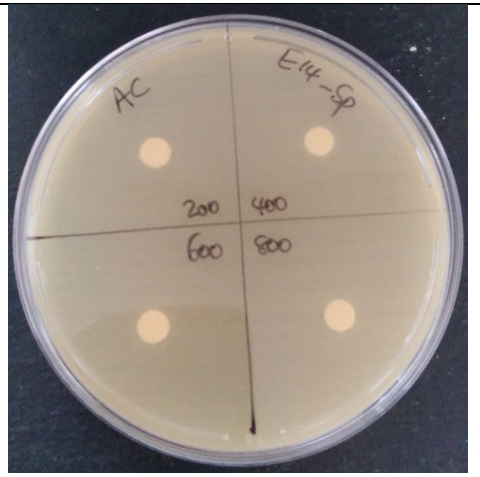
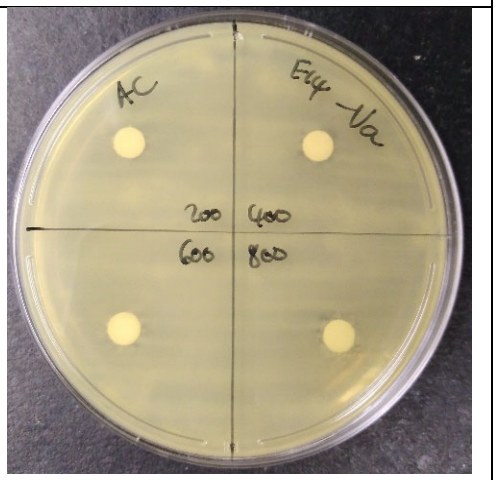
Ac_E13_*P. aeruginosa*

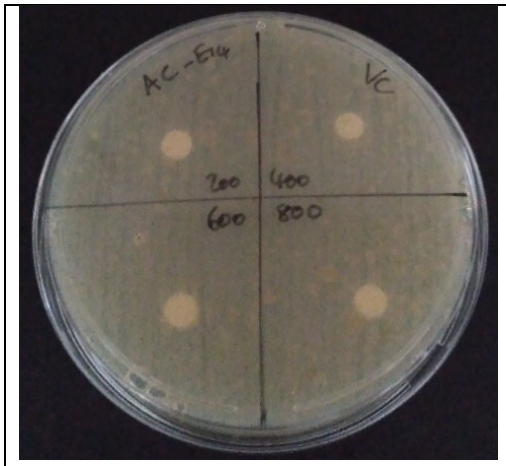
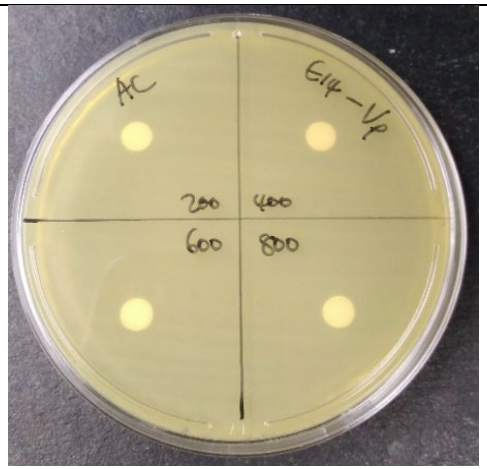
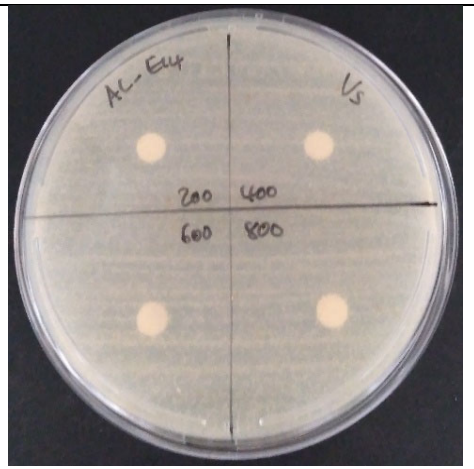
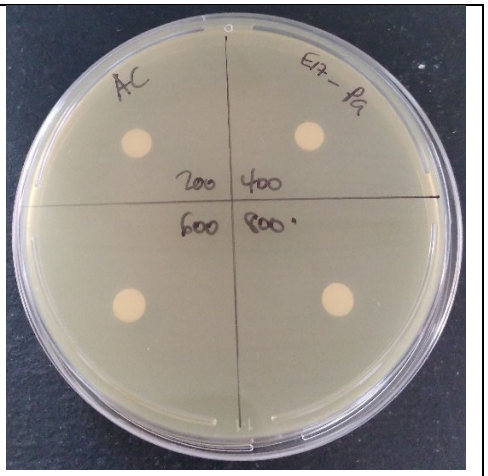
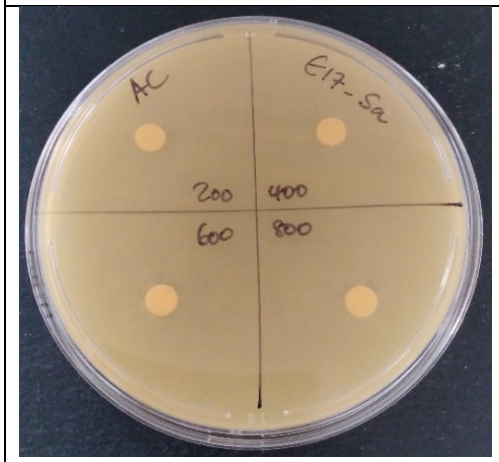
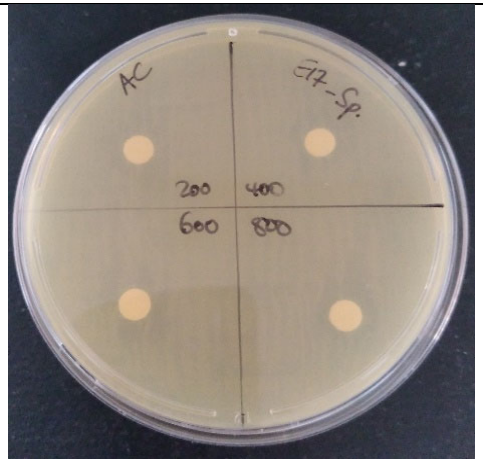
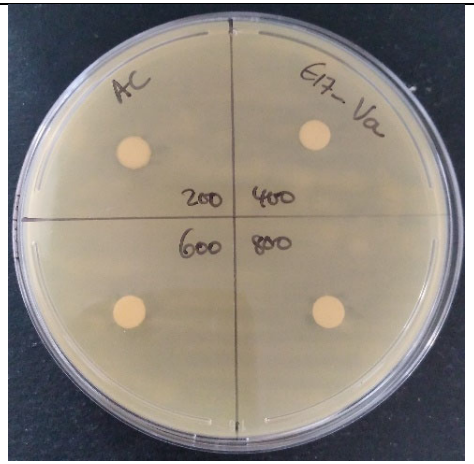
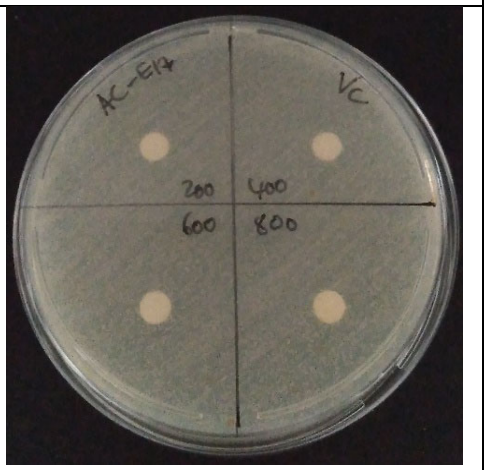


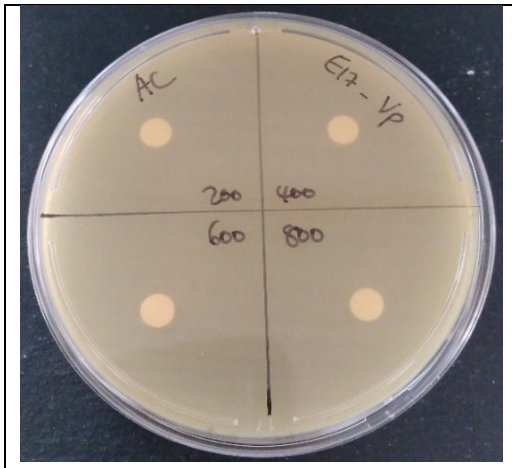
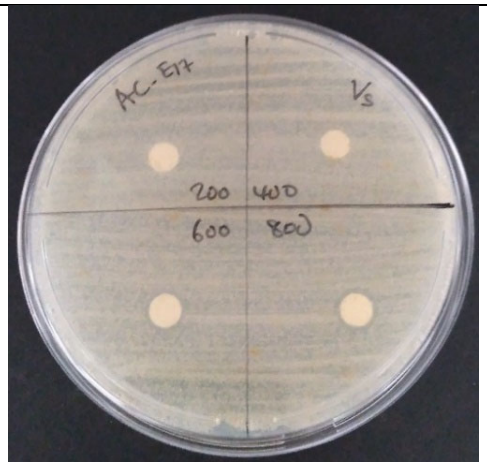
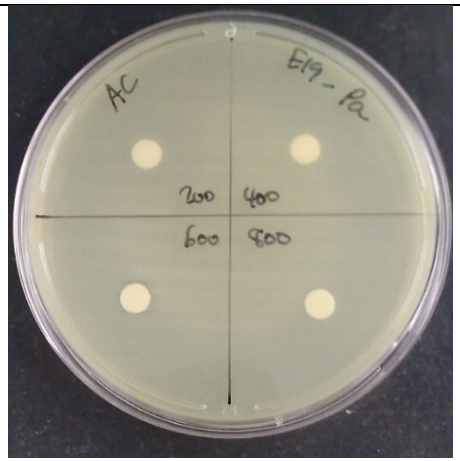
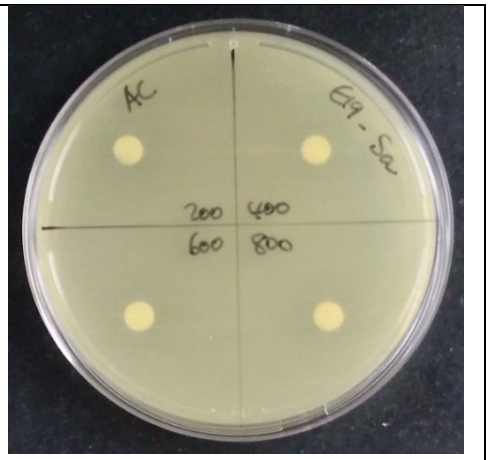
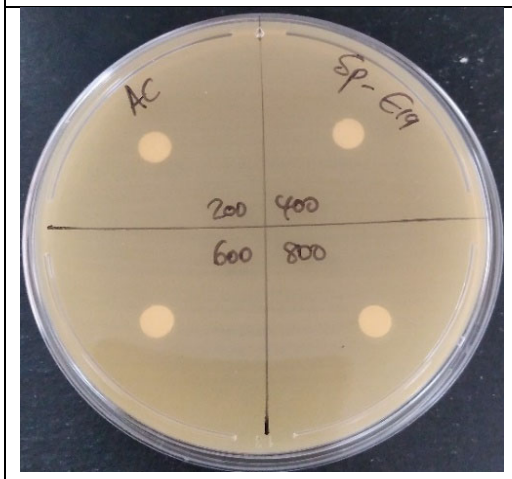
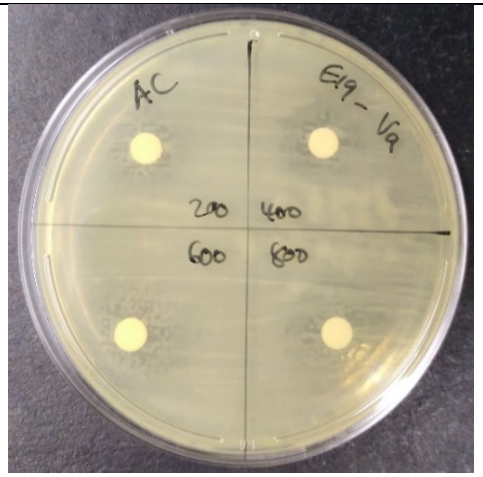
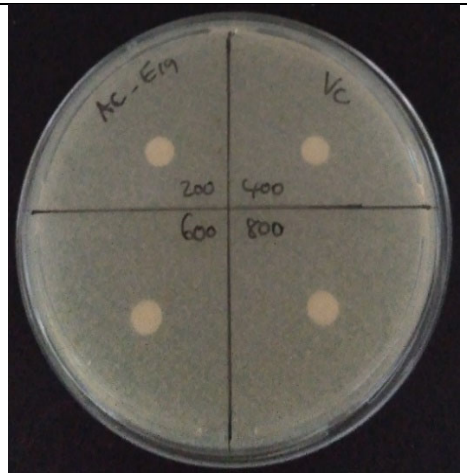
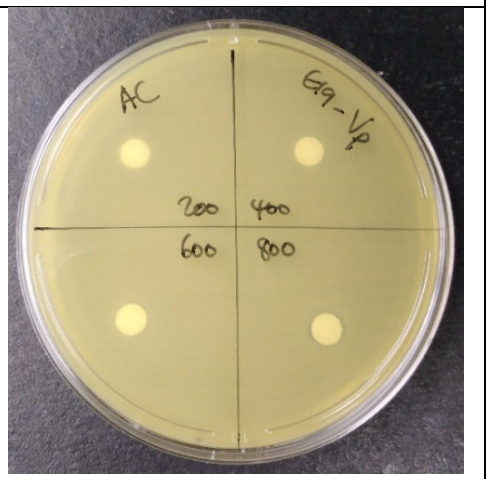
Ac_E13_*S. aureus*

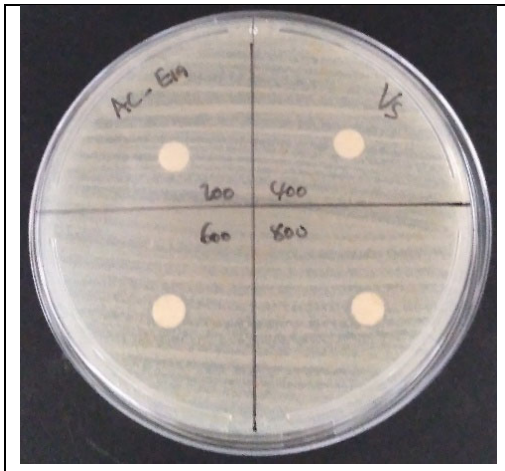
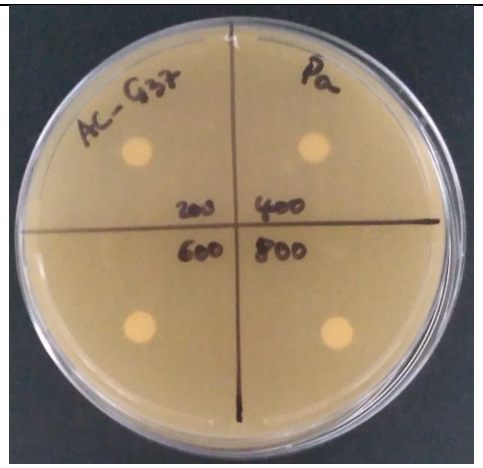
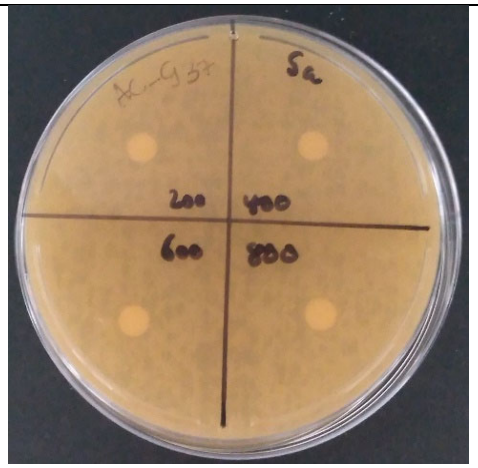
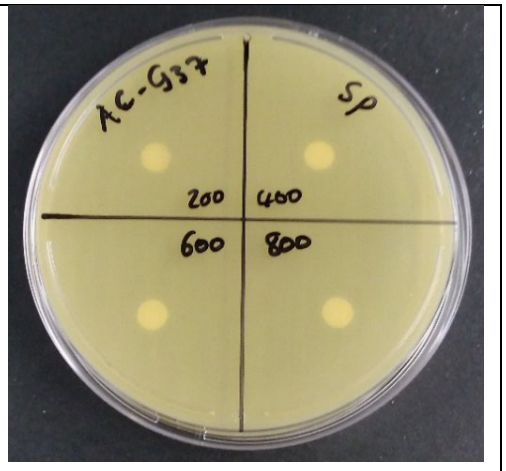
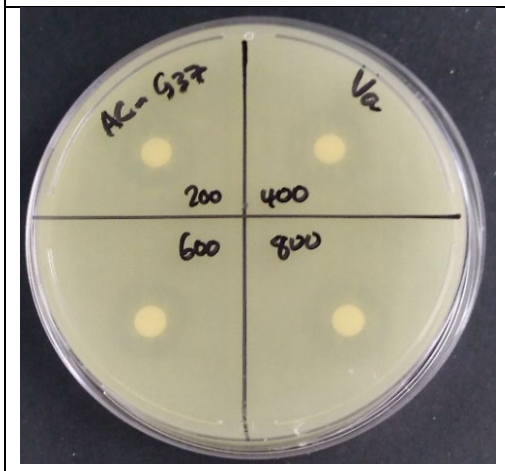
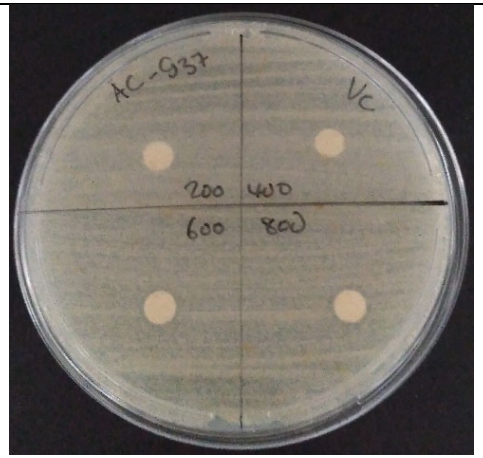
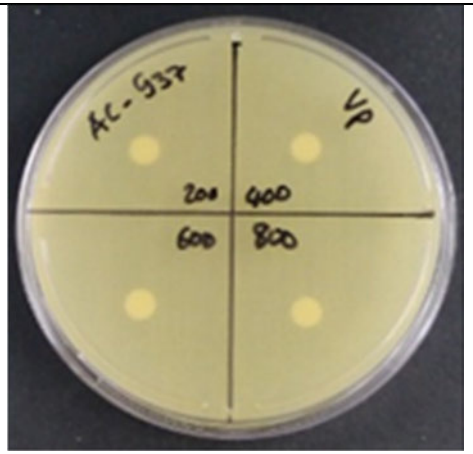
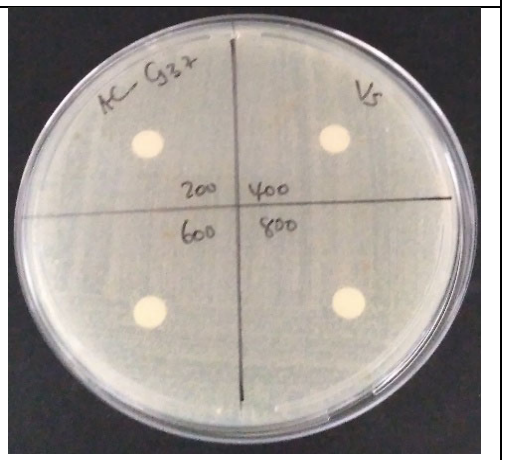


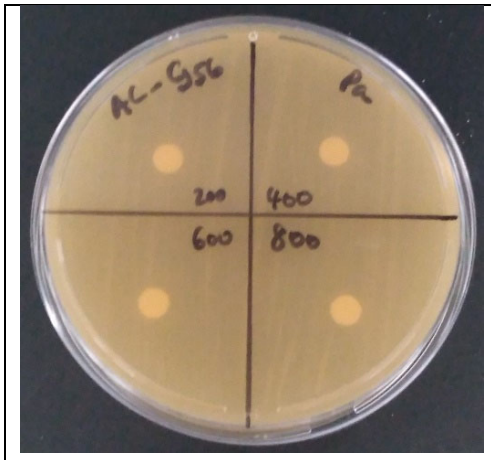
Ac_E13_*S. putrefaciens*

			
Ac_E13_*V. alginolyticus*	**Ac_E13**_*V. corallilyticus*	**Ac_E13**_*V. parahaemolyticus*	**Ac_E13**_*V. shilonii*
			
Ac_E14_*P. aeruginosa*	**Ac_E14**_*S. aureus*	**Ac_E14**_*S. putrefaciens*	**Ac_E14**_*V. alginolyticus*

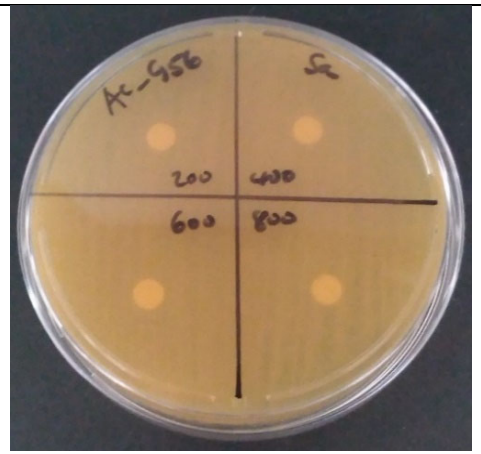
			
Ac_E14_*V. corallilyticus*	**Ac_E14**_*V. parahaemolyticus*	**Ac_E14**_*V. shilonii*	**Ac_E17**_*P. aeruginosa*
			
Ac_E17_*S. aureus*	**Ac_E17**_*S. putrefaciens*	**Ac_E17**_*V. alginolyticus*	**Ac_E17**_*V. corallilyticus*

			
Ac_E17_*V. parahaemolyticus*	**Ac_E17**_*V. shilonii*	**Ac_E19**_*P. aeruginosa*	**Ac_E19**_*S. aureus*
			
Ac_E19_*S. putrefaciens*	**Ac_E19**_*V. alginolyticus*	**Ac_E19**_*V. corallilyticus*	**Ac_E19**_*V. parahaemolyticus*

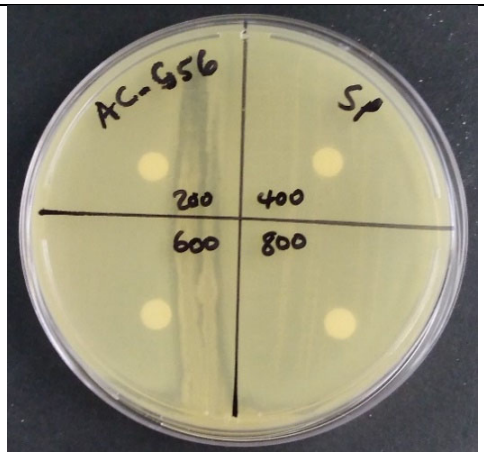
			
Ac_E19_*V. shilonii*	**Ac_G37**_*P. aeruginosa*	**Ac_G37**_*S. aureus*	**Ac_G37**_*S. putrefaciens*
			
Ac_G37_*V. alginolyticus*	**Ac_G37**_*V. corallilyticus*	**Ac_G37**_*V. parahaemolyticus*	**Ac_G37**_*V. shilonii*



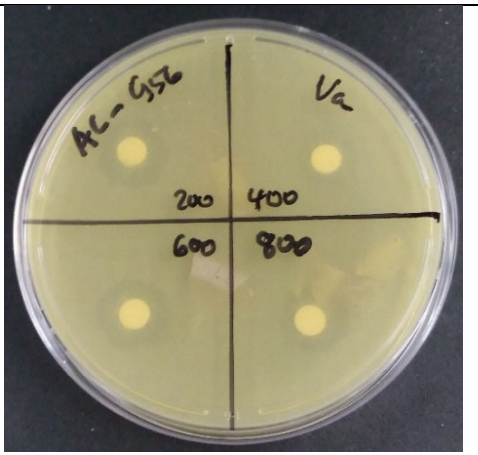
Ac_G56_*P. aeruginosa*



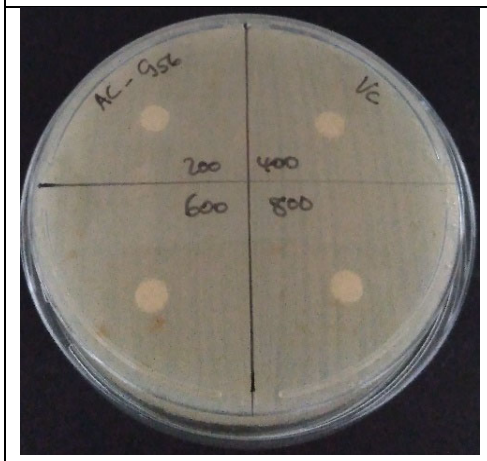
Ac_G56_*S. aureus*



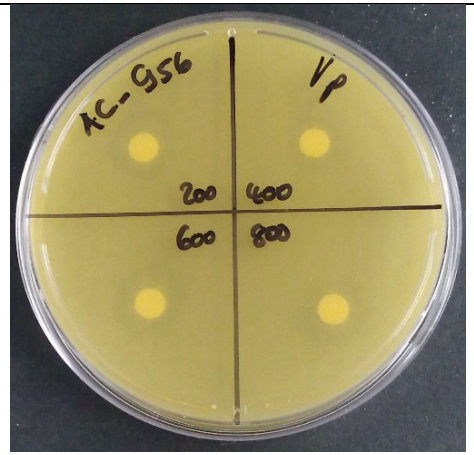
Ac_G56_*S. putrefaciens*



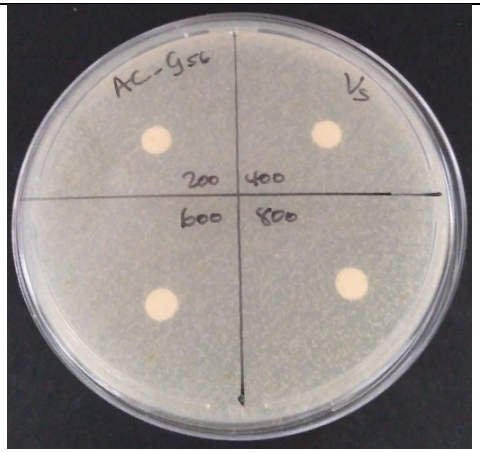
Ac_G56_*V. alginolyticus*



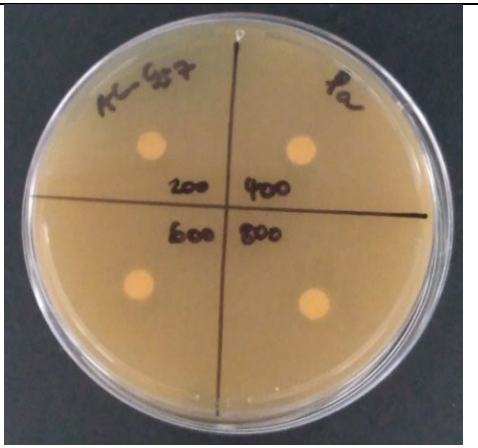
Ac_G56_*V. corallilyticus*



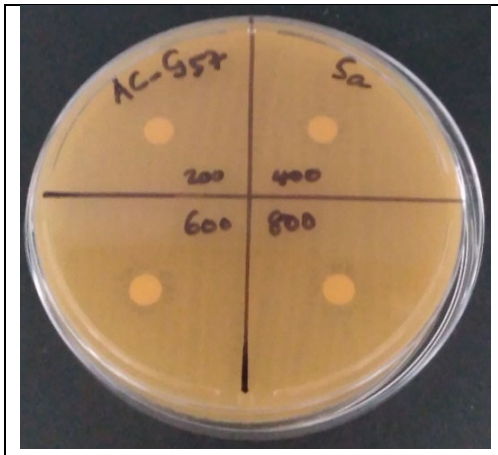
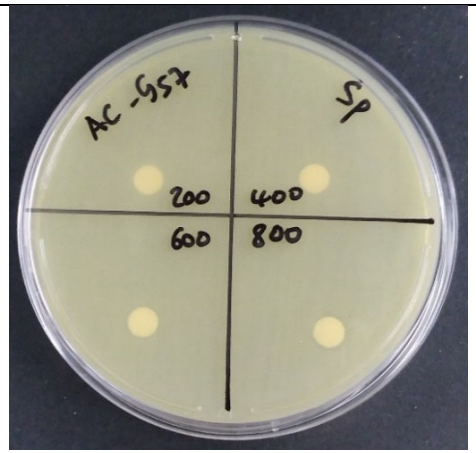
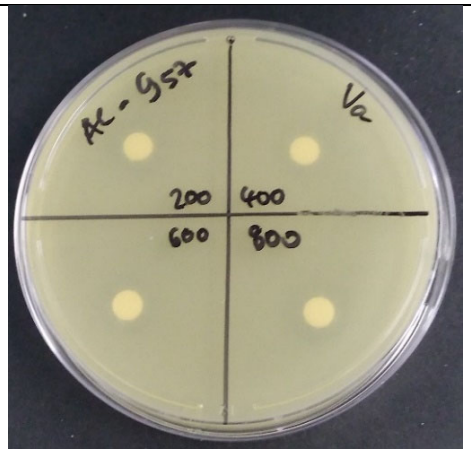
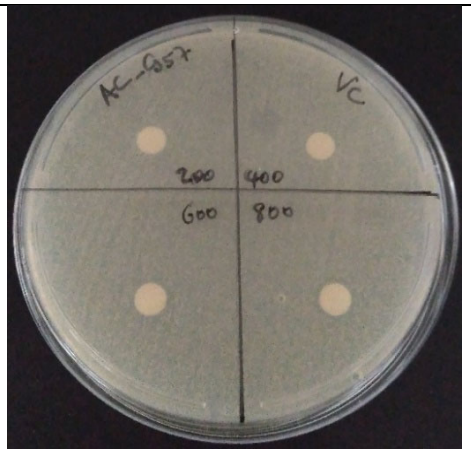
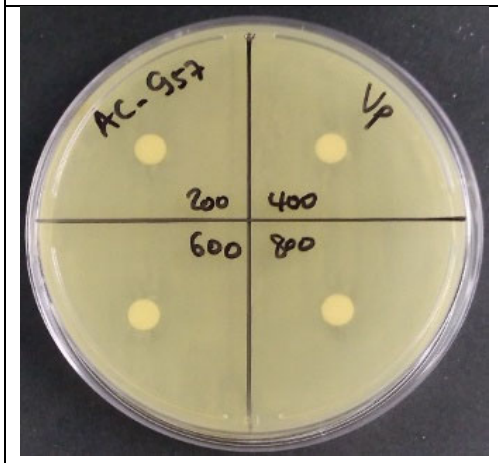
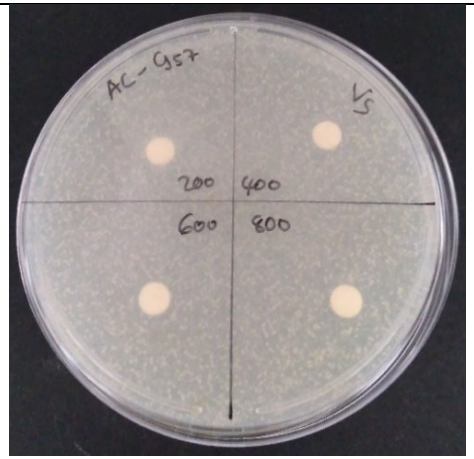
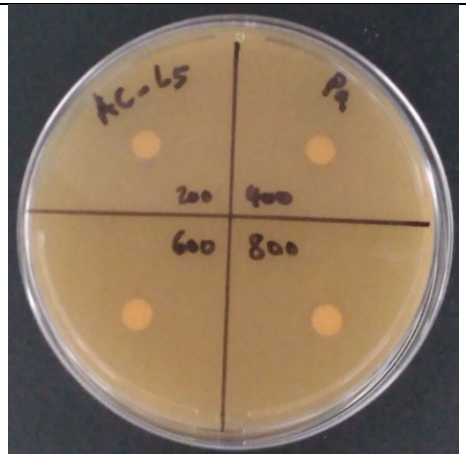
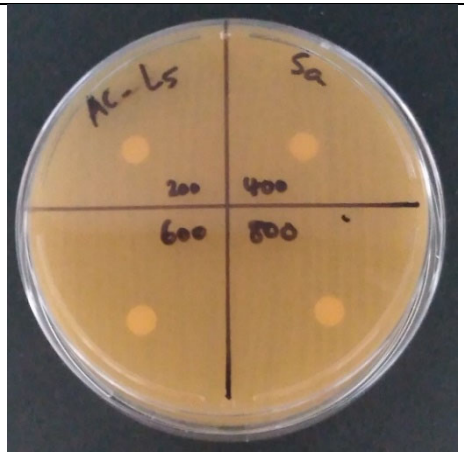
Ac_G56_*V. parahaemolyticus*

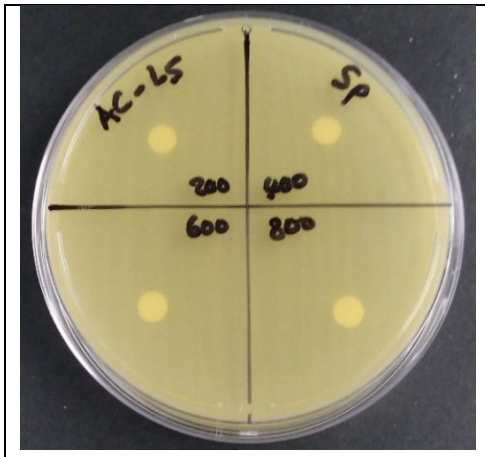


Ac_G56_*V. shilonii*

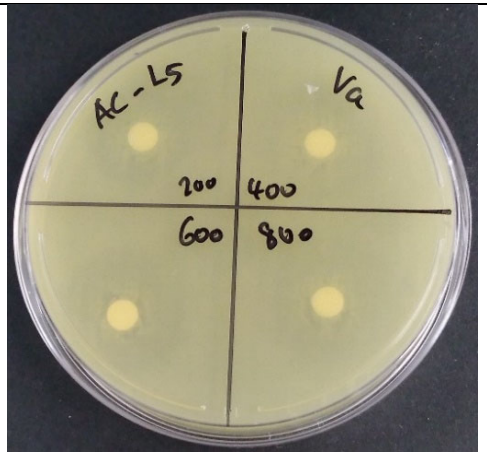


Ac_G57_*P. aeruginosa*

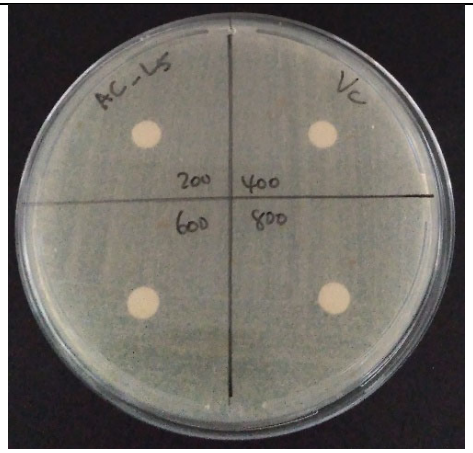
			
Ac_G57_*S. aureus*	**Ac_G57**_*S. putrefaciens*	**Ac_G57**_*V. alginolyticus*	**Ac_G57**_*V. corallilyticus*
			
Ac_G57_*V. parahaemolyticus*	**Ac_G57**_*V. shilonii*	**Ac_L5**_*P. aeruginosa*	**Ac_L5**_*S. aureus*



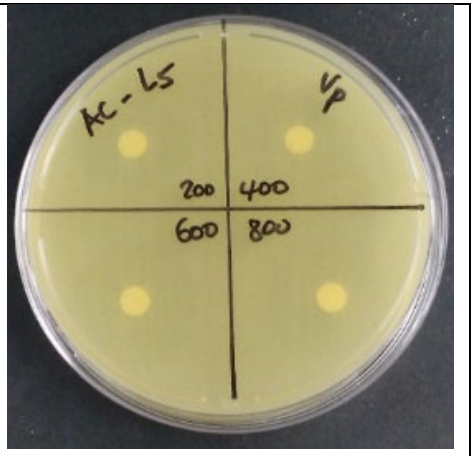
Ac_L5_*S. putrefaciens*



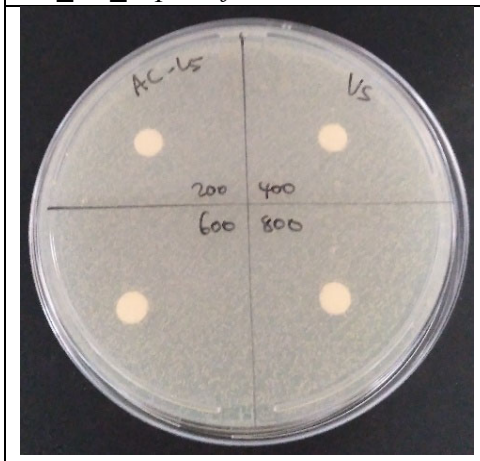
Ac_L5 *V. alginolyticus*



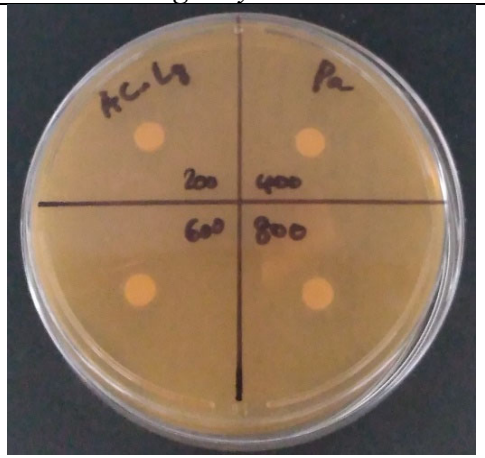
Ac_L5_*V. corallilyticus*



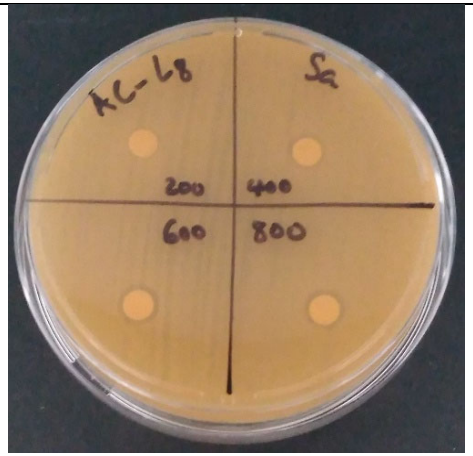
Ac_L5_*V. parahaemolyticus*



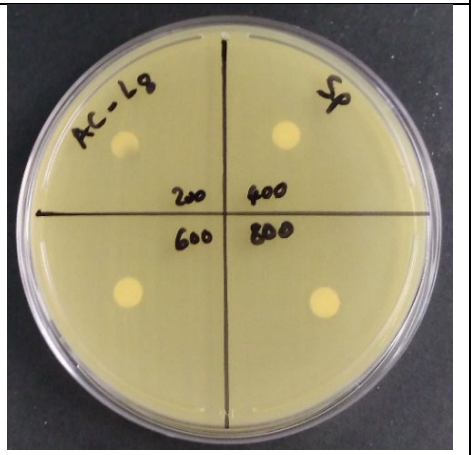
Ac_L5_*V. shilonii*



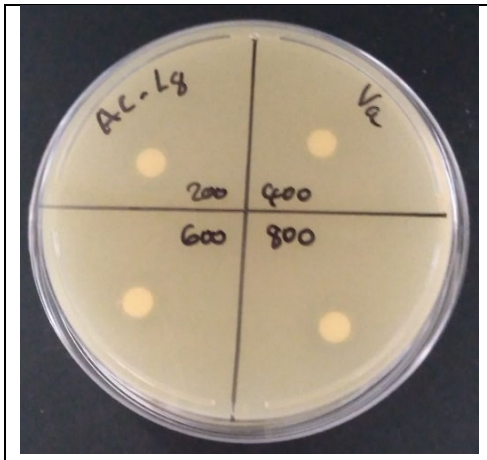
Ac_L8_*P. aeruginosa*



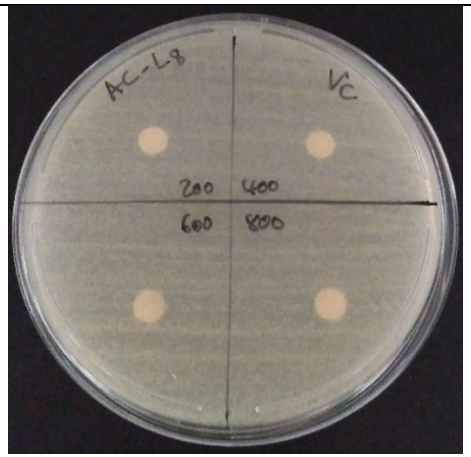
Ac_L8_*S. aureus*



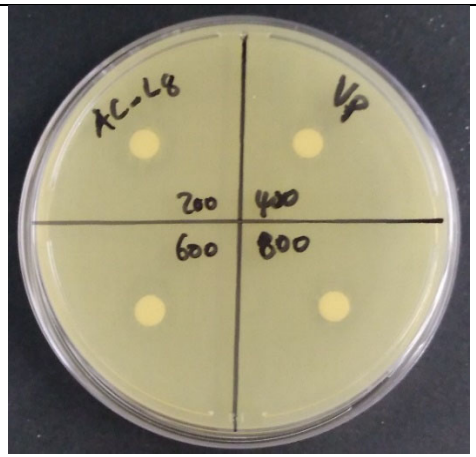
Ac_L8_*S. putrefaciens*



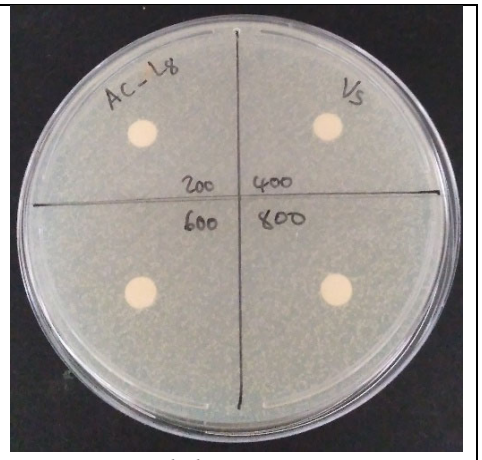
Ac L8 *V. alginolyticus*



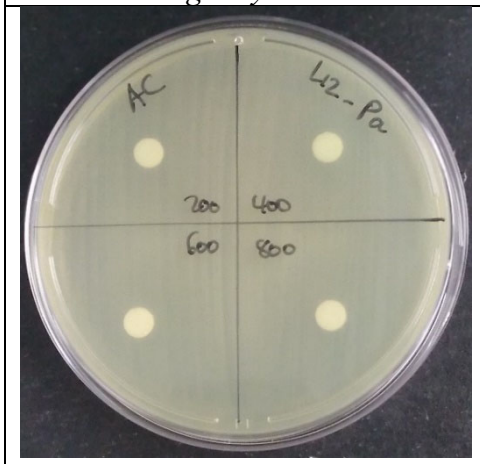
Ac L8 *V. corallilyticus*



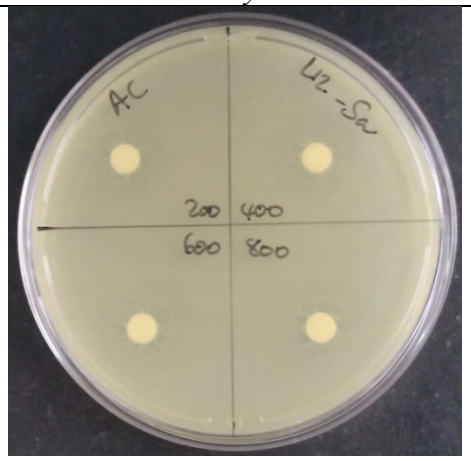
Ac L8 *V. parahaemolyticus*



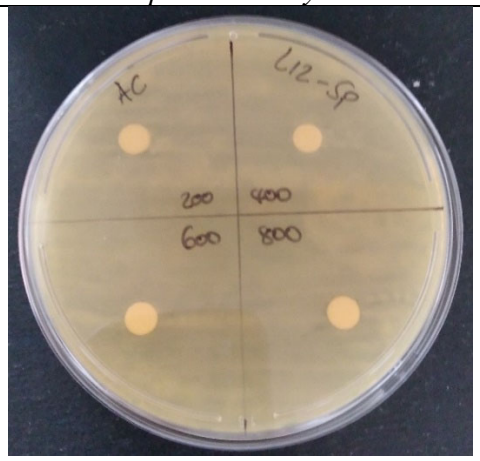
Ac_L8_*V. shilonii*



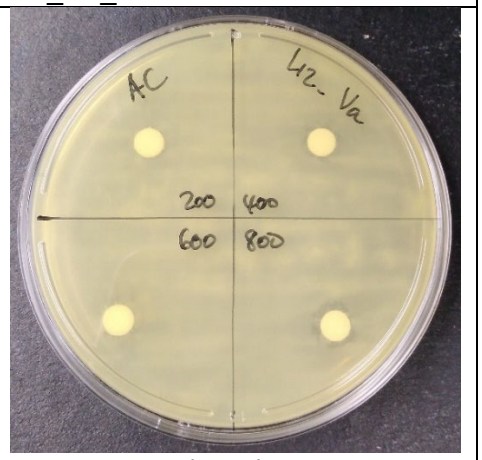
Ac_L12_*P. aeruginosa*



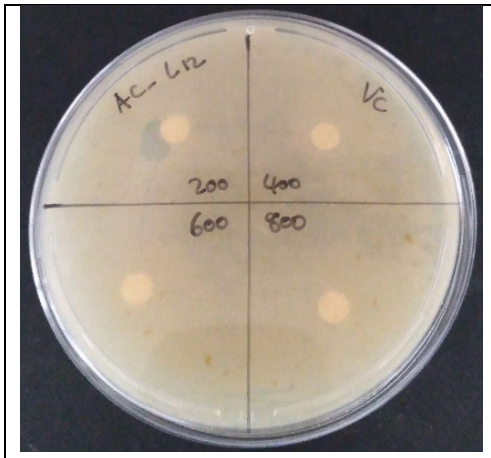
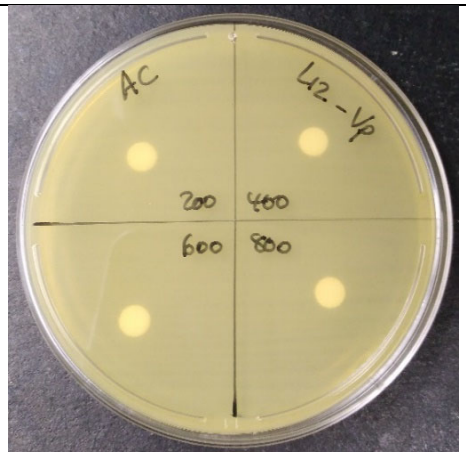
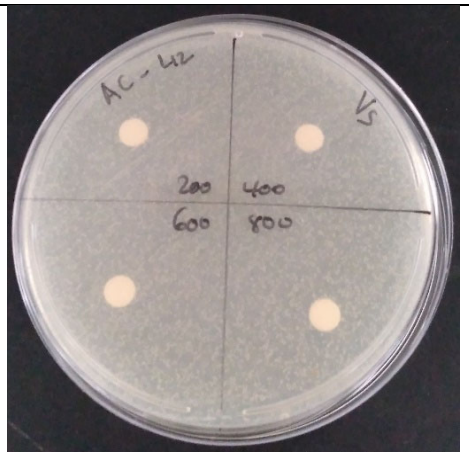
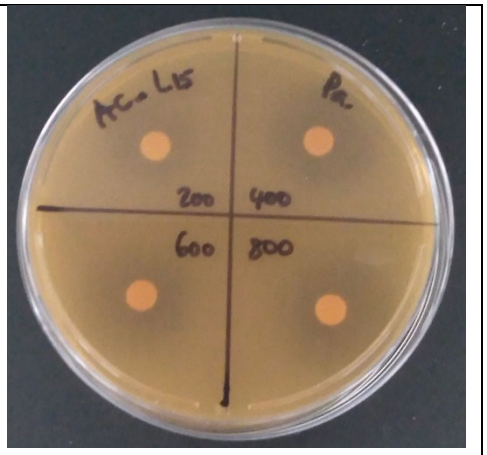
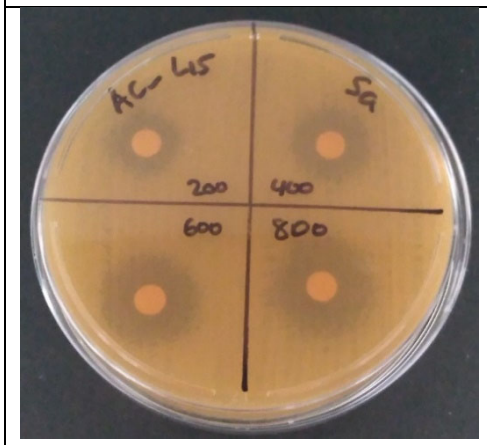
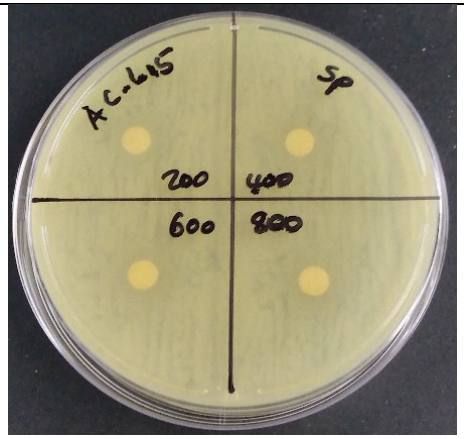
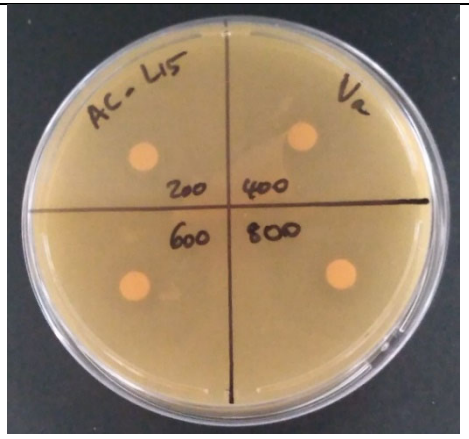
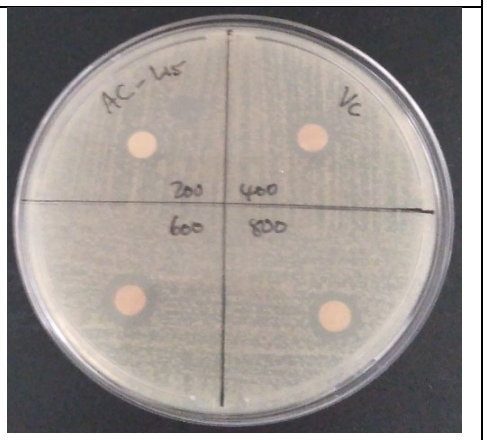
Ac_L12_*S. aureus*

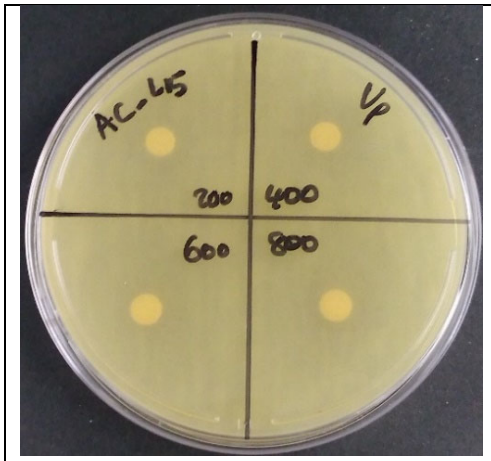
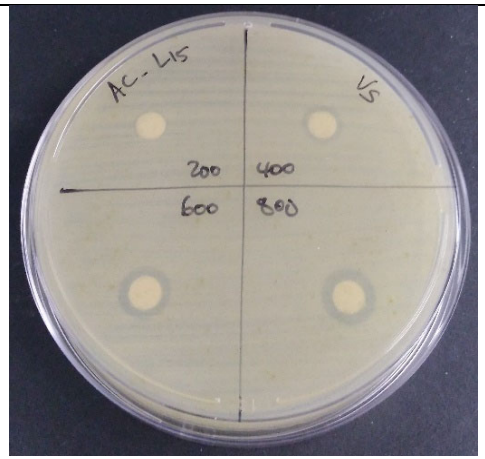
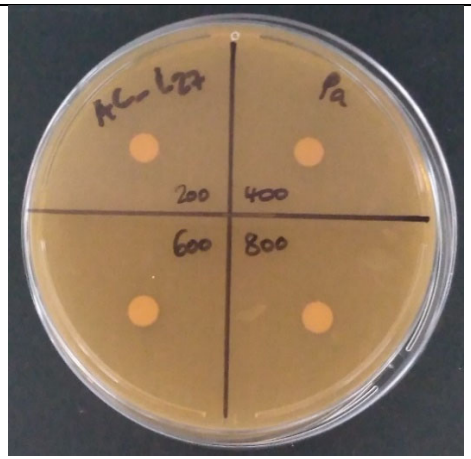
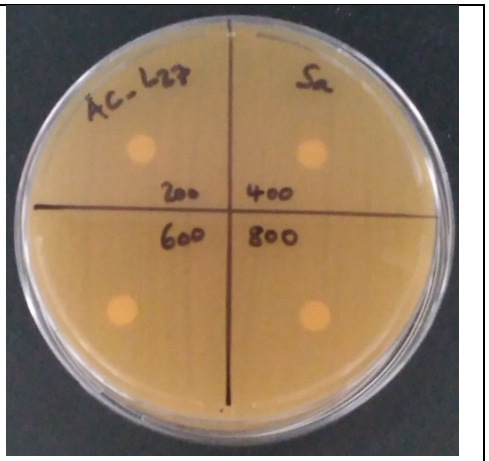
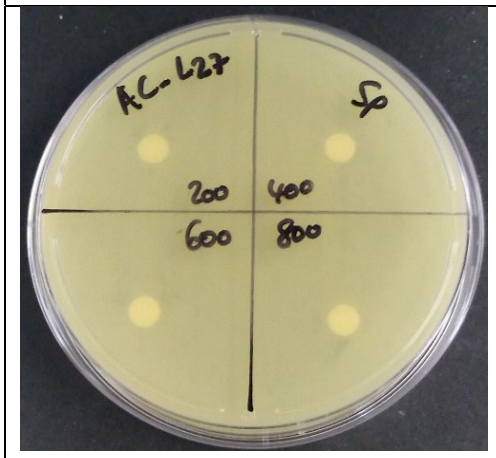
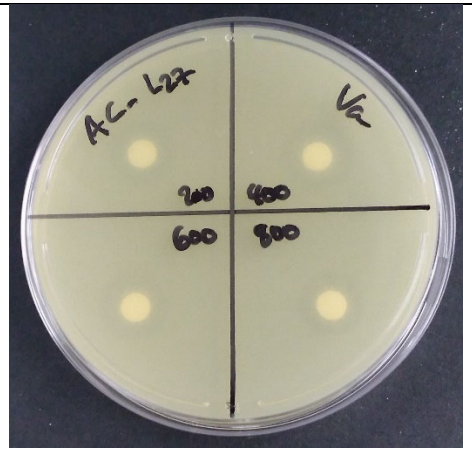
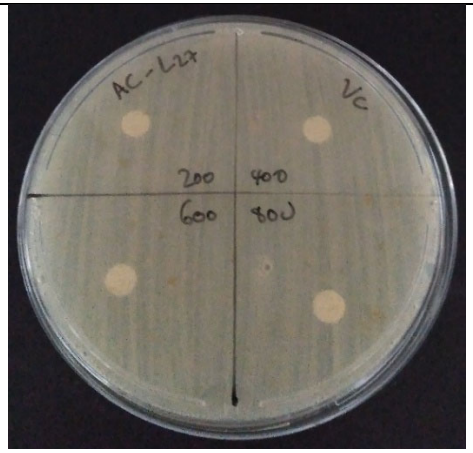
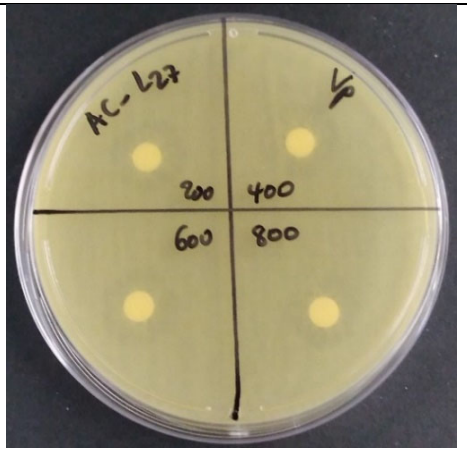


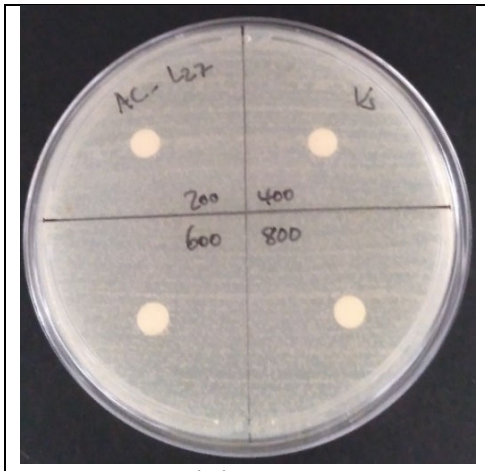
Ac_L12_*S. putrefaciens*



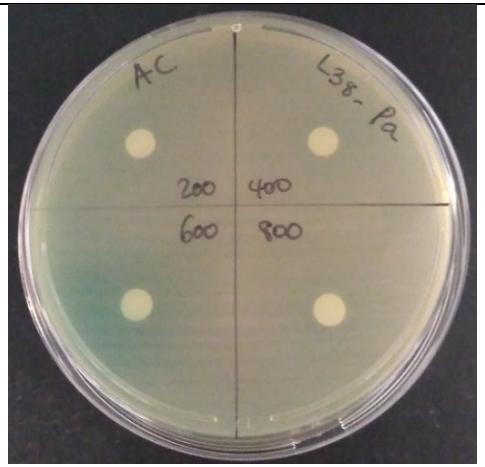
Ac_L12_*V. alginolyticus*

			
Ac_L12_*V. corallilyticus*	**Ac_L12**_*V. parahaemolyticus*	**Ac_L12**_*V. shilonii*	**Ac_L15**_*P. aeruginosa*
			
Ac_L15_*S. aureus*	**Ac_L15**_*S. putrefaciens*	**Ac_L15**_*V. alginolyticus*	**Ac_L15**_*V. corallilyticus*

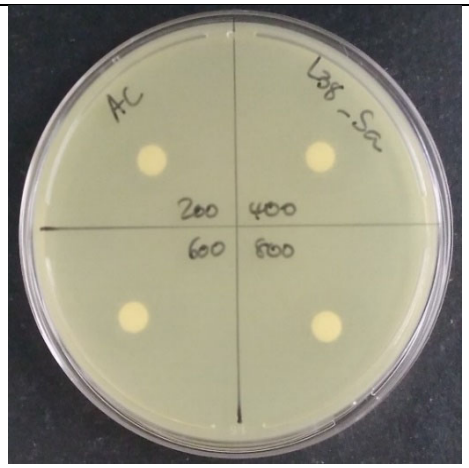
			
Ac_L15_*V. parahaemolyticus*	**Ac_L15**_*V. shilonii*	**Ac_L27**_*P. aeruginosa*	**Ac_L27**_*S. aureus*
			
Ac_L27_*S. putrefaciens*	**Ac_L27**_*V. alginolyticus*	**Ac_L27**_*V. corallilyticus*	**Ac_L27**_*V. parahaemolyticus*



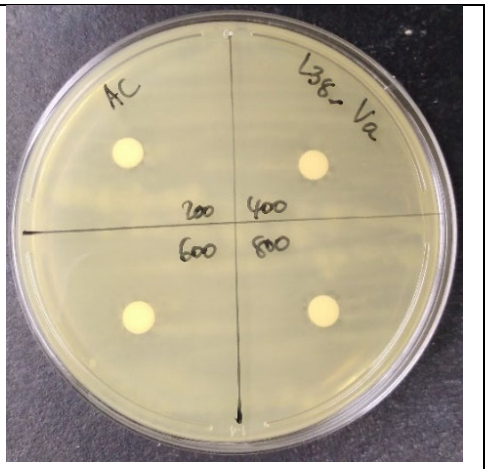
Ac_L27_*V. shilonii*



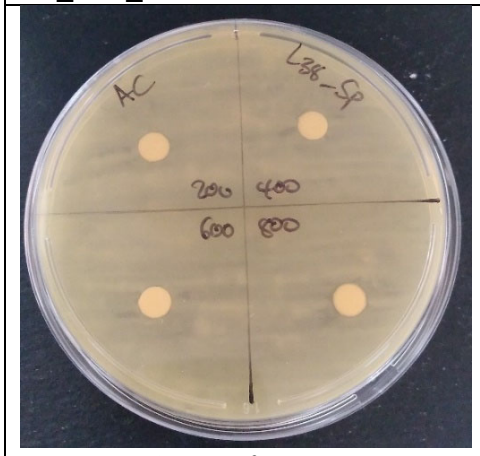
Ac_L38 *P. aeruginosa*



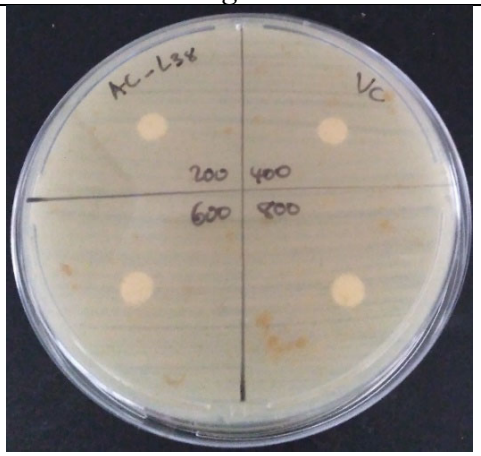
Ac_L38_*S. aureus*



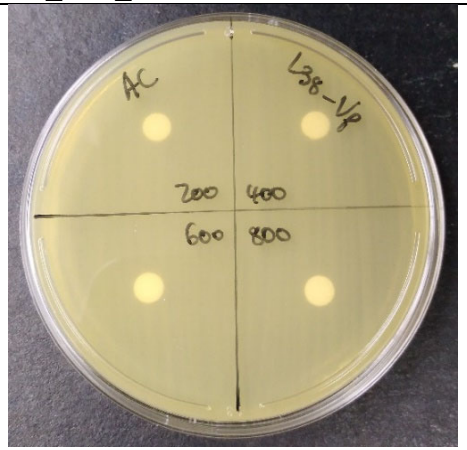
Ac_L38_*V. alginolyticus*



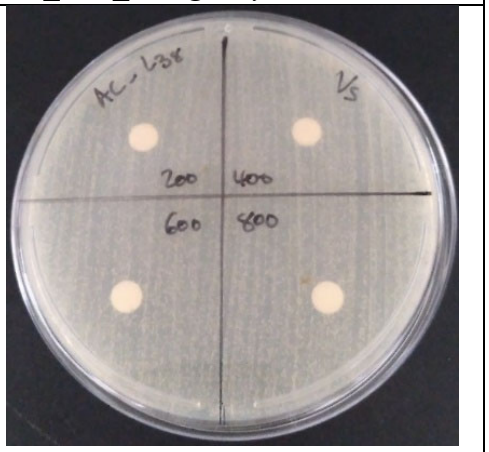
Ac_L38_*S. putrefaciens*



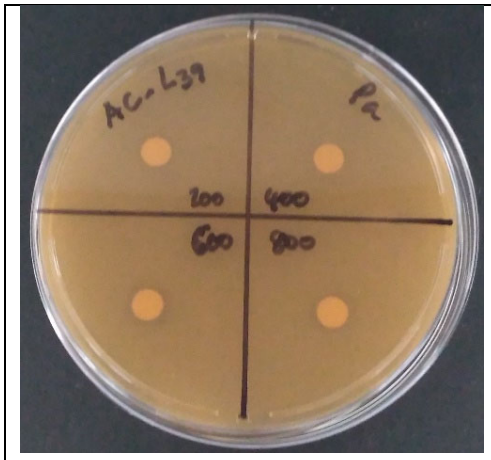
Ac_L38_*V. corallilyticus*



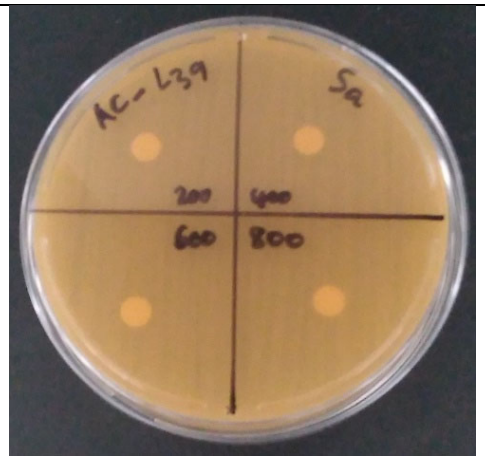
Ac_L38_*V. parahaemolyticus*



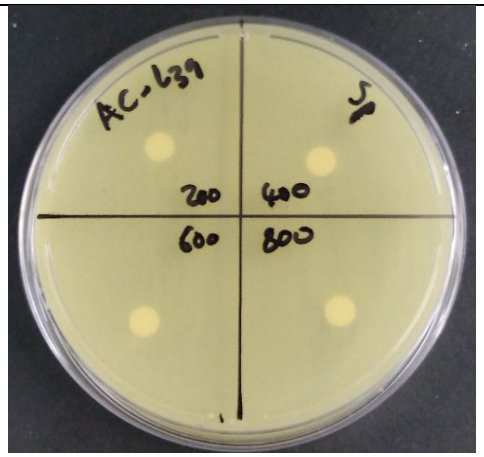
Ac_L38_*V. shilonii*



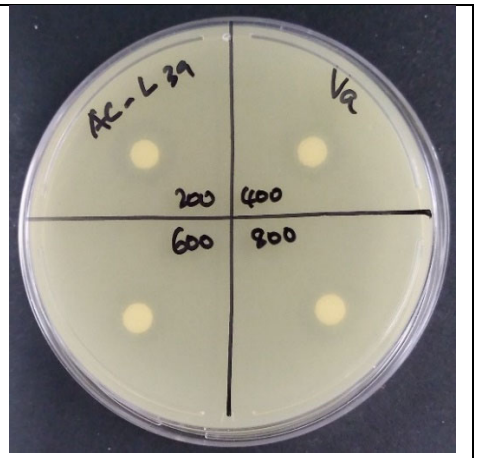
Ac_L39_*P. aeruginosa*



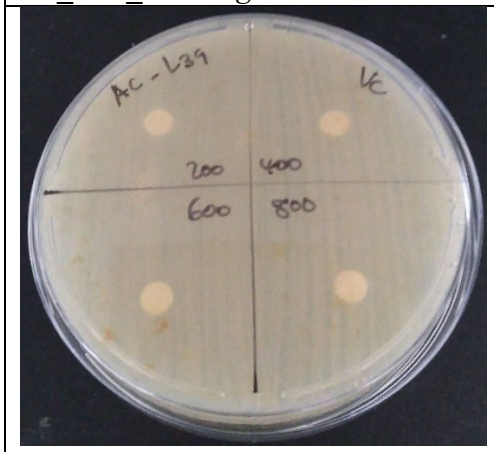
Ac_L39_*S. aureus*



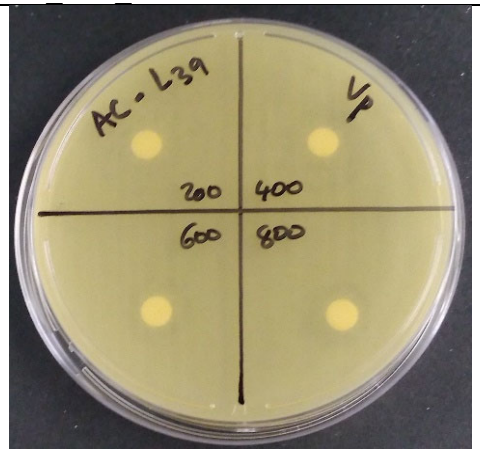
Ac_L39_*S. putrefaciens*



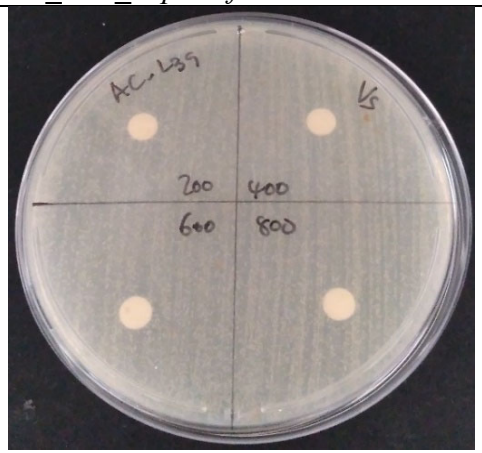
Ac_L39_*V. alginolyticus*



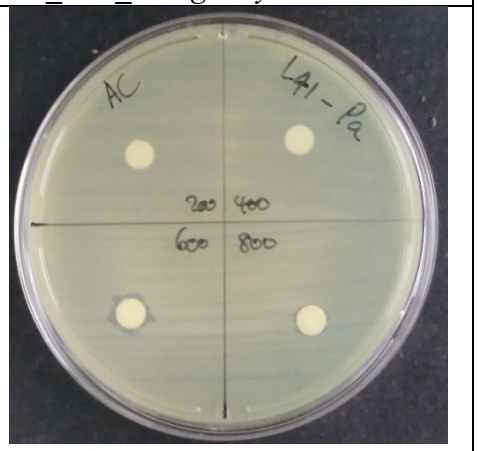
Ac_L39_*V. corallilyticus*



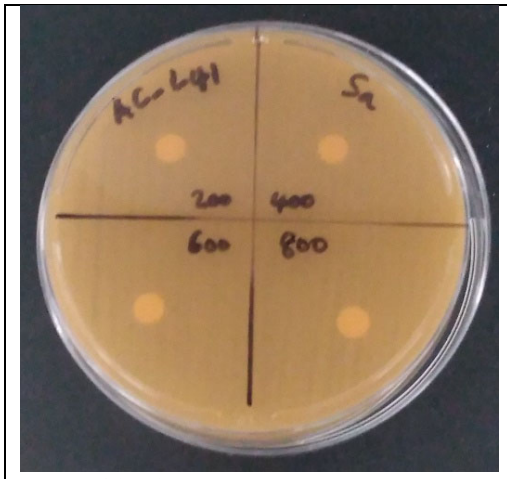
Ac_L39_*V. parahaemolyticus*



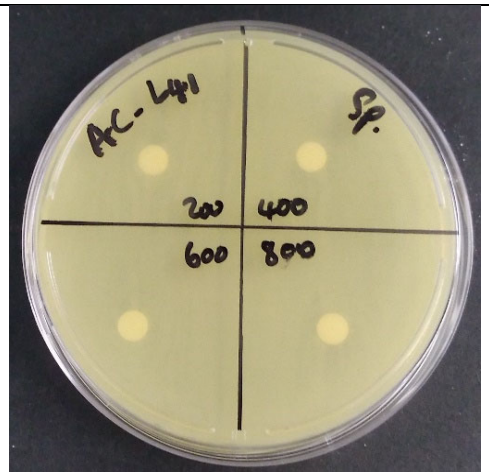
Ac_L39_*V. shilonii*



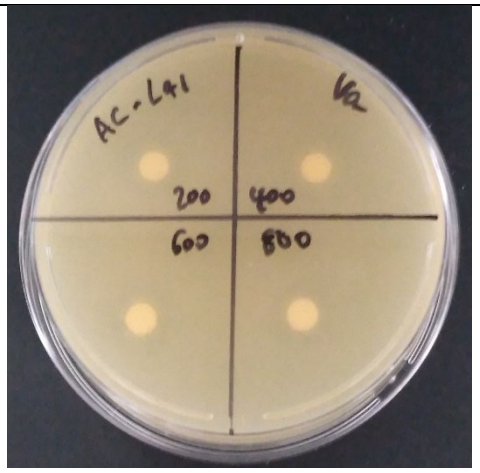
Ac_L41_*P. aeruginosa*



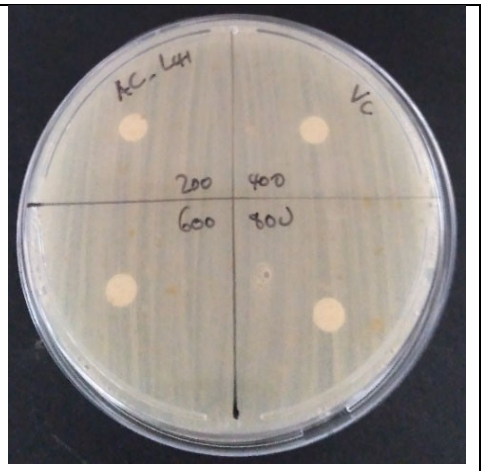
Ac_L41 *S. aureus*



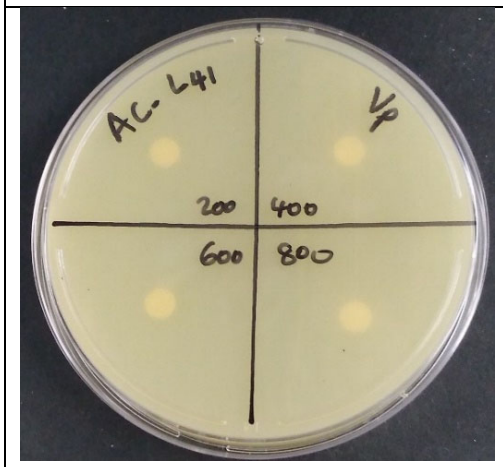
Ac_L41 *S. putrefaciens*



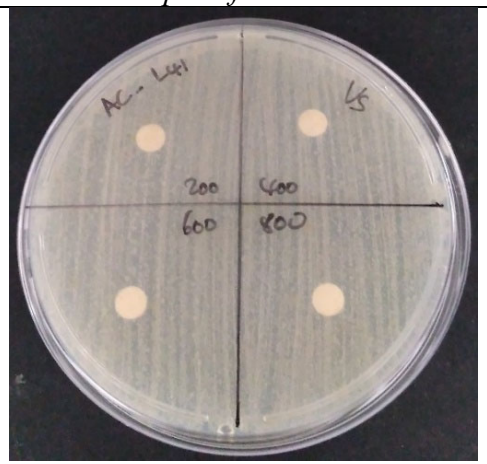
Ac_L41_*V. alginolyticus*



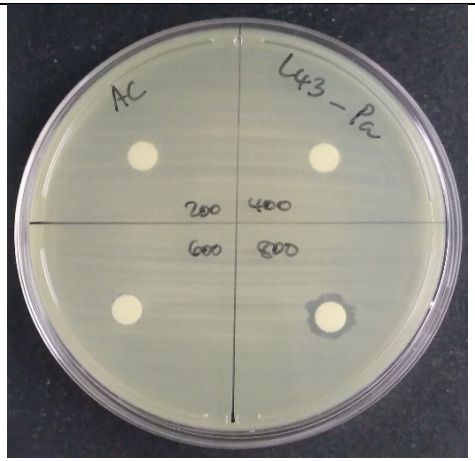
Ac_L41_*V. corallilyticus*



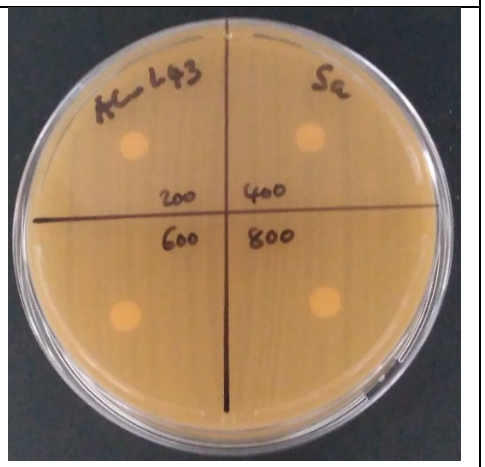
Ac_L41_*V. parahaemolyticus*



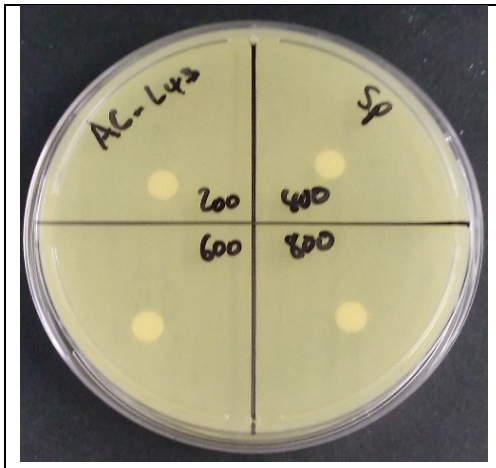
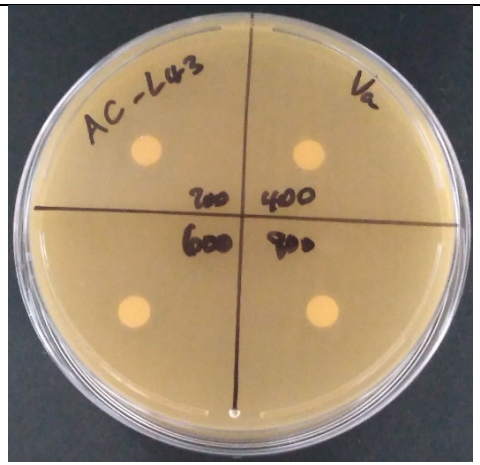
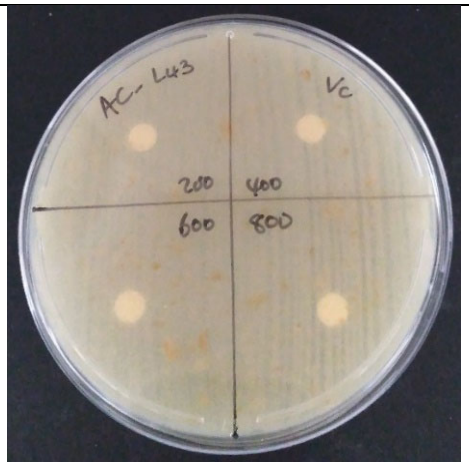
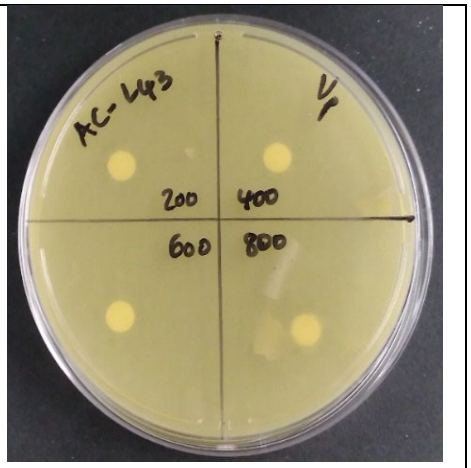
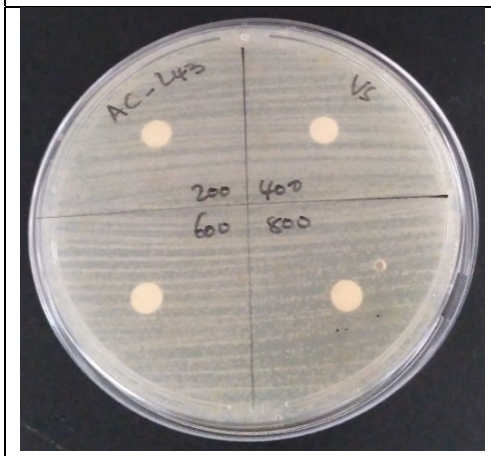
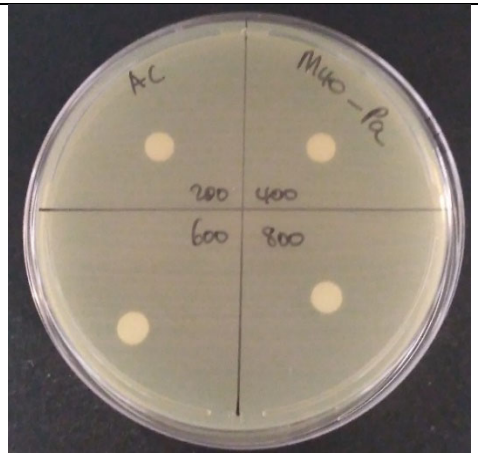
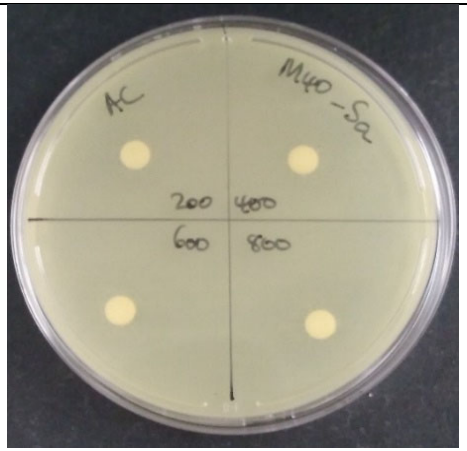
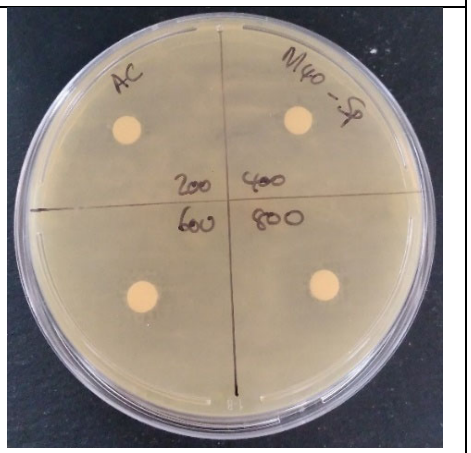
Ac_L41_*V. shilonii*

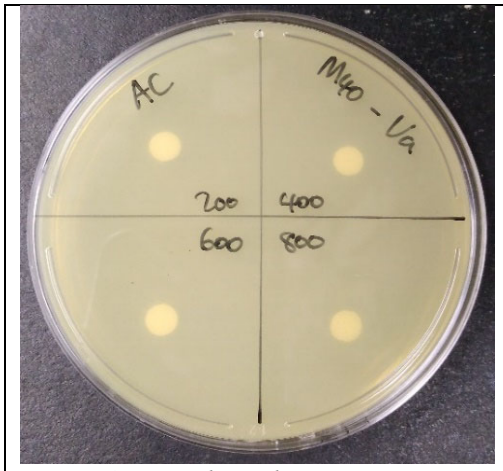


Ac_L43_*P. aeruginosa*

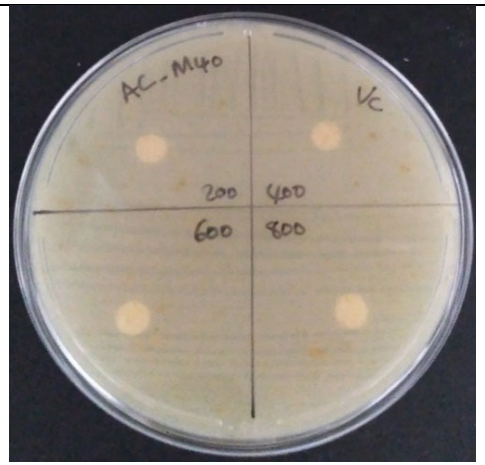


Ac_L43_*S. aureus*

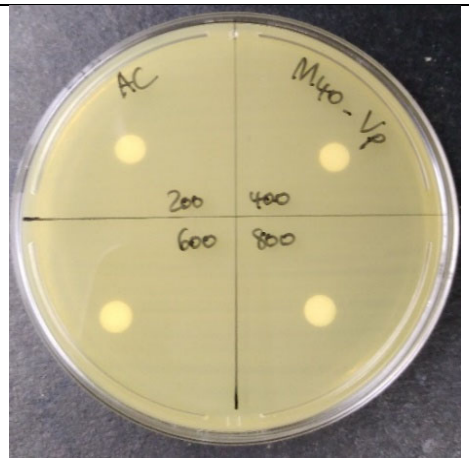
 **Ac_L43**_*S. putrefaciens*	 **Ac_L43**_*V. alginolyticus*	 **Ac_L43**_*V. corallilyticus*	 **Ac_L43**_*V. parahaemolyticus*
 **Ac_L43**_*V. shilonii*	 **Ac_M40**_*P. aeruginosa*	 **Ac_M40**_*S. aureus*	 **Ac_M40**_*S. putrefaciens*



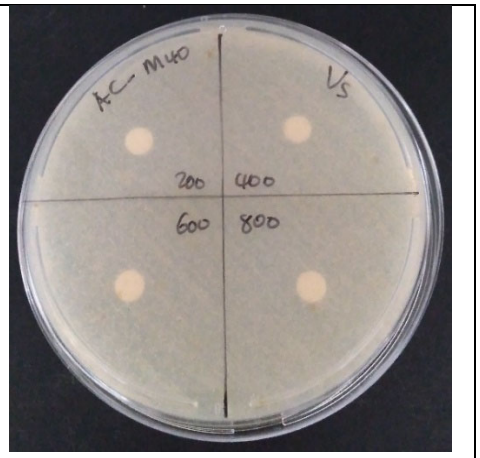
Ac_M40_*V. alginolyticus*



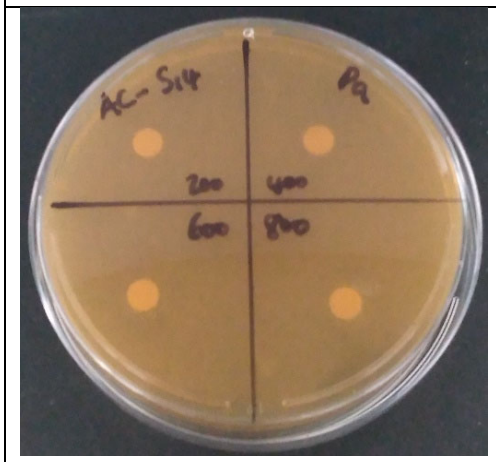
Ac_M40_*V. corallilyticus*



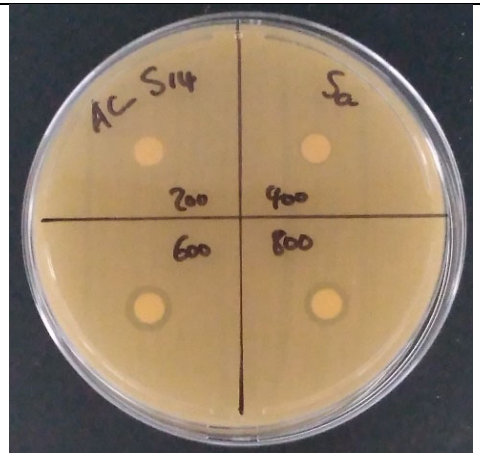
Ac_M40_*V. parahaemolyticus*



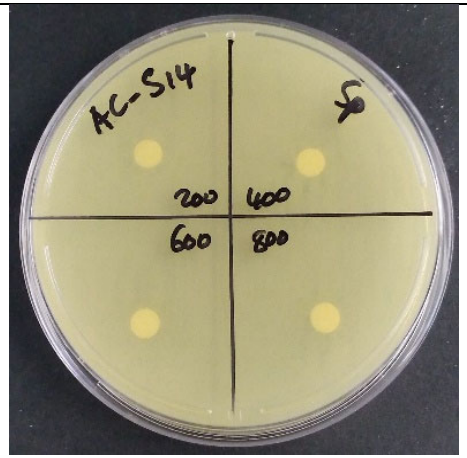
Ac_M40_*V. shilonii*



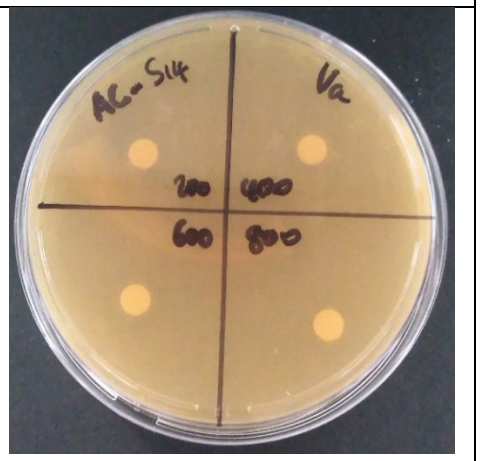
Ac_S14_*P. aeruginosa*



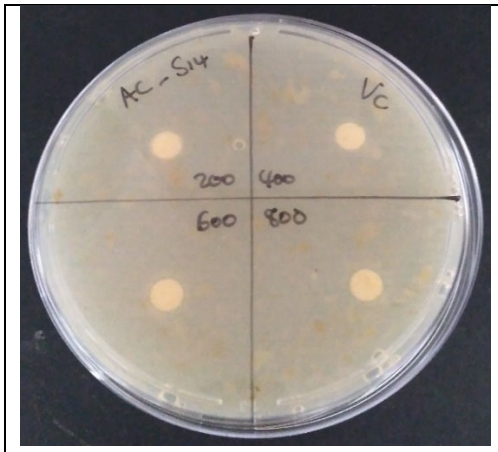
Ac_S14_*S. aureus*



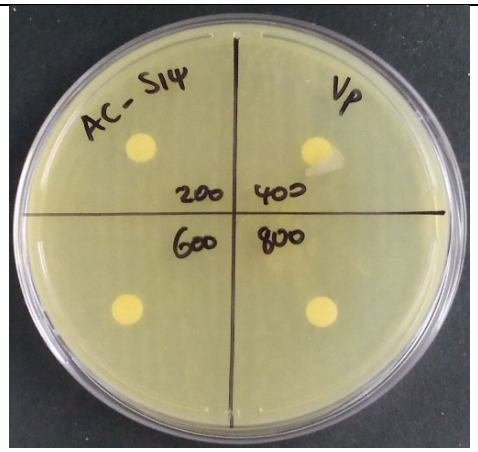
Ac_S14_*S. putrefaciens*



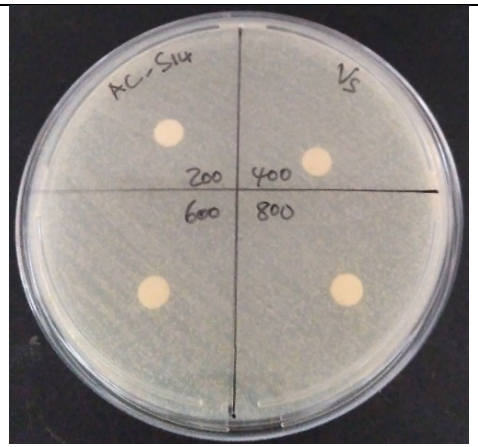
Ac_S14_*V. alginolyticus*



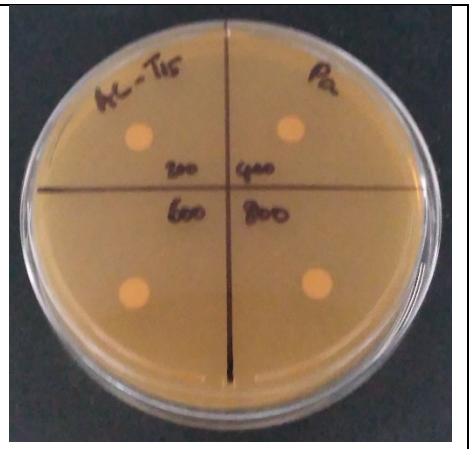
Ac_S14_*V. corallilyticus*



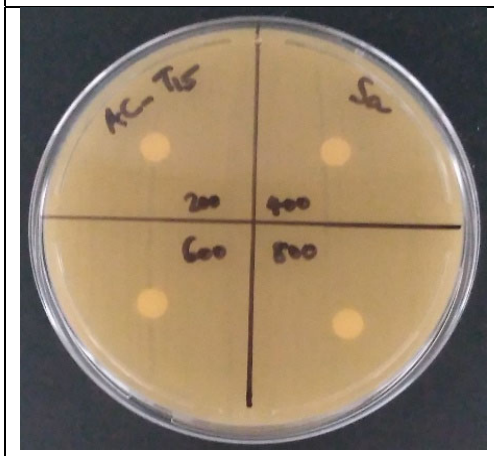
Ac_S14_*V. parahaemolyticus*



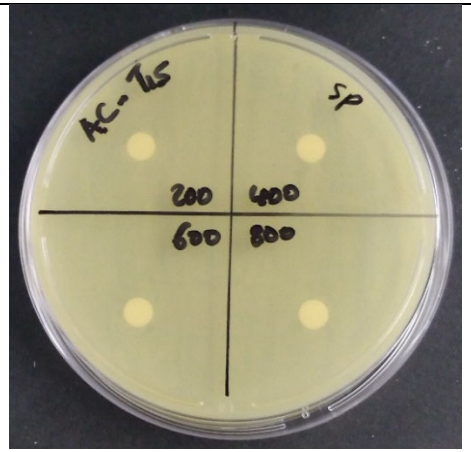
Ac_S14_*V. shilonii*



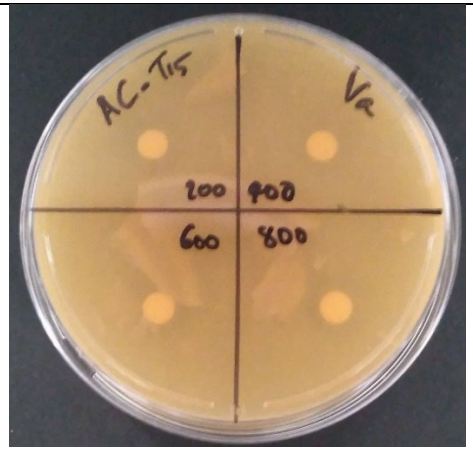
Ac_T15_*P. aeruginosa*



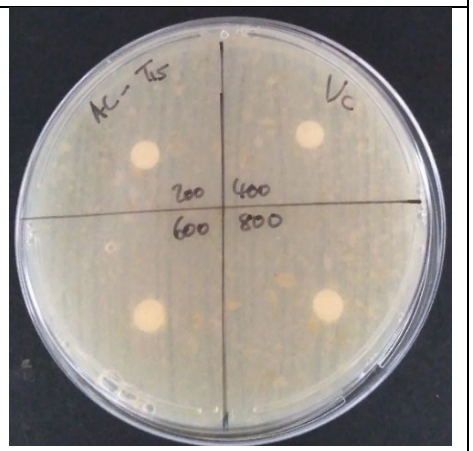
Ac_T15_*S. aureus*



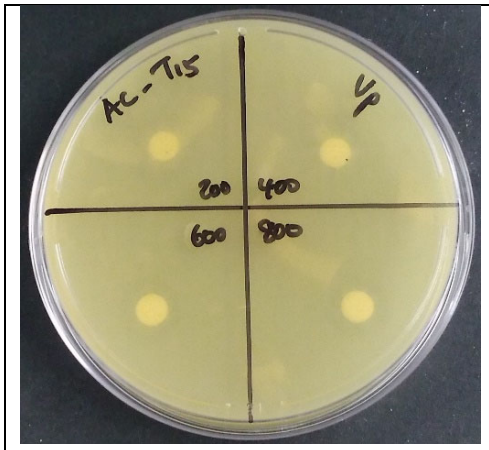
Ac_T15_*S. putrefaciens*



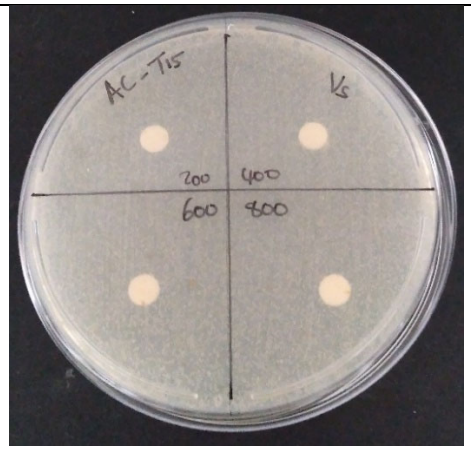
Ac_T15_*V. alginolyticus*



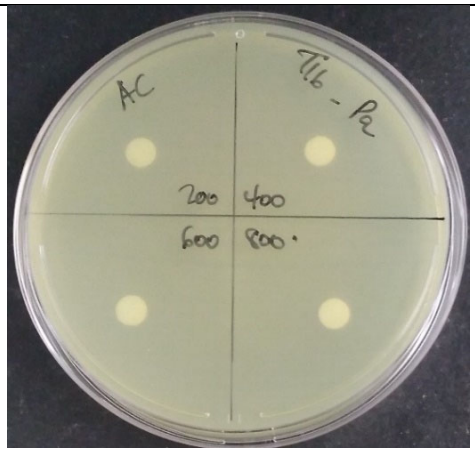
Ac_T15_*V. corallilyticus*



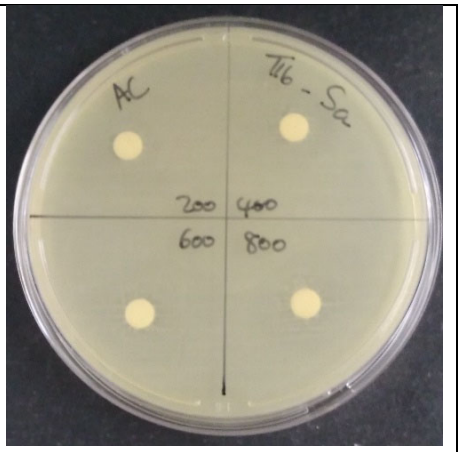
Ac_T15_*V. parahaemolyticus*



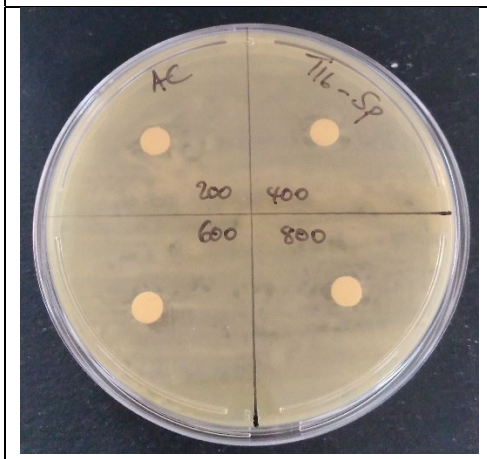
Ac_T15_*V. shilonii*



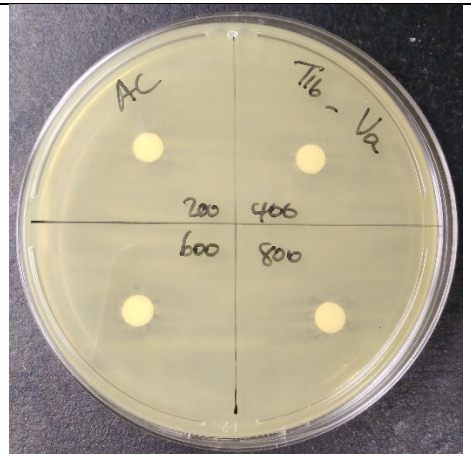
Ac_T16_*P. aeruginosa*



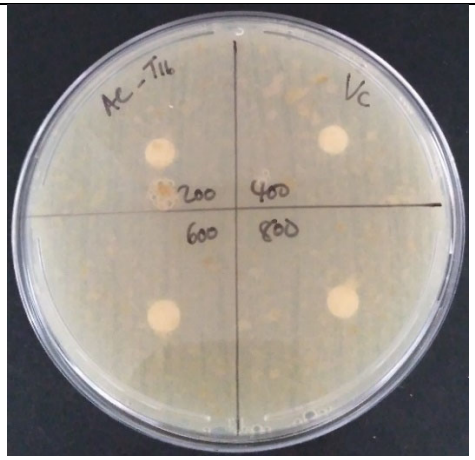
Ac_T16_*S. aureus*



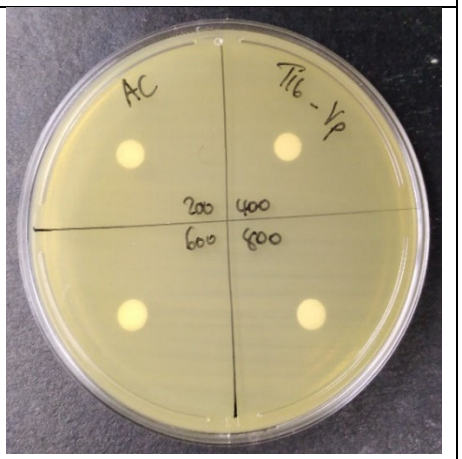
Ac_T16_*S. putrefaciens*



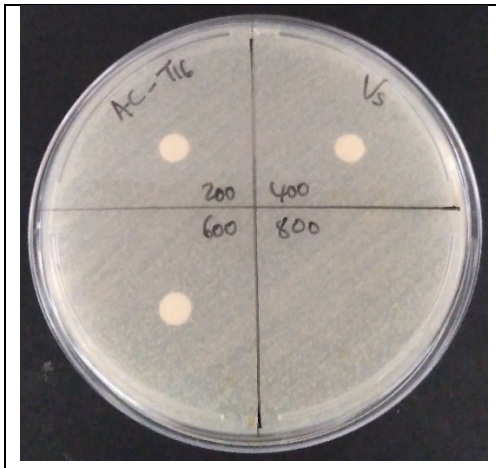
Ac_T16_*V. alginolyticus*



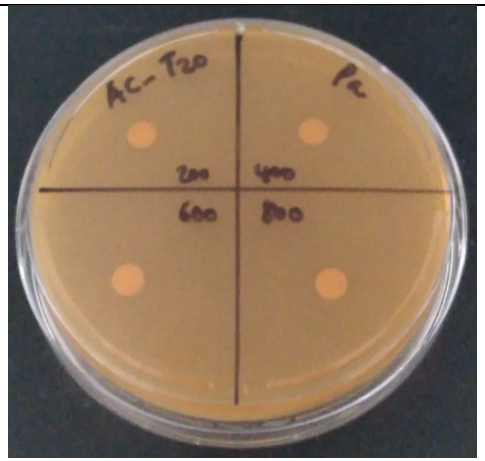
Ac_T16_*V. corallilyticus*



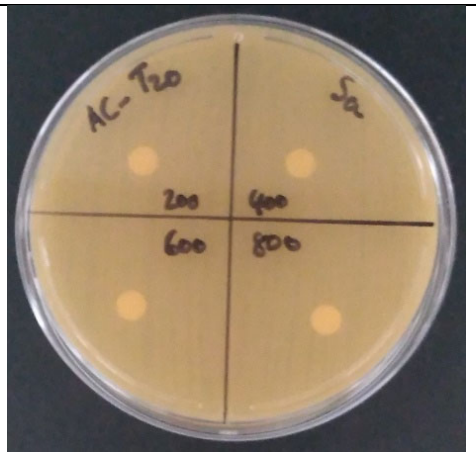
Ac_T16_*V. parahaemolyticus*



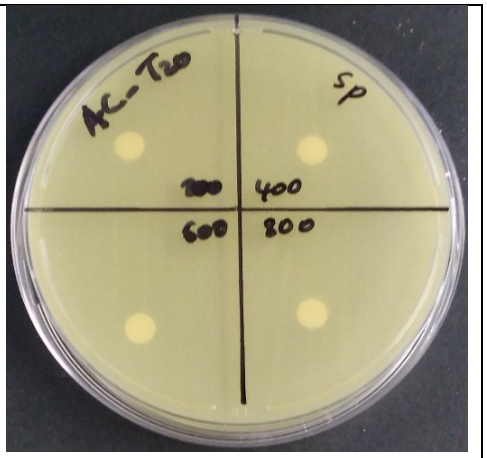
Ac_T16_*V. shilonii*



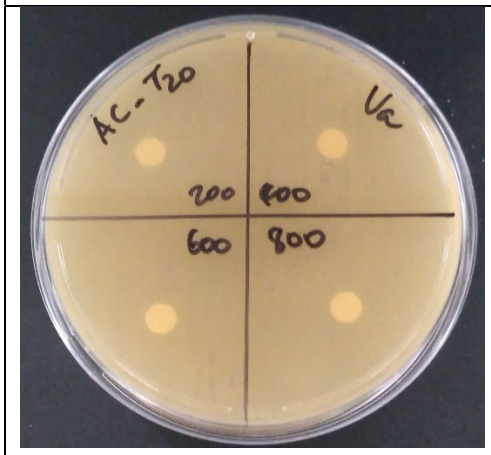
Ac_T20_*P. aeruginosa*



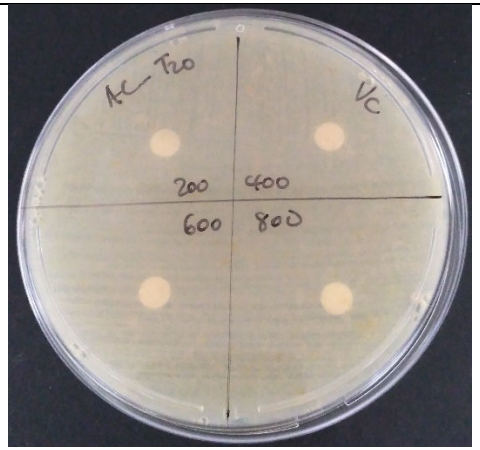
Ac_T20_*S. aureus*



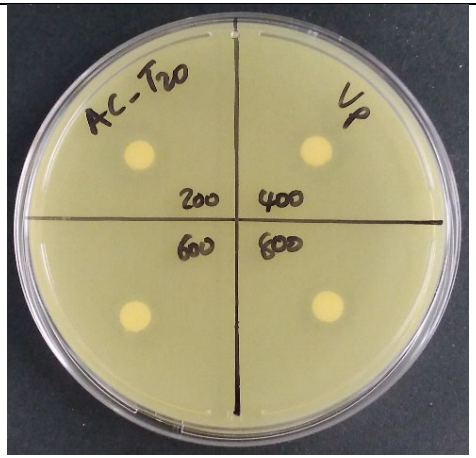
Ac_T20_*S. putrefaciens*



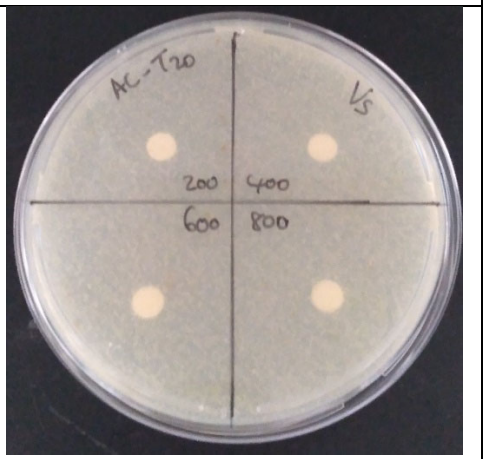
Ac_T20_*V. alginolyticus*



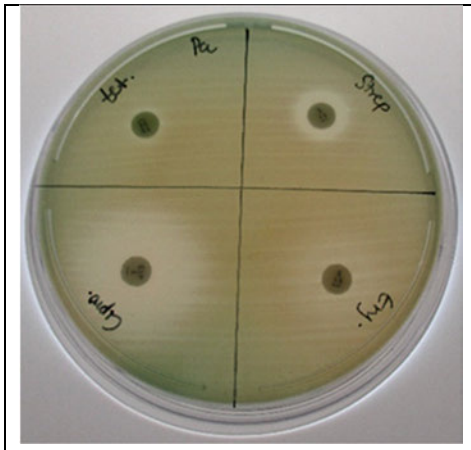
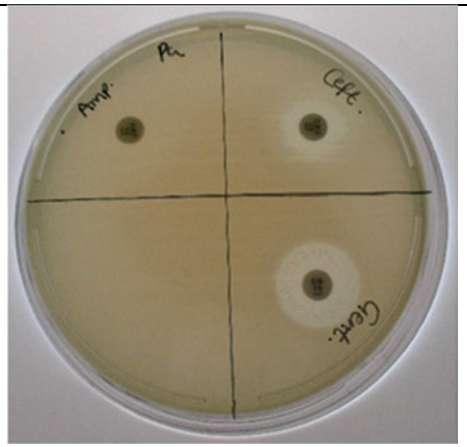
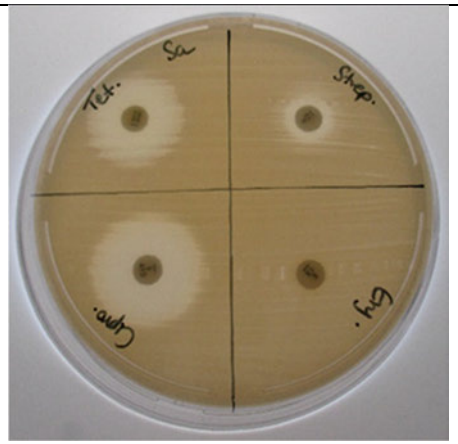
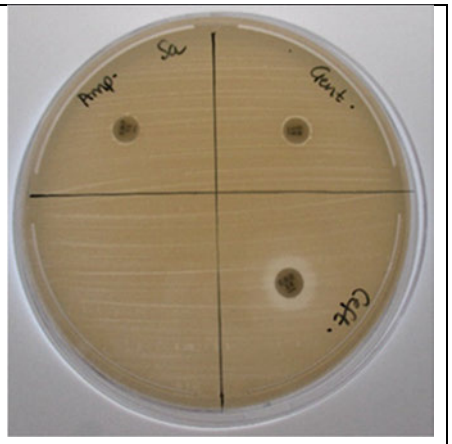
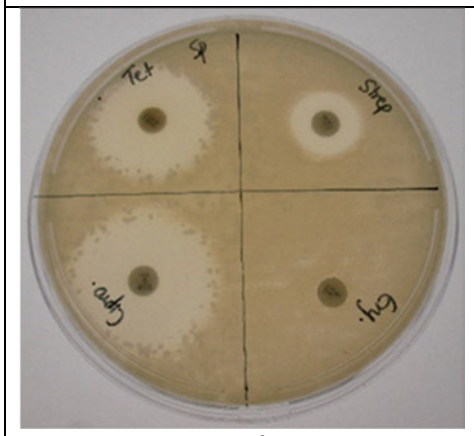
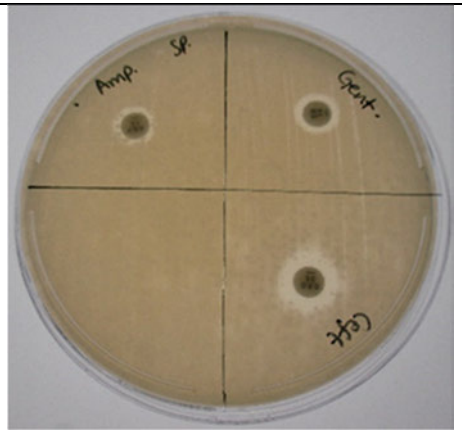
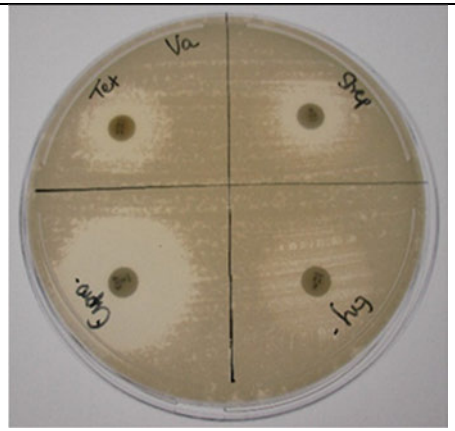
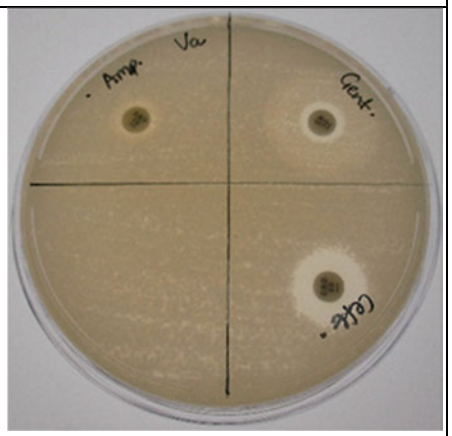
Ac_T20_*V. corallilyticus*

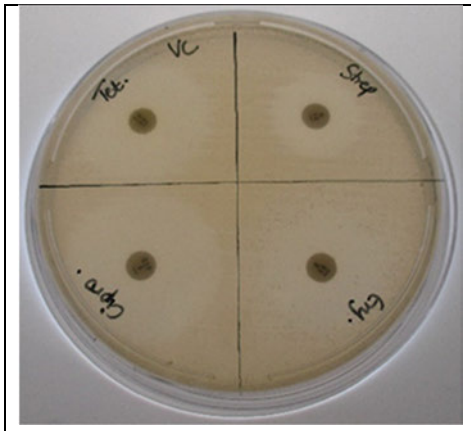


Ac_T20_*V. parahaemolyticus*

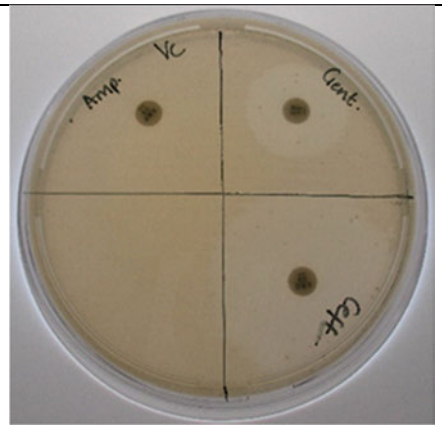


Ac_T20_*V. shilonii*

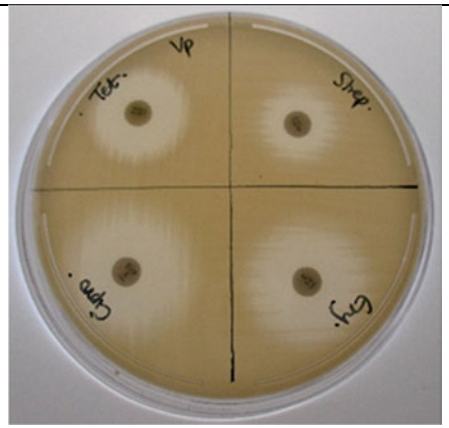
 **Control**_*P. aeruginosa*	 **Control**_ *P. aeruginosa*	 **Control**_ *S. aureus*	 **Control**_ *S. aureus*
 **Control**_ *S. putrefaciens*	 **Control**_ *S. putrefaciens*	 **Control**_*V. alginolyticus*	 **Control**_*V. alginolyticus*



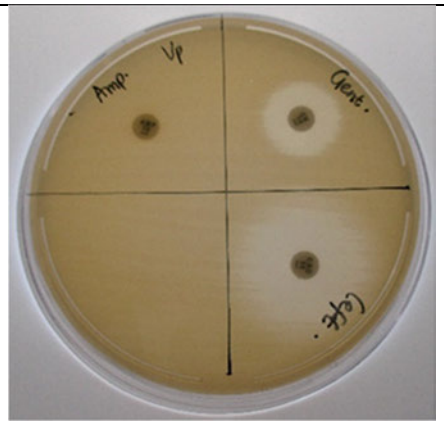
Control_ *V. corallilyticus*



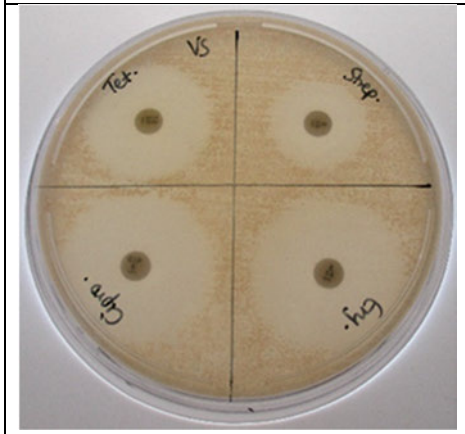
Control_ *V. corallilyticus*



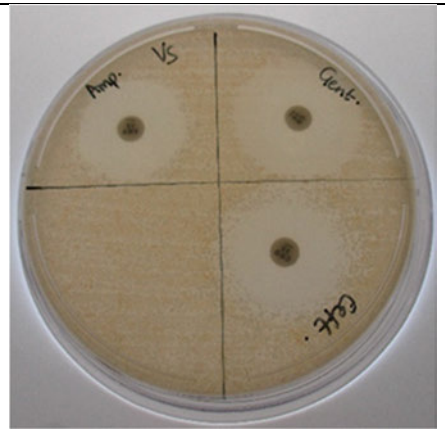
Control_*V. parahaemolyticus*



Control_*V. parahaemolyticus*



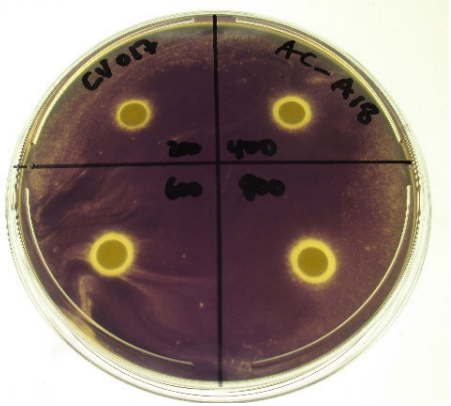
Control *V. shilonii*



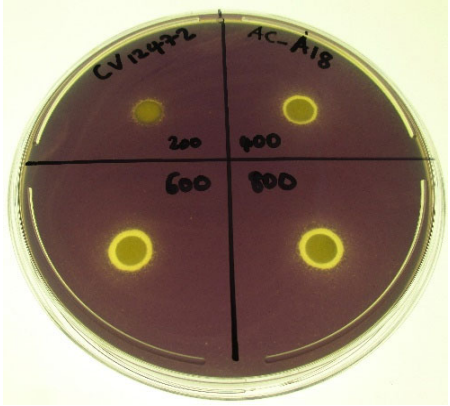
Control *V. shilonii*

Figure S4.2: Twenty-four *Acropora* CAB extracts were subjected to secondary screening using the disc diffusion assay against clinical indicators, multidrug-resistant *Pseudomonas aeruginosa* ATCC 27853, methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300, marine bacteria *Shewanella putrefaciens* ATCC 8071, *Vibrio alginolyticus* ATCC 17749, *Vibrio parahaemolyticus* ATCC 17802, and marine pathogens *Vibrio coralliilyticus* ATCC_BAA 450, *Vibrio shilonii* ATCC_BAA 91. Antimicrobial activity was denoted by the appearance of clear and opaque zones around the discs, while clear zones were indicative of killing activity.

4.6.3 Anti-quorum sensing screening of 24 *Acropora* CAB extracts.



Ac_A18_CV017



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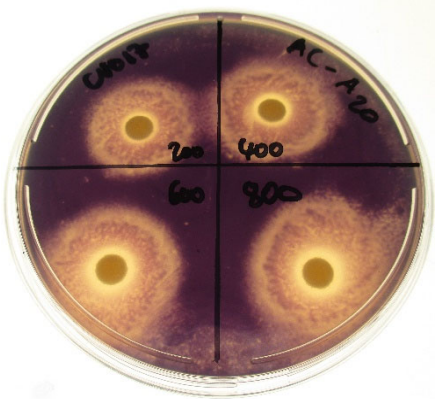

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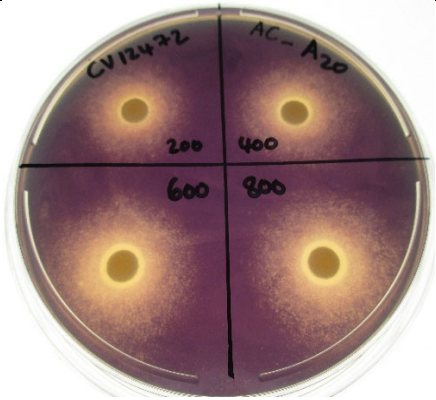

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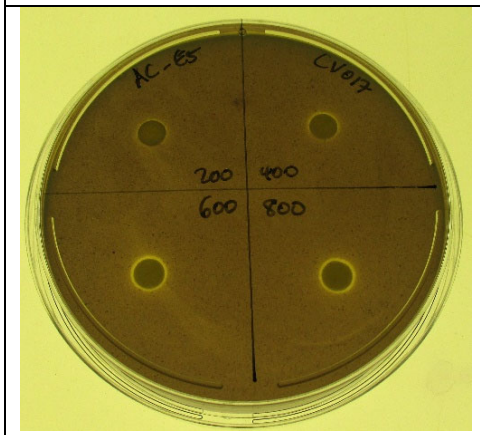

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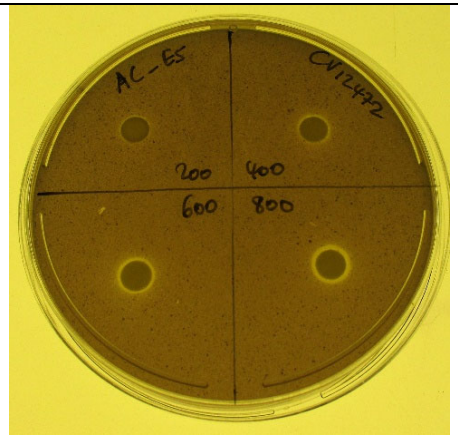

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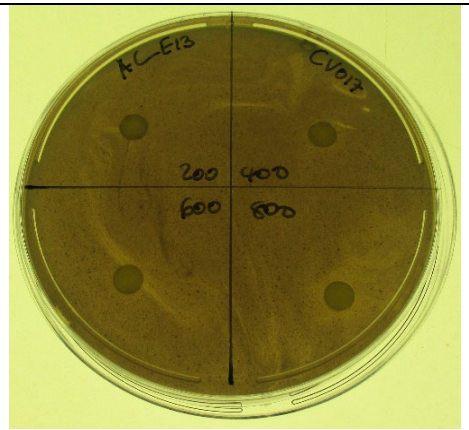

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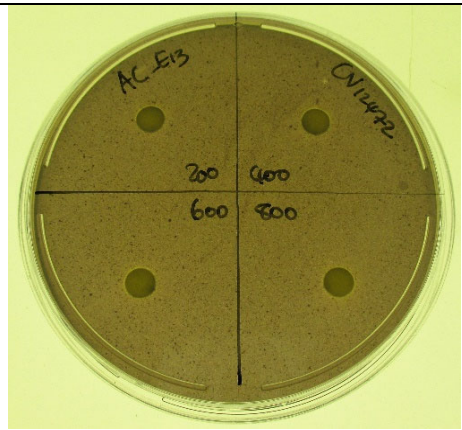

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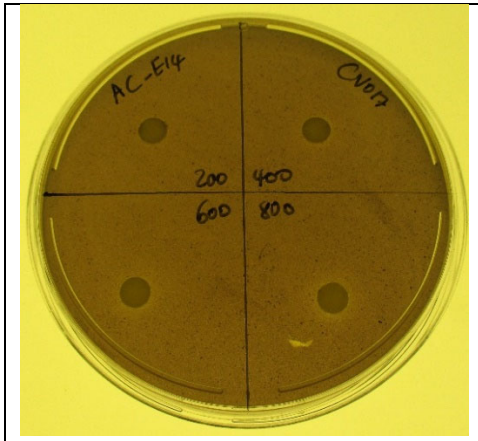

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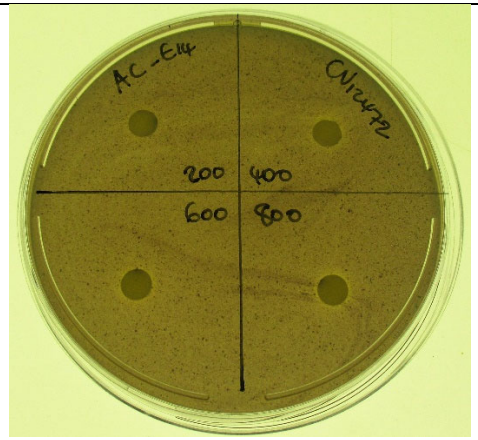

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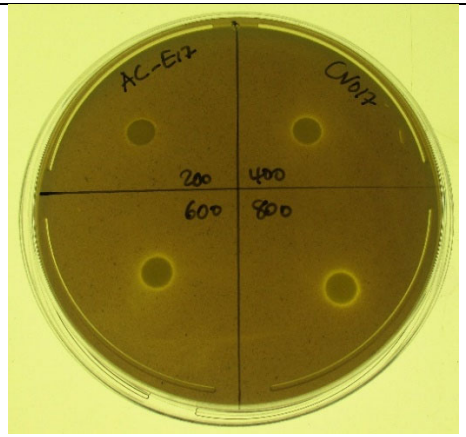

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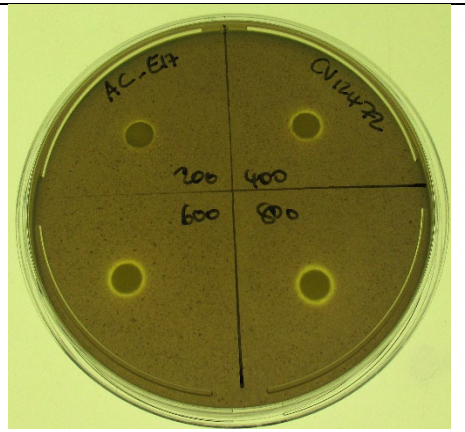

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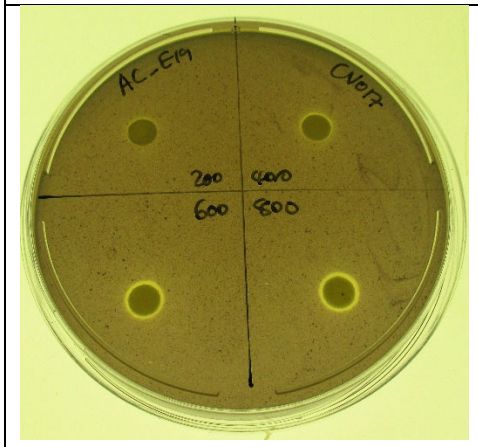

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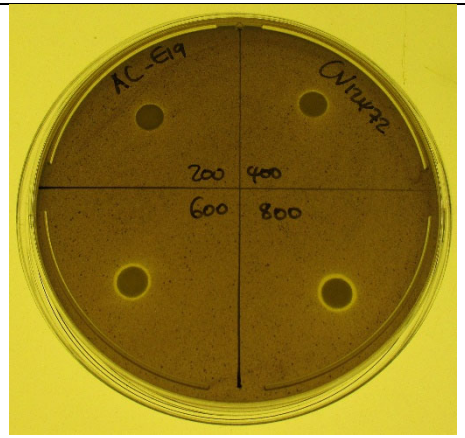

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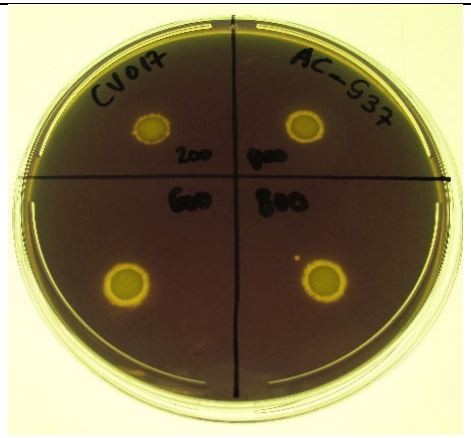

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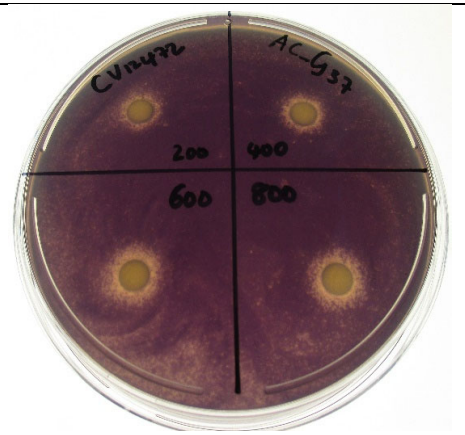

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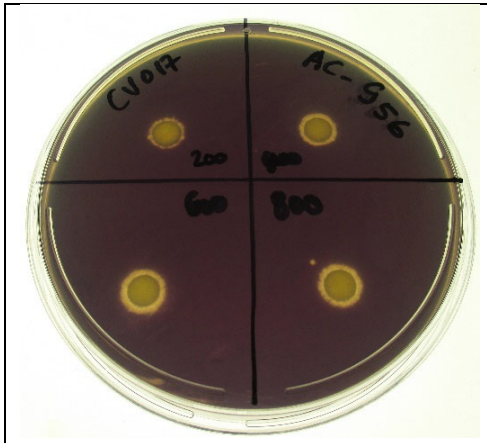
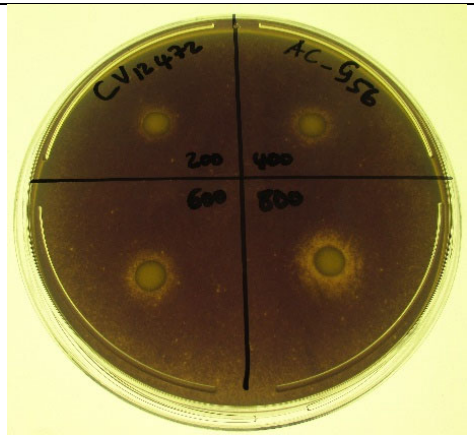
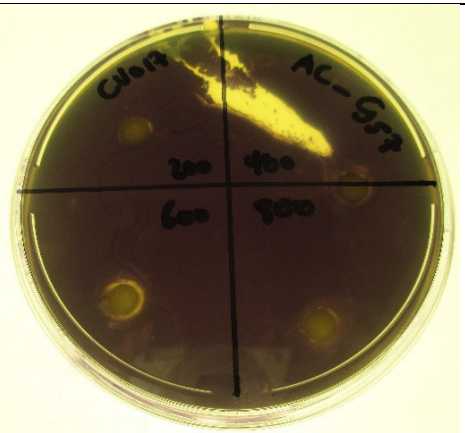
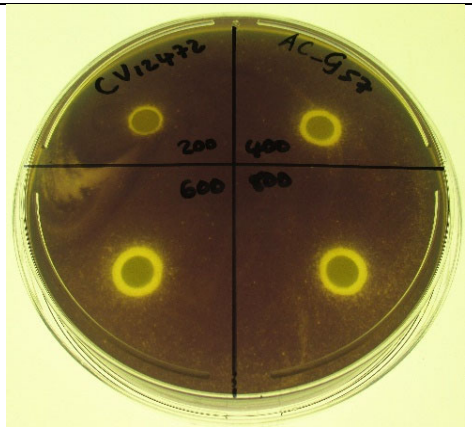
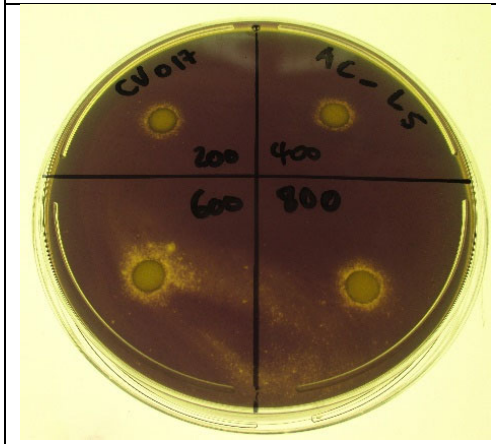
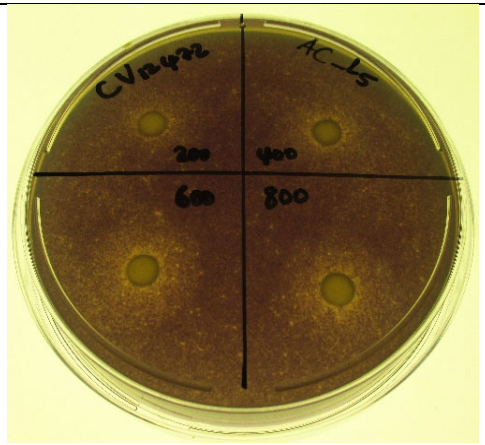
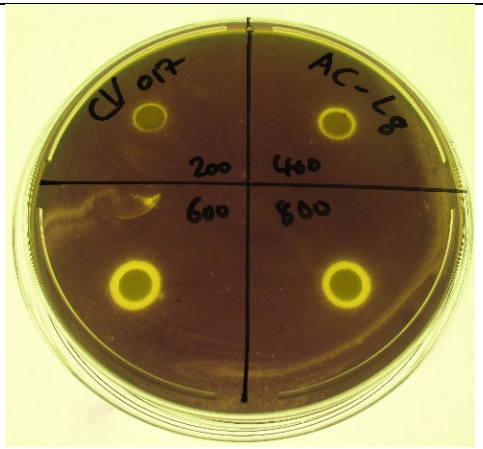
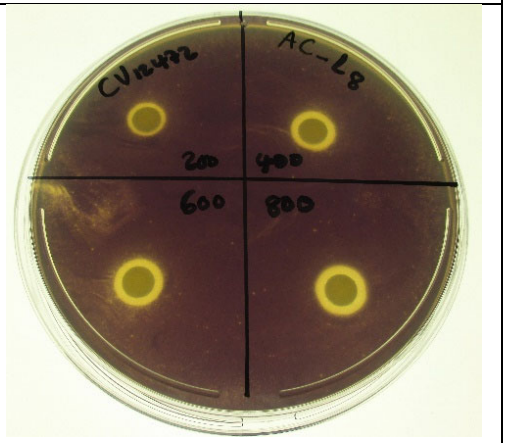
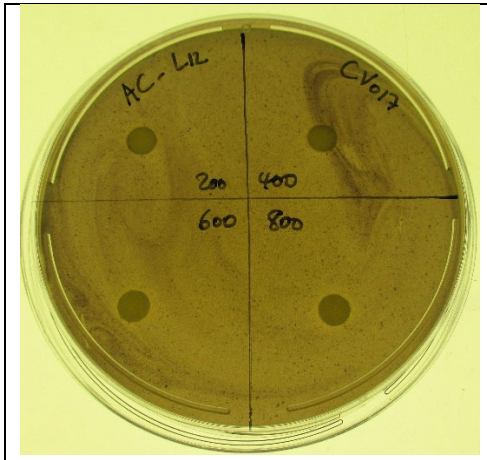
 Ac_G56_CV017	 Ac_G56_CV12472	 Ac_G57_CV017	 Ac_G57_CV12472
 Ac_L5_CV017	 Ac_L5_CV12472	 Ac_L8_CV017	 Ac_L8_CV12472



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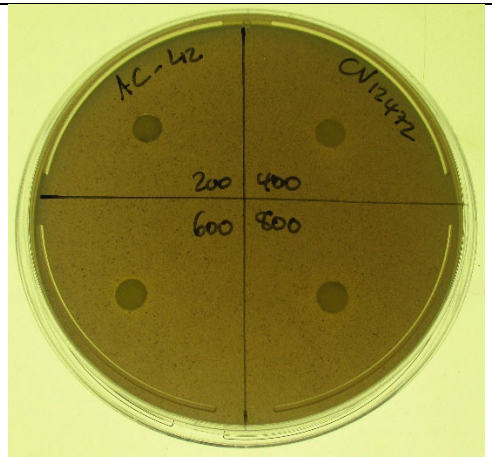

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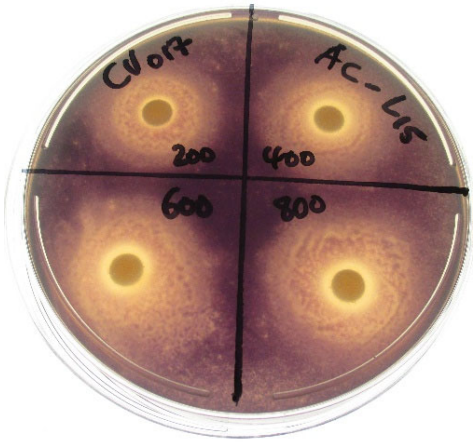

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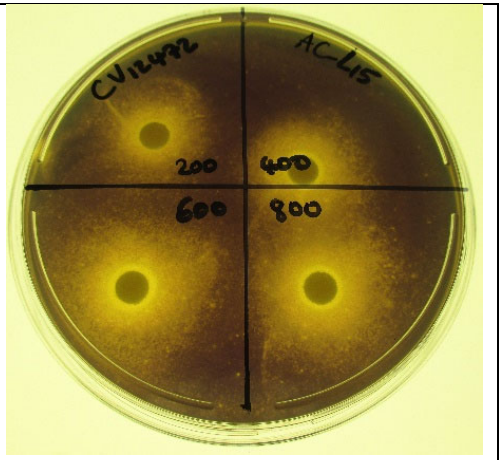

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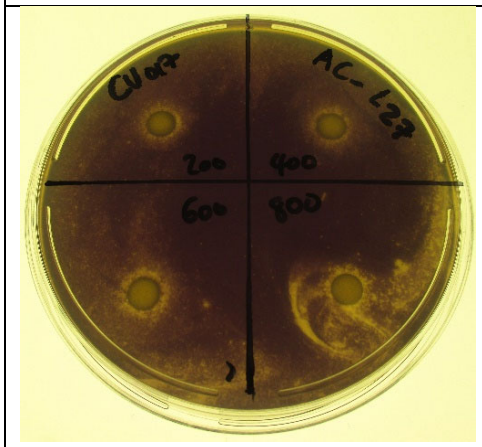

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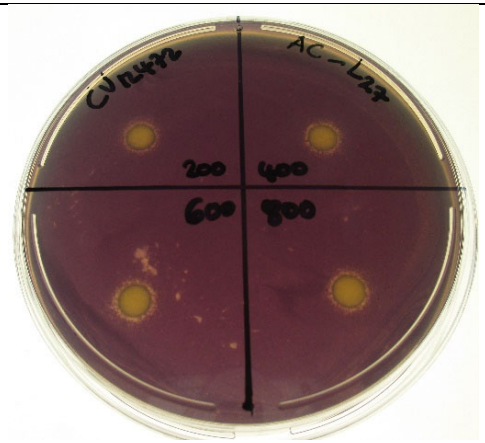

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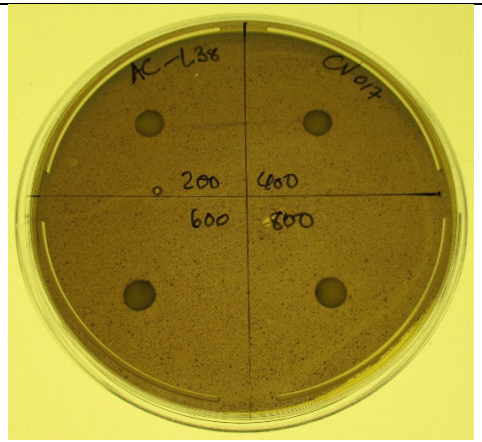

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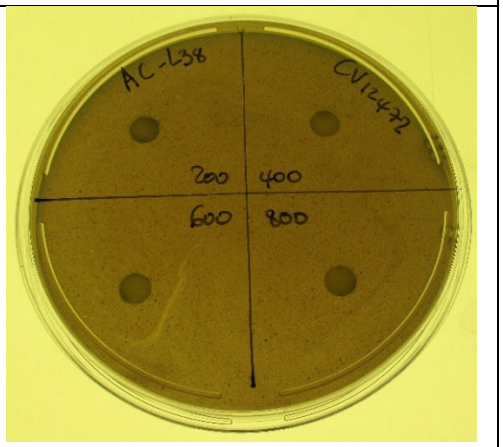

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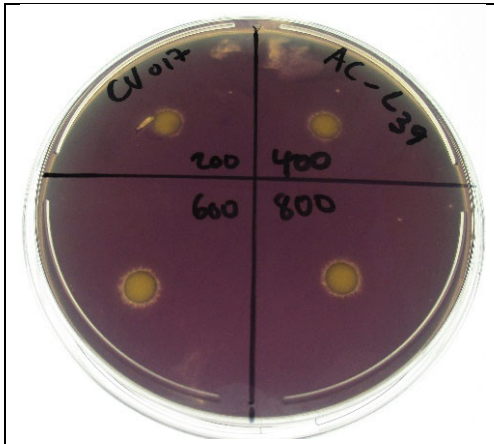

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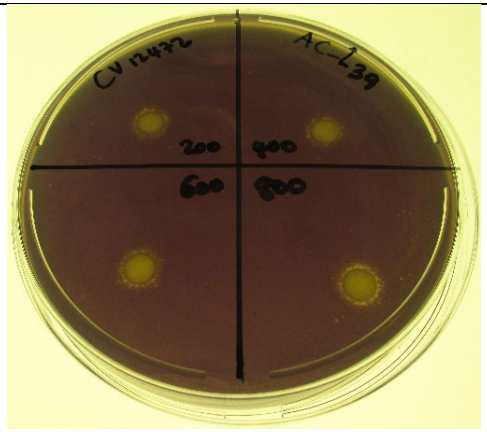

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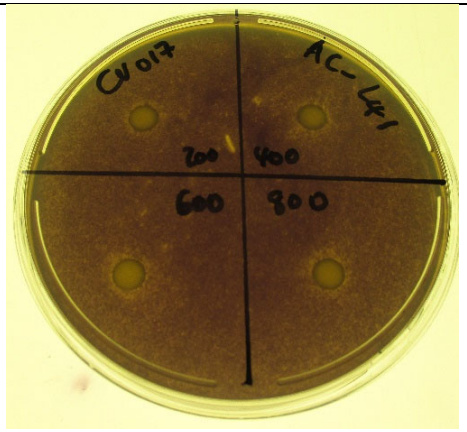

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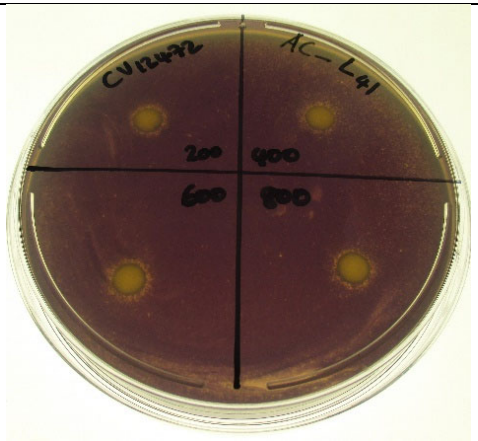

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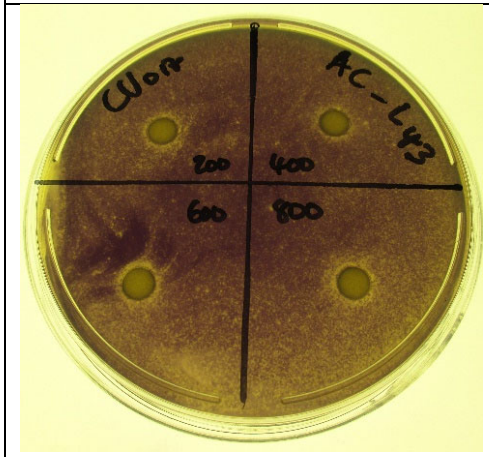

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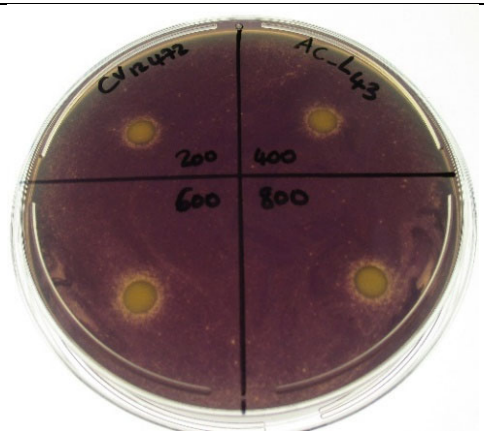

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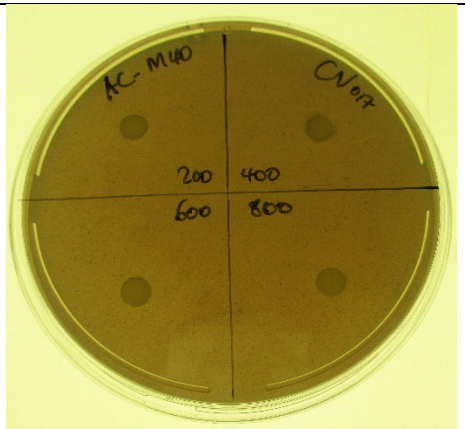

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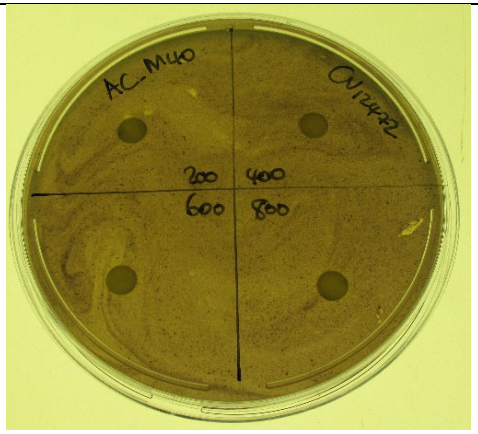

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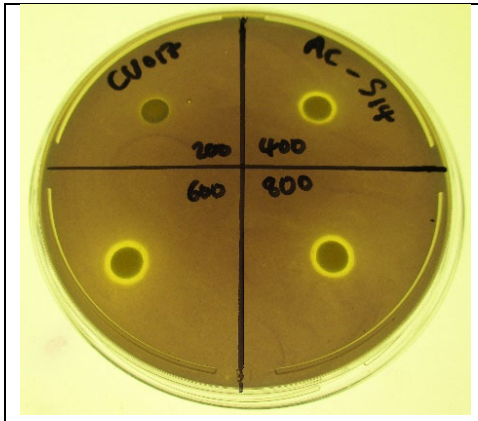
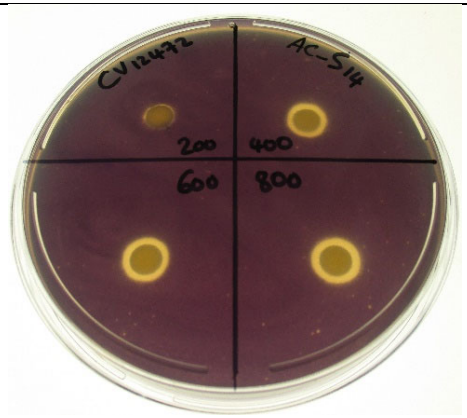
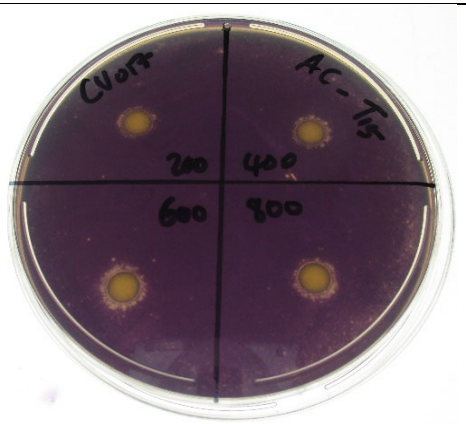
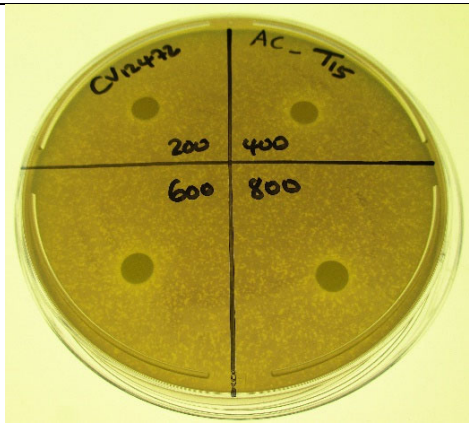
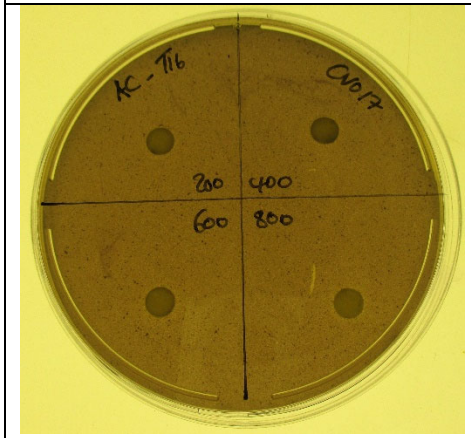
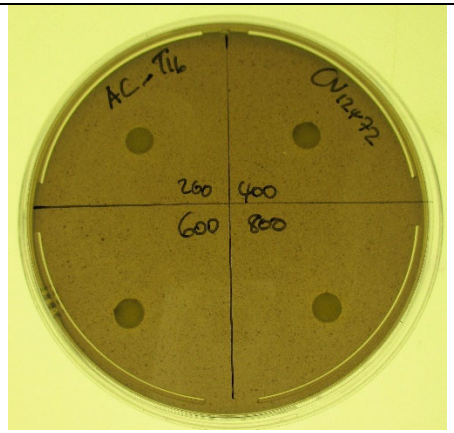
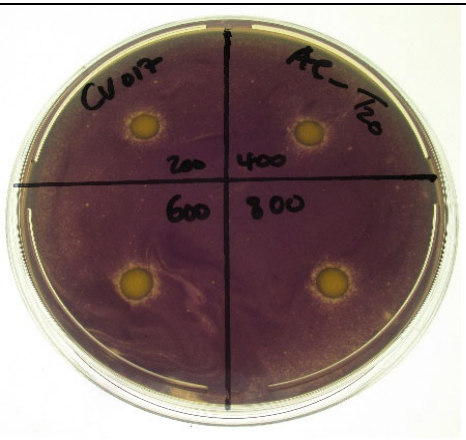
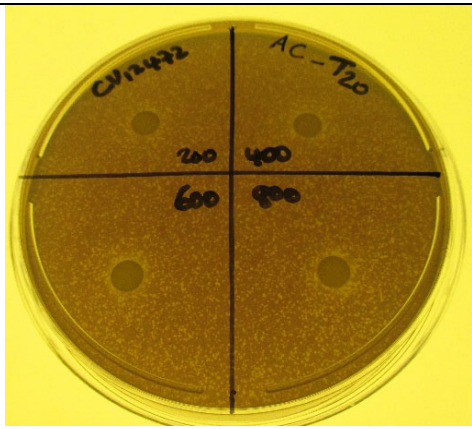
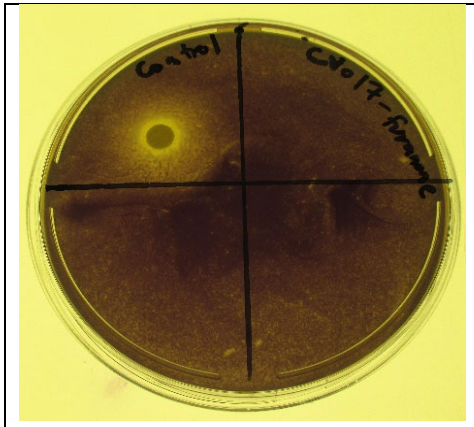
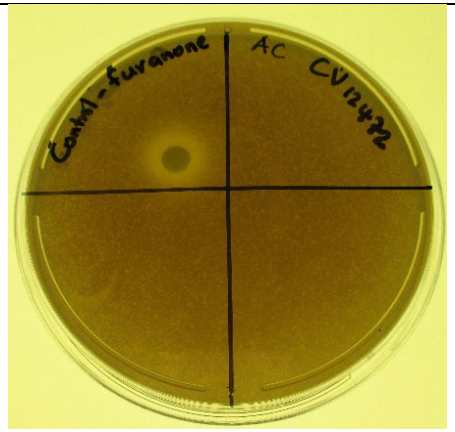
 Ac_S14_CV017	 Ac_S14_CV12472	 Ac_T15_CV017	 Ac_T15_CV12472
 Ac_T16_CV017	 Ac_T16_CV12472	 Ac_T20_CV017	 Ac_T20_CV12472

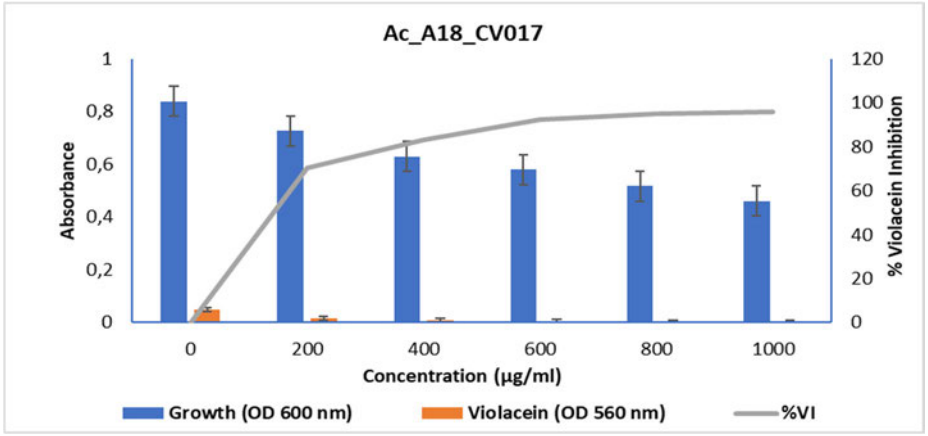


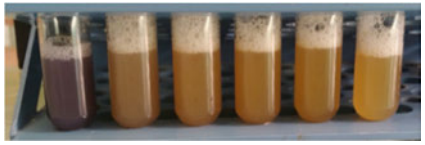
Control_CV017

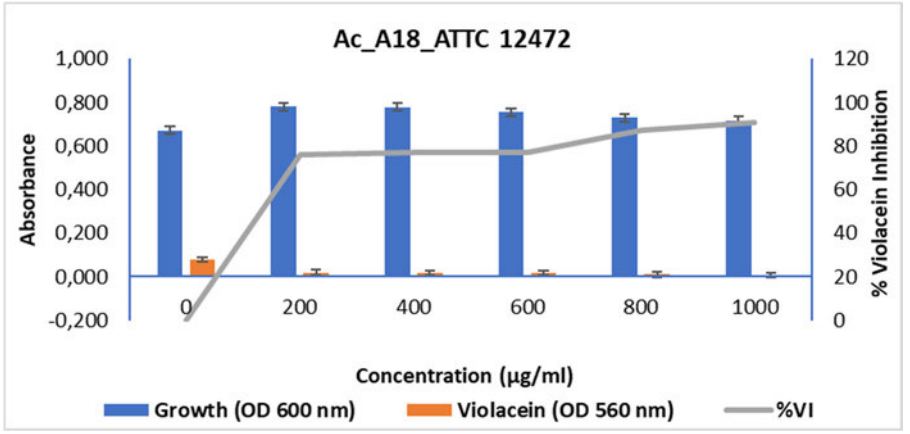


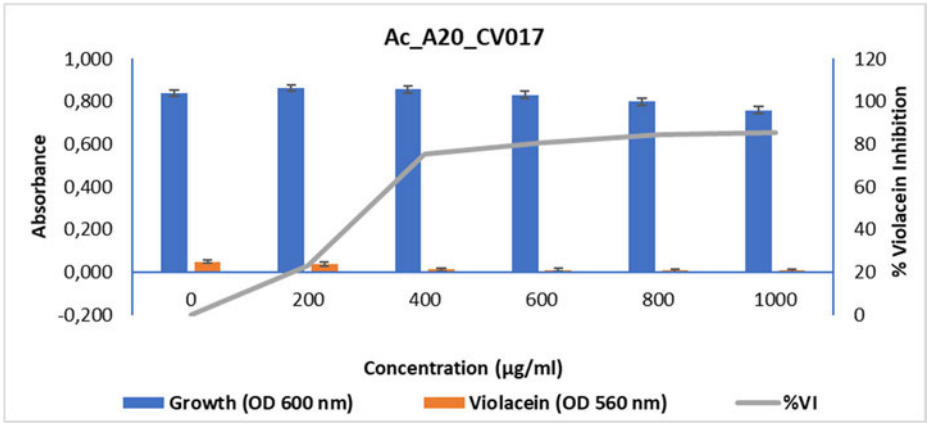
Control_CV12472

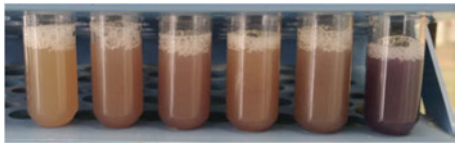
Figure S4.3: Anti-quorum sensing (QS) inhibition of selected *Acropora* CAB extracts against *Chromobacterium substugae* CV017 and *Chromobacterium violaceum* ATCC 12472. The inhibition of the violacein pigment production appearing as opaque zones was indicative of QS inhibition and clear zones around the discs were indicative of killing.

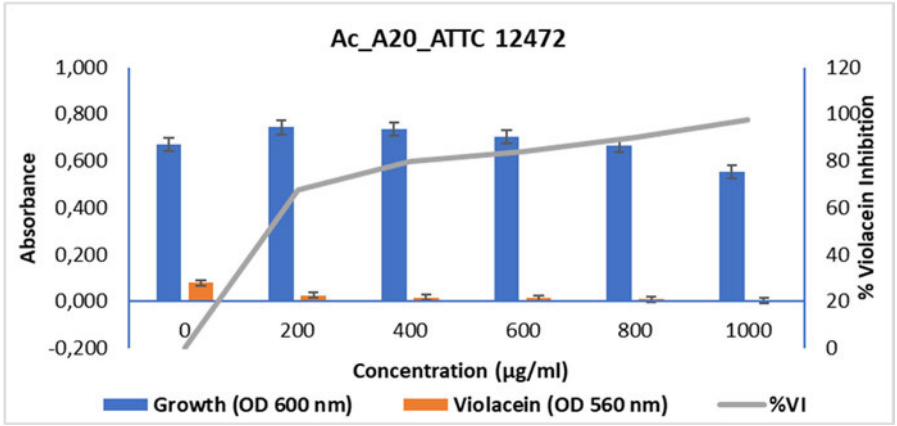

Ac_A18_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

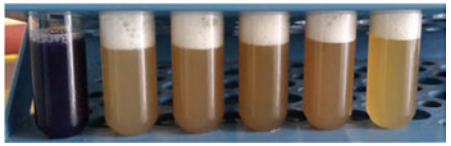


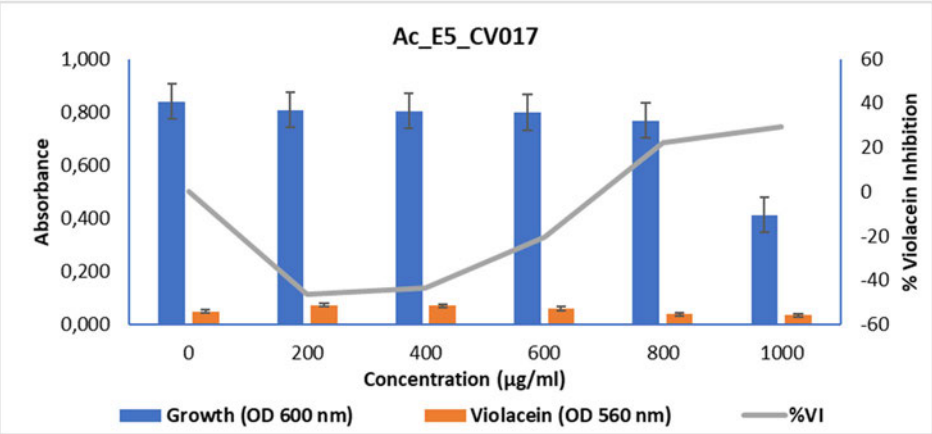

Ac_A18_ATTC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

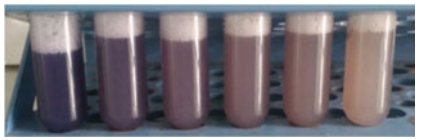

Ac_A20_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

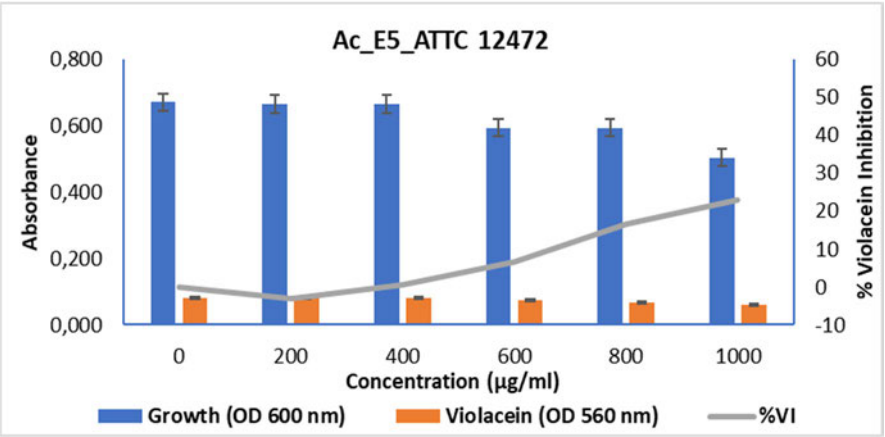


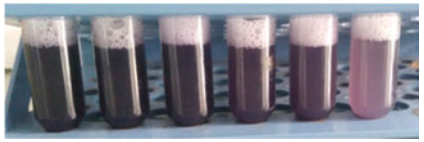

Ac_A20_ATTC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

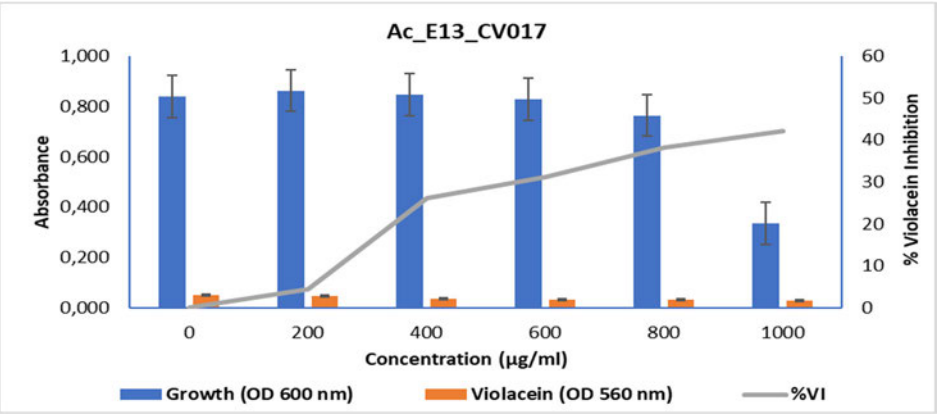


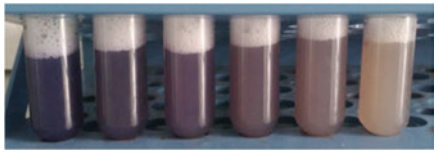

Ac_E5_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

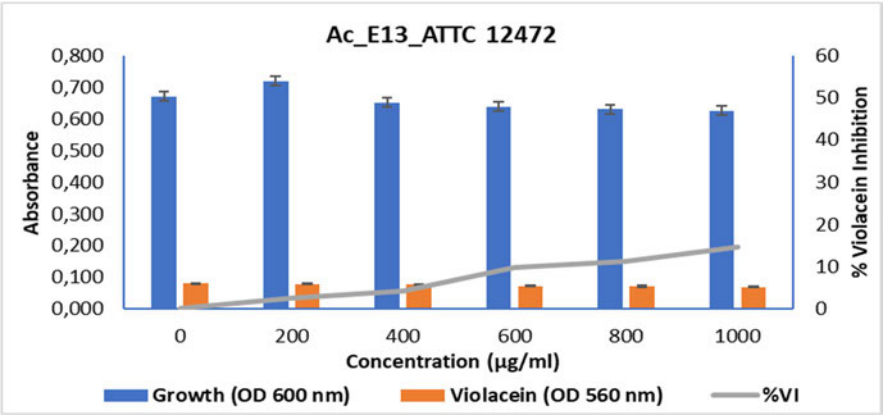


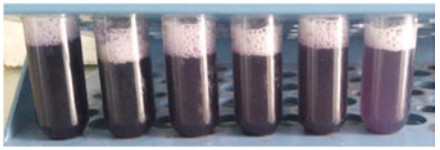

Ac_E5_ATTC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

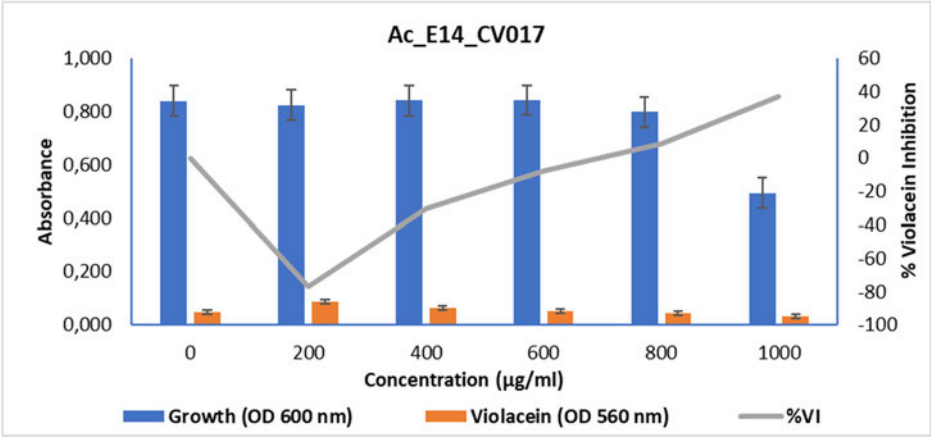


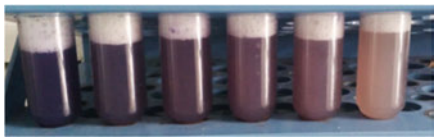

Ac_E13_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

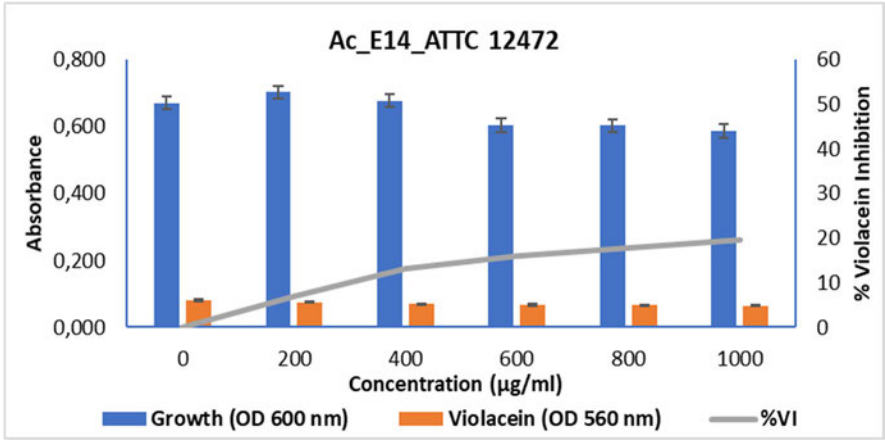


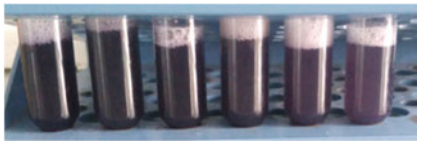

Ac_E13_ATTC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

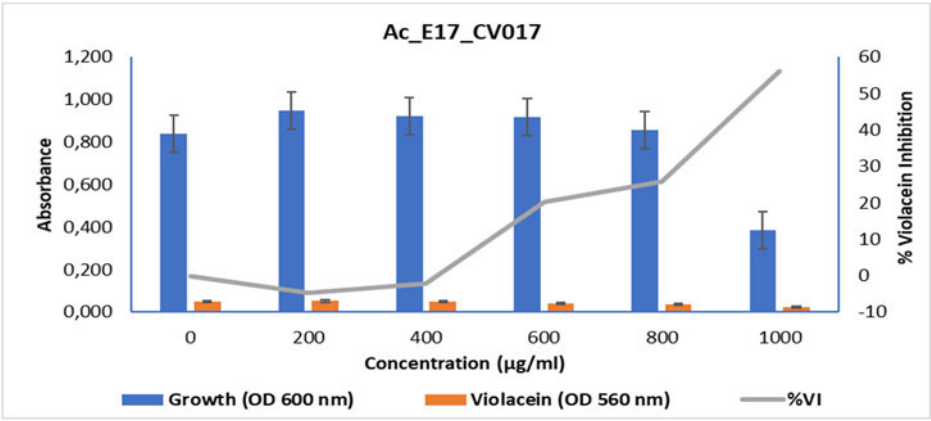


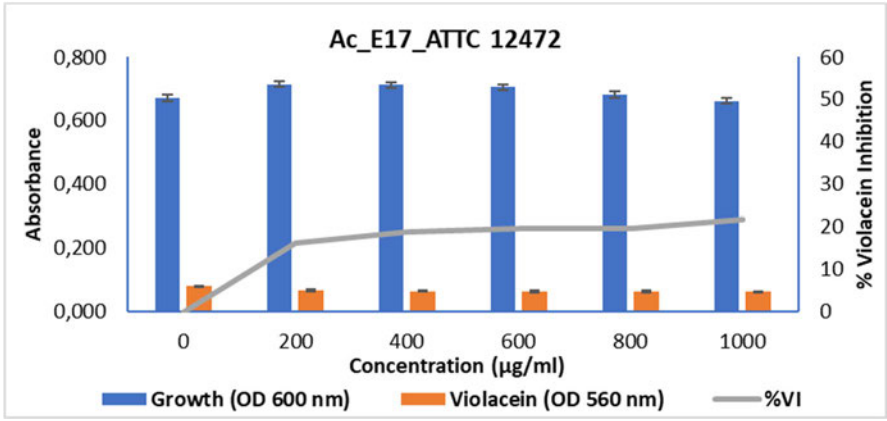

Ac_E14_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

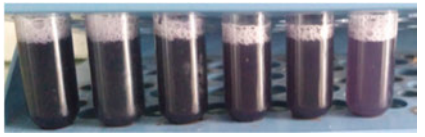


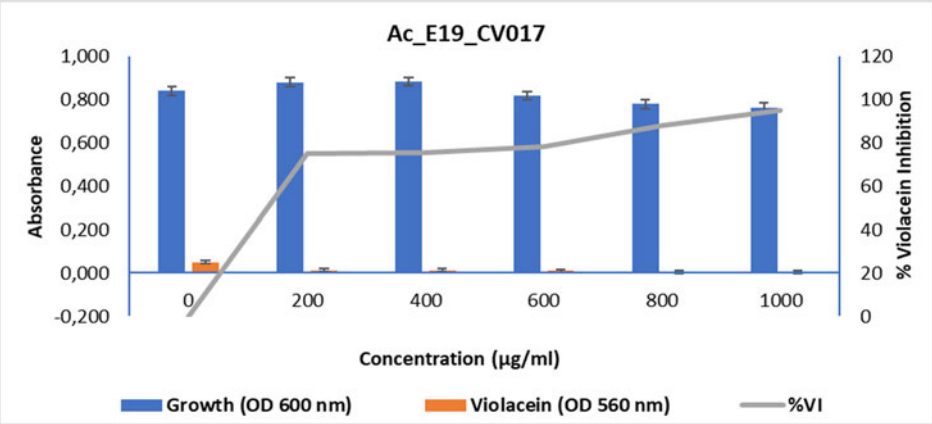

Ac_E14_ATTC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

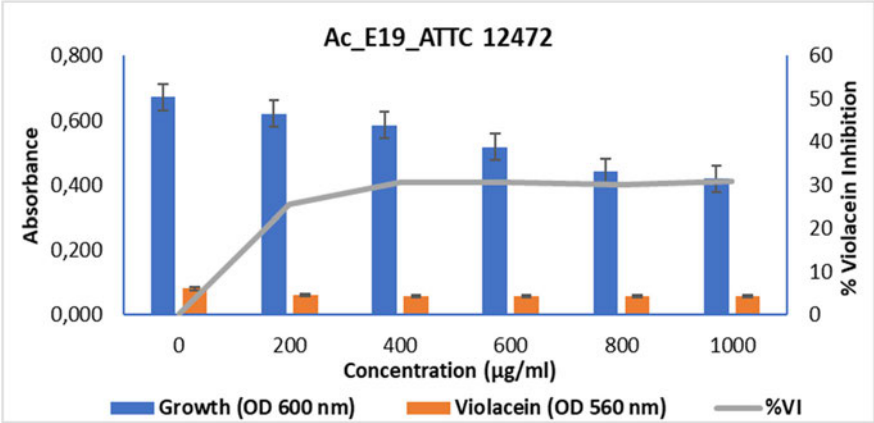


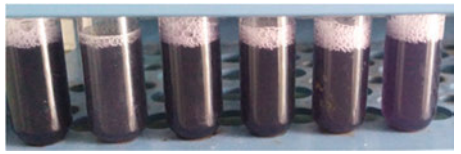

Ac_E17_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

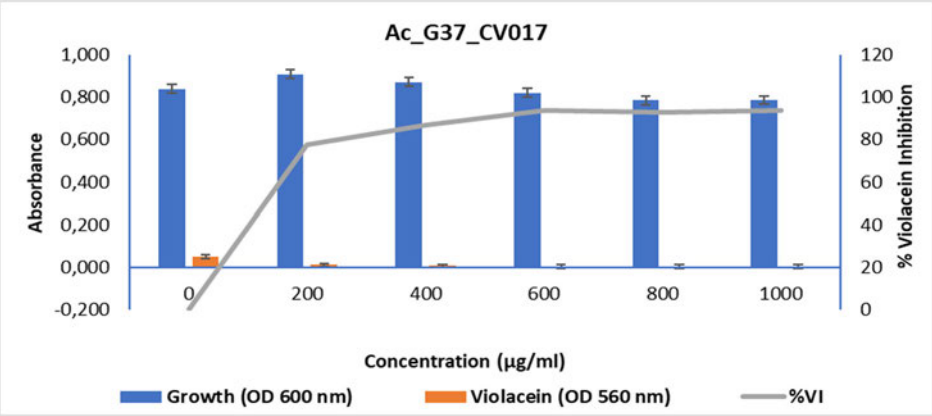

Ac_E17_ATTC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

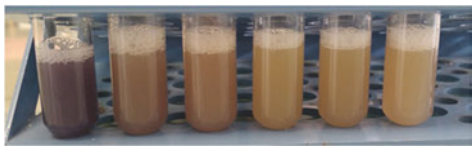


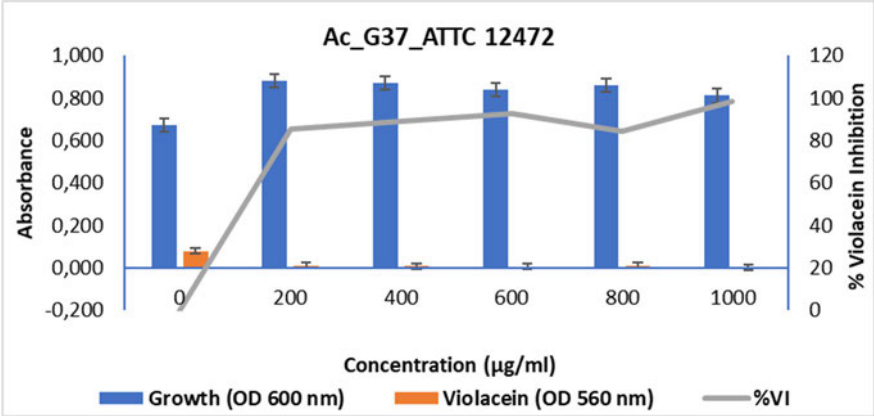

Ac_E19_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

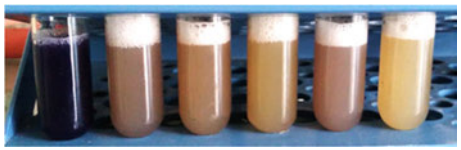

Ac_E19_ATTC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

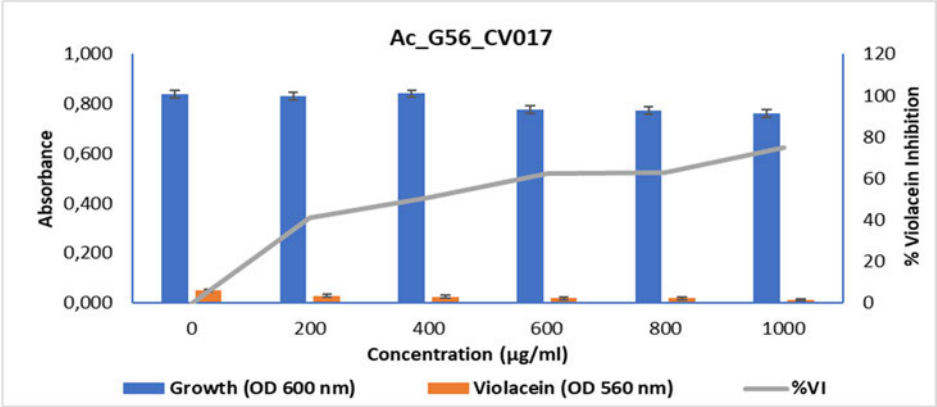


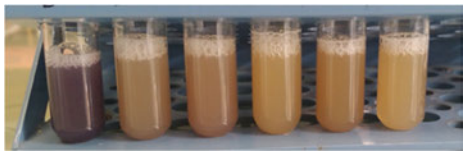

Ac_G37_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

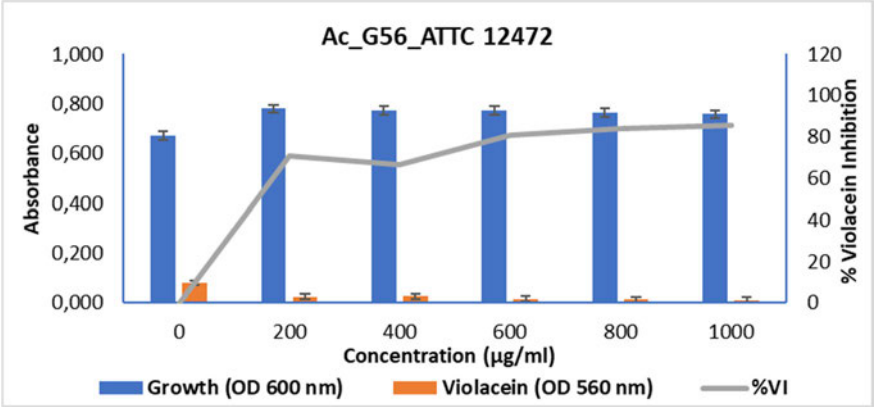


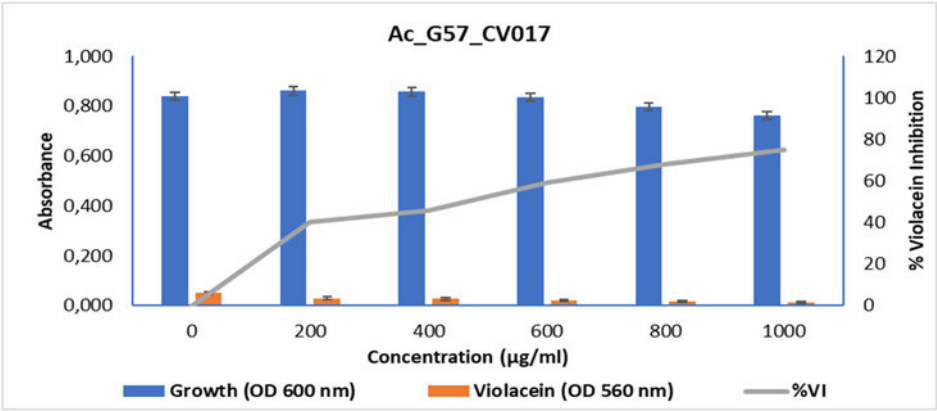

Ac_G37_ATTC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

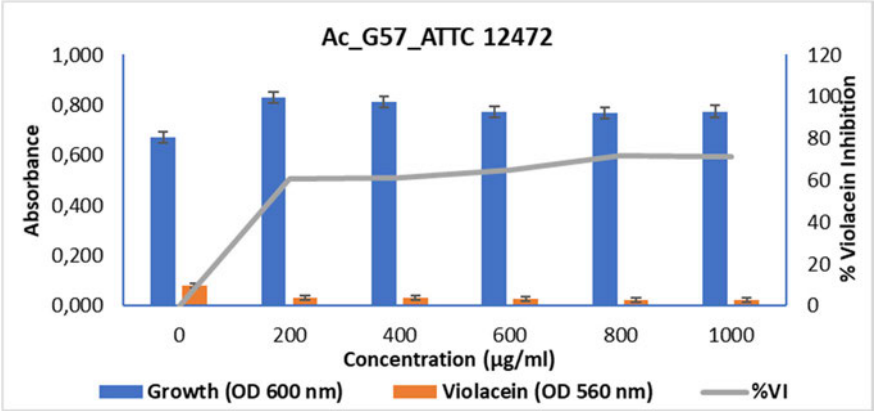


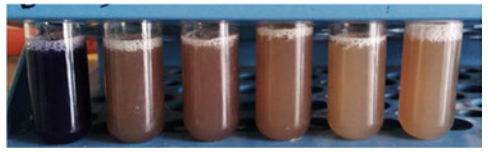

Ac_G56_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

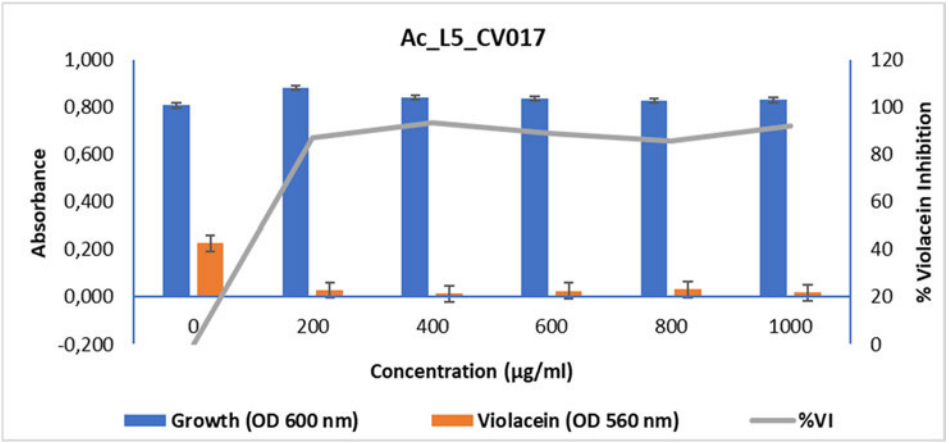


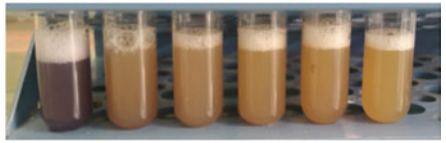

Ac_G56_ATTC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

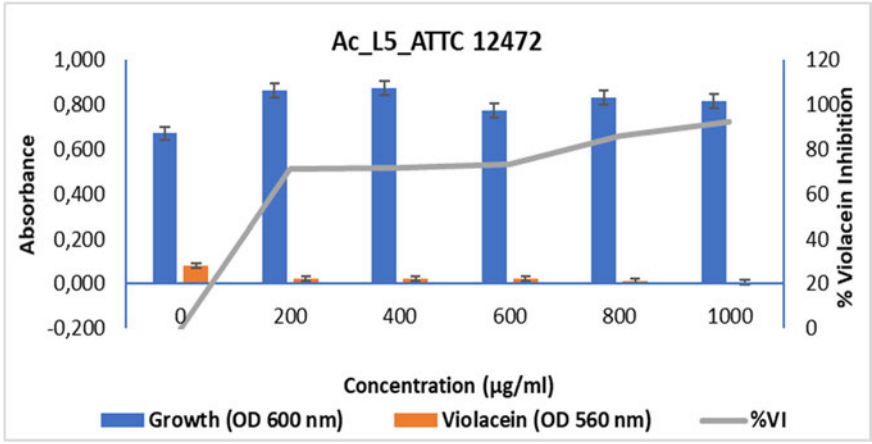

Ac_G57_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

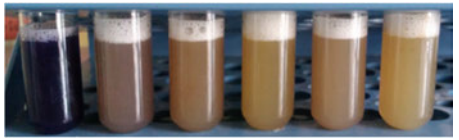

Ac_G57_ATTC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

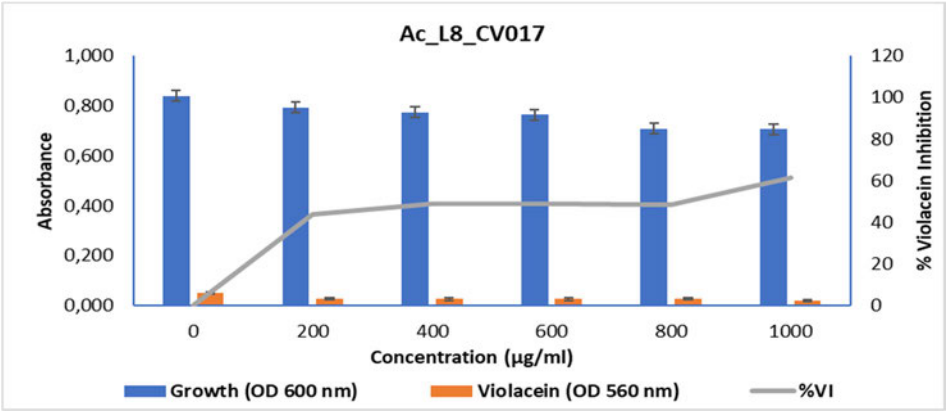


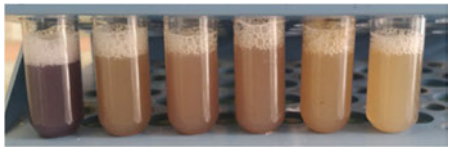

Ac_L5_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

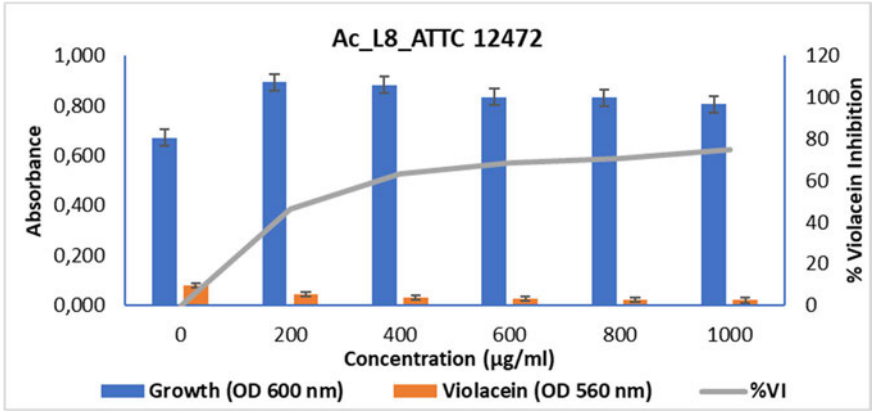


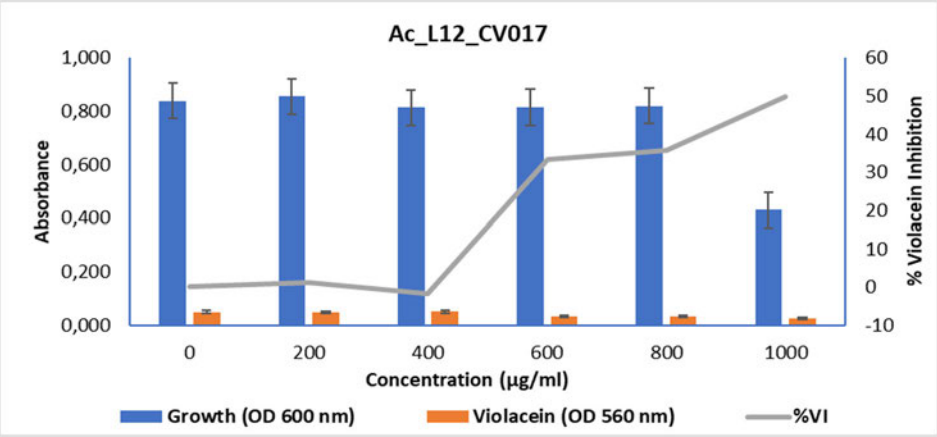

Ac_L5_ATTC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

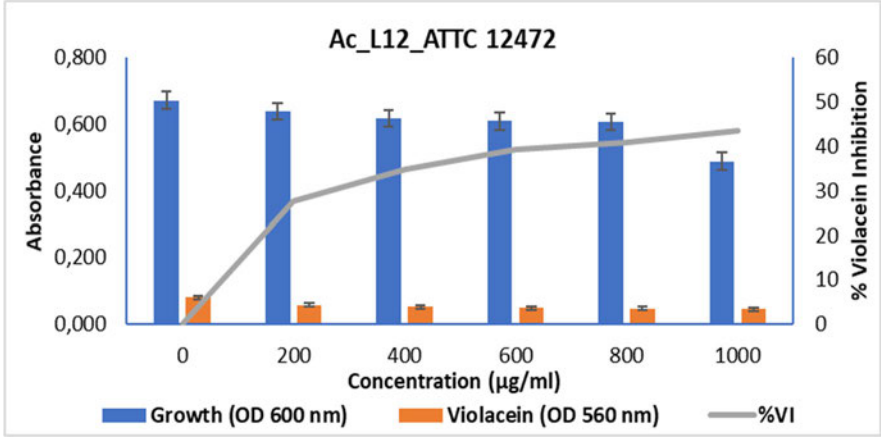


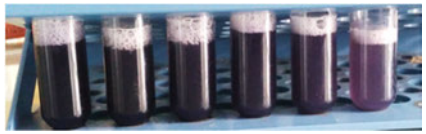

Ac_L8_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

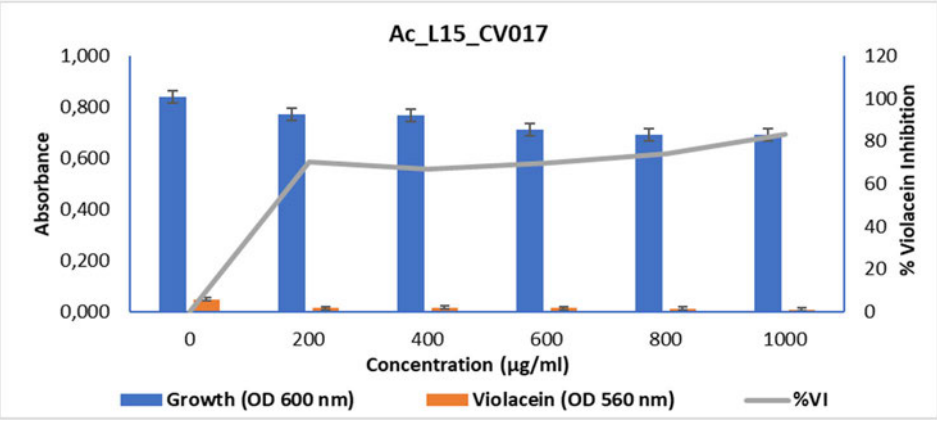


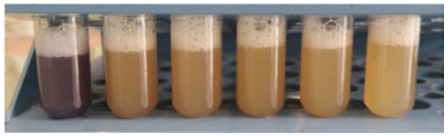

Ac_L8_ATTC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

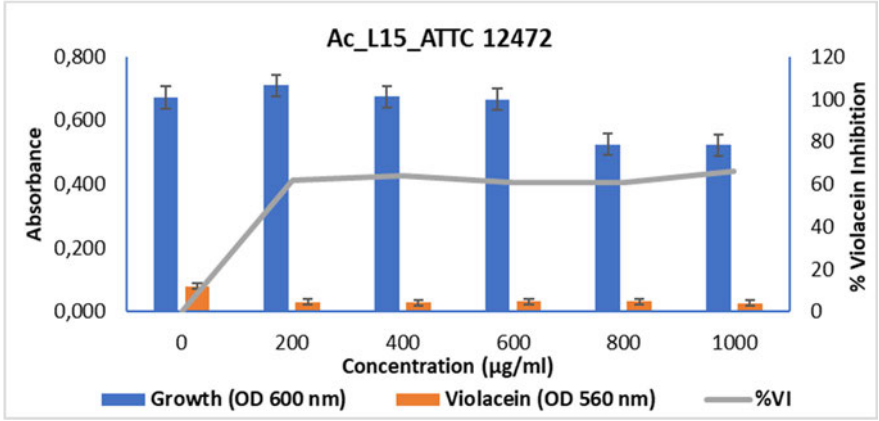

Ac_L12_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

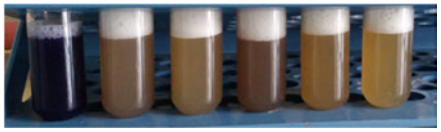

Ac_L12_ATTC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

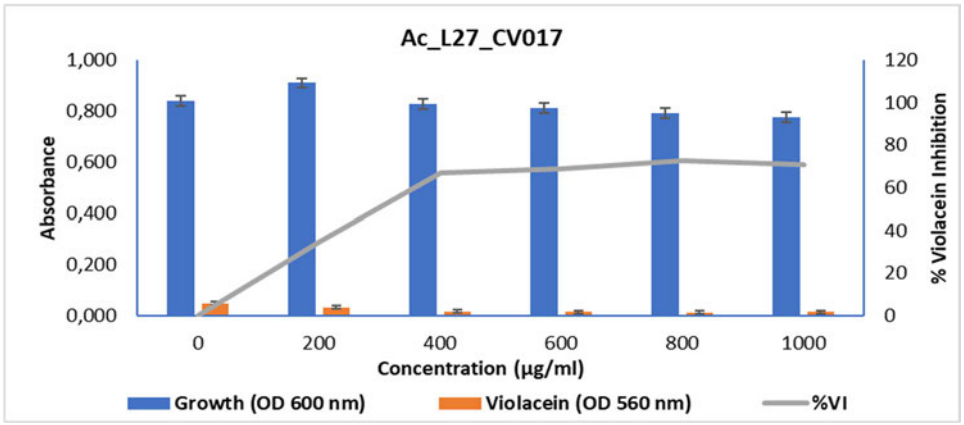


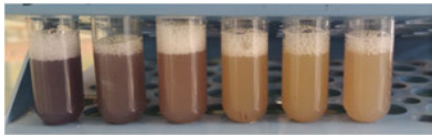

Ac_L15_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

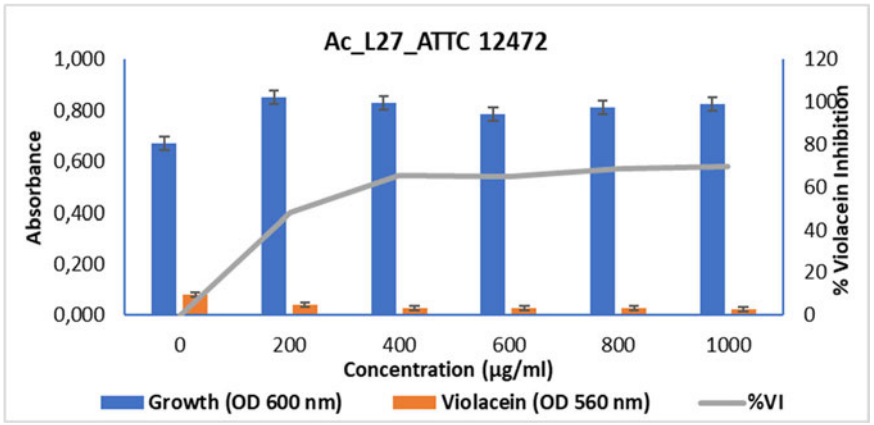


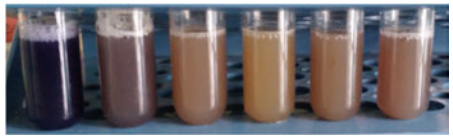

Ac_L15_ATTC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

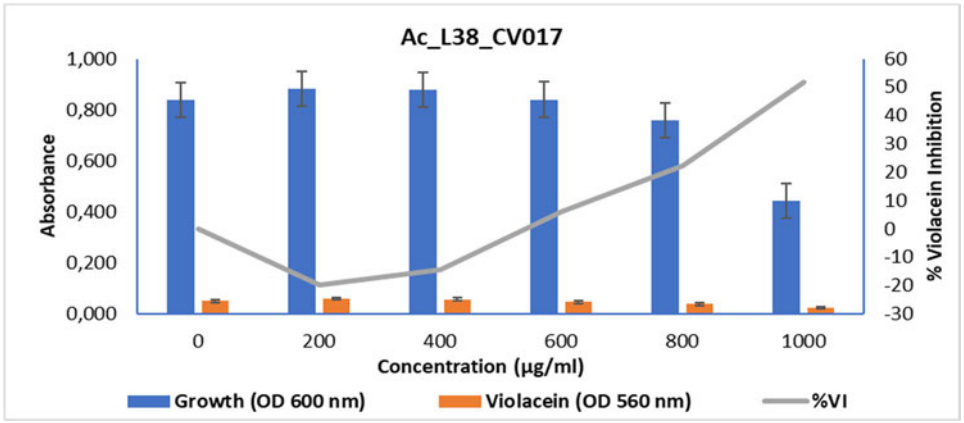


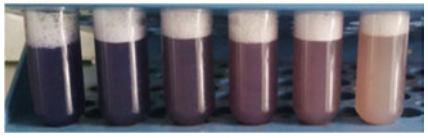

Ac_L27_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

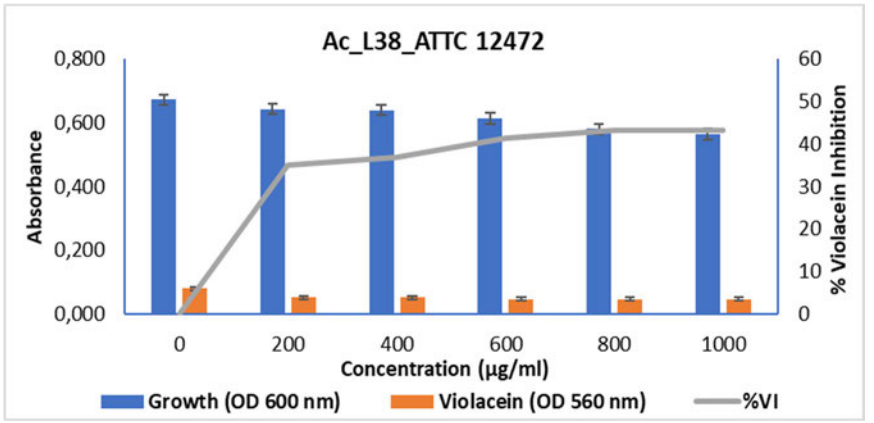


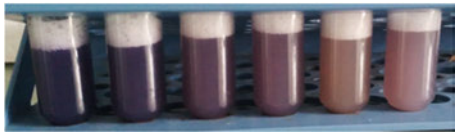

Ac_L27_ATTC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

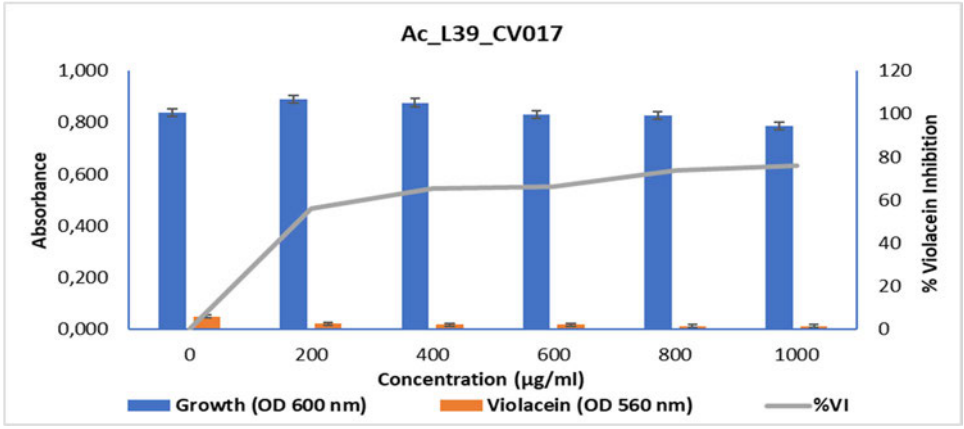


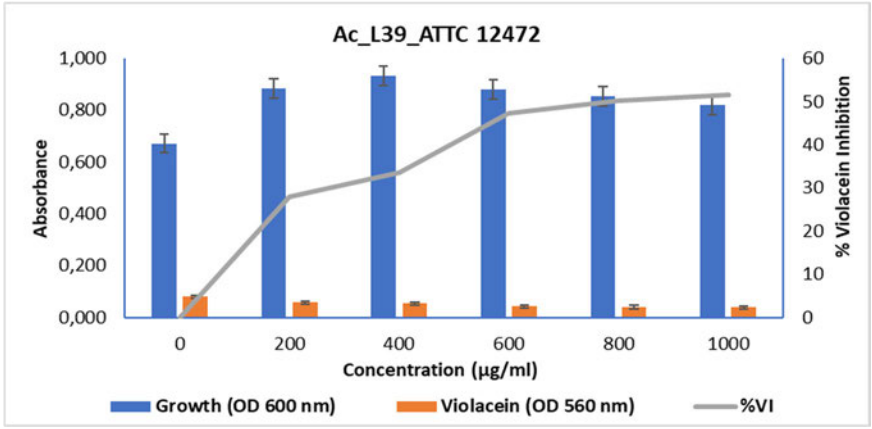

Ac_L38_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

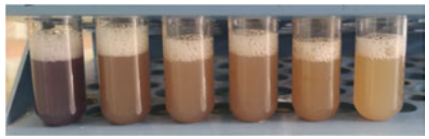


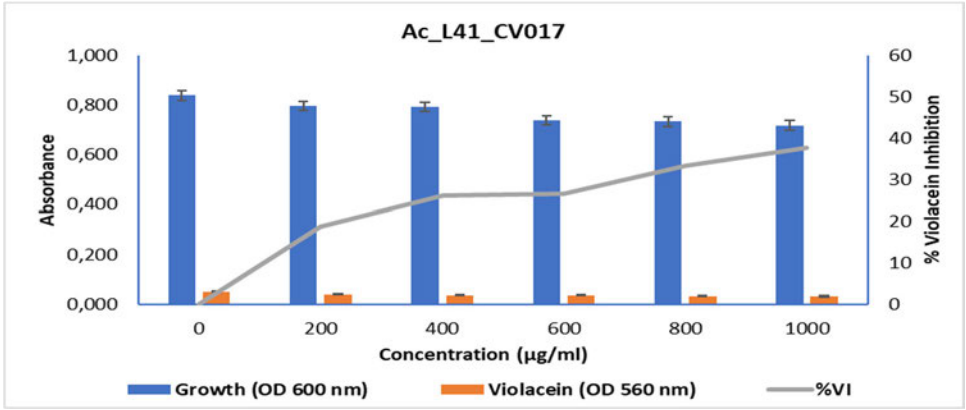

Ac_L38_ATTC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

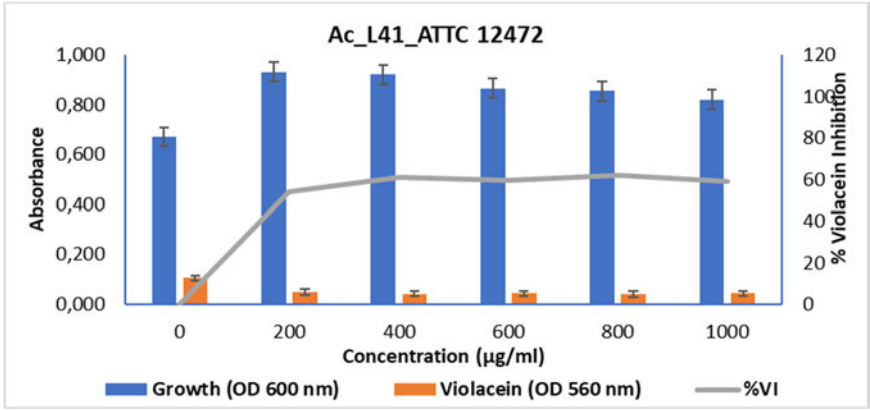


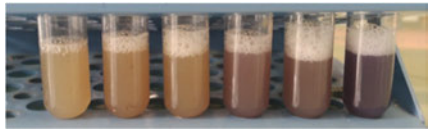

Ac_L39_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

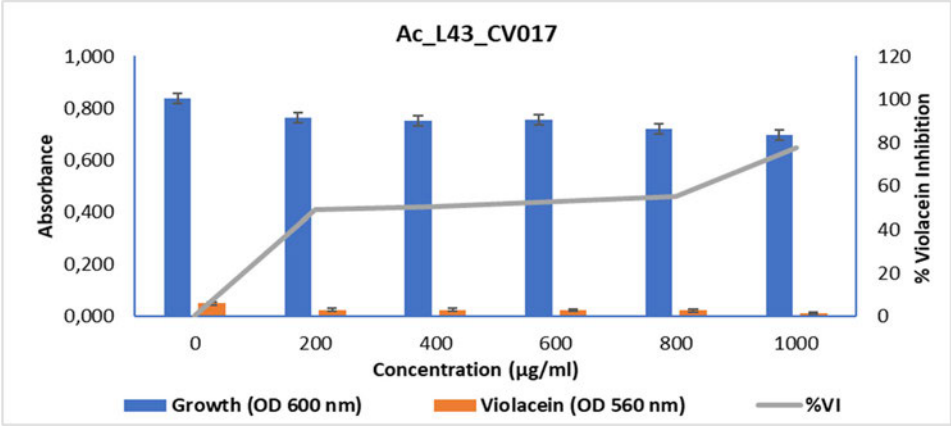

Ac_L39_ATTC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

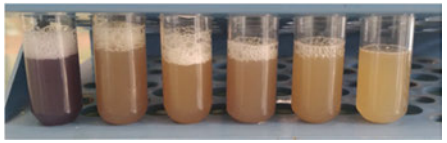


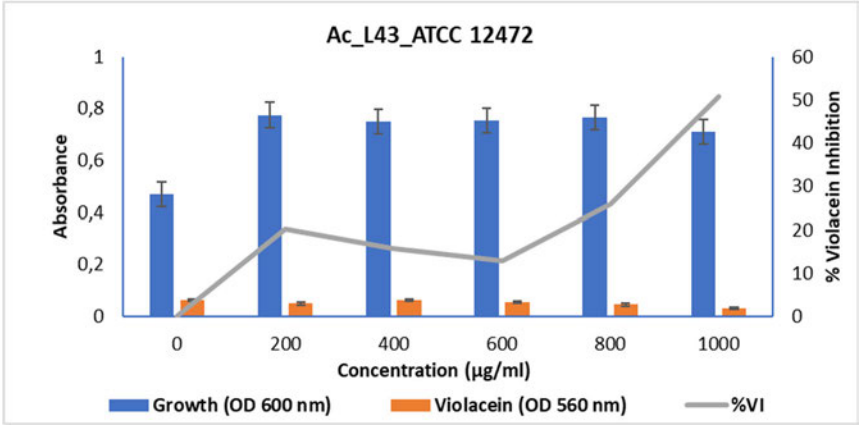

Ac_L41_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

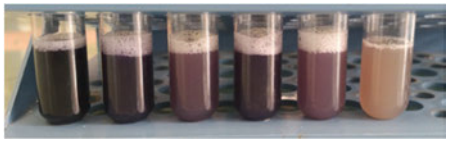

Ac_L41_ATTC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

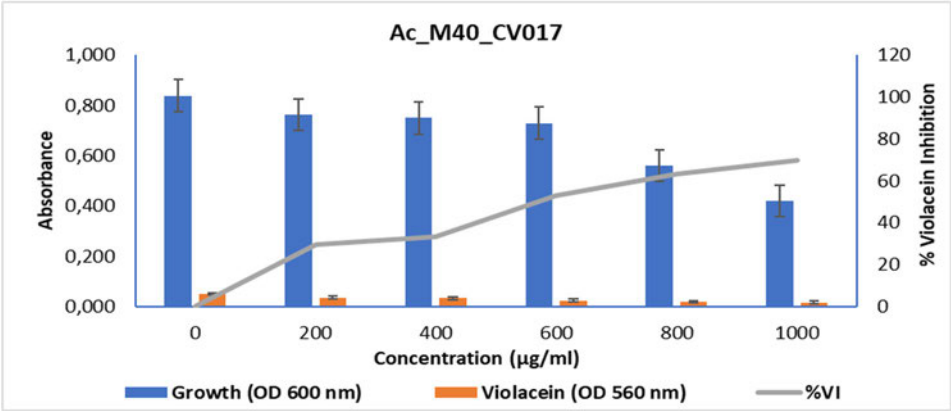


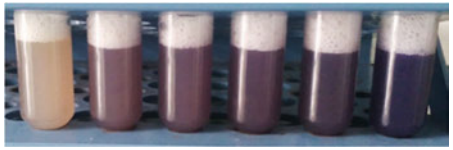

Ac_L43_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

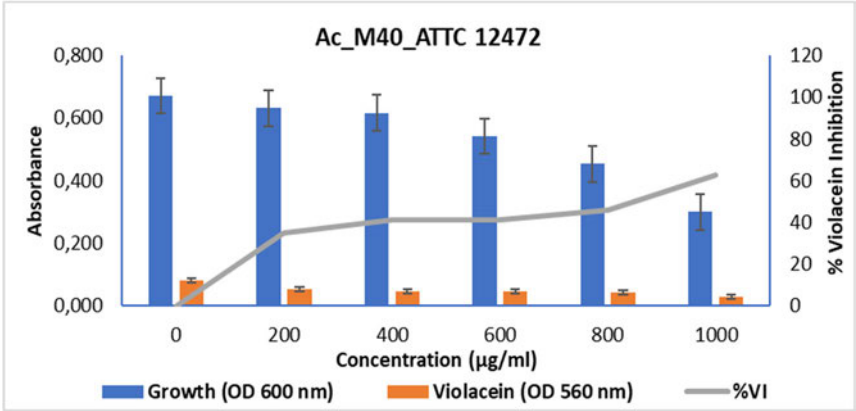


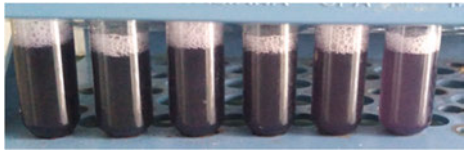

Ac_L43_ATCC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

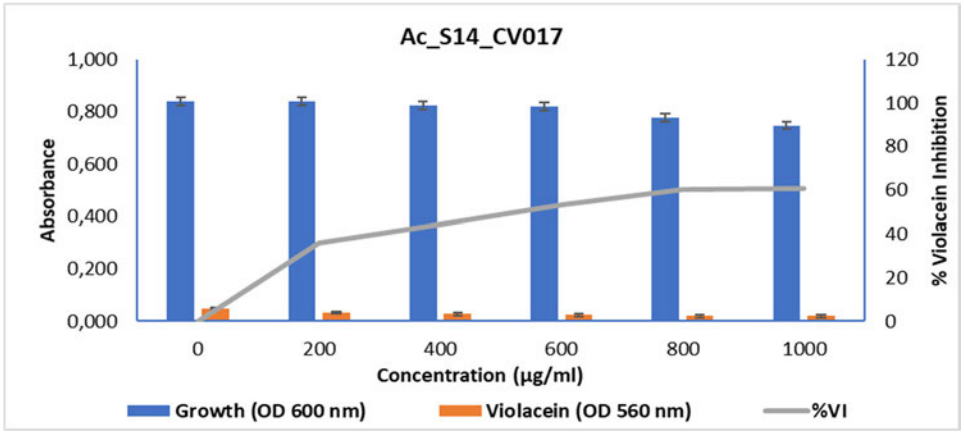


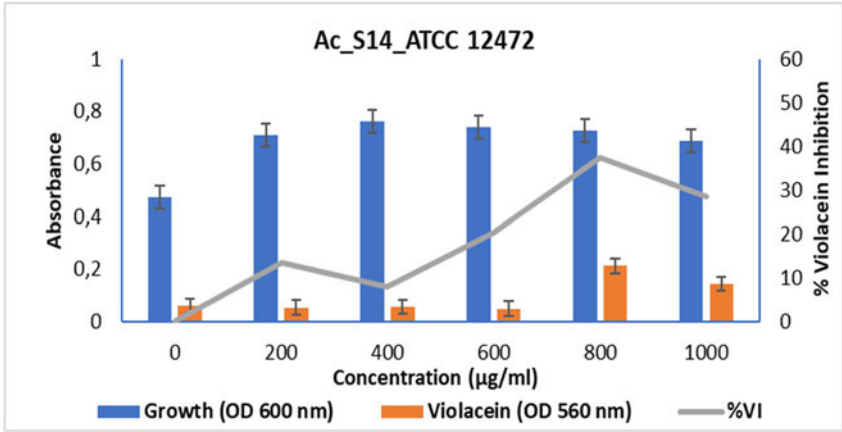

Ac_M40_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

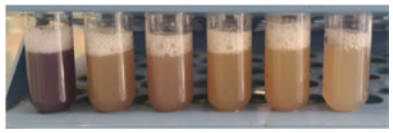


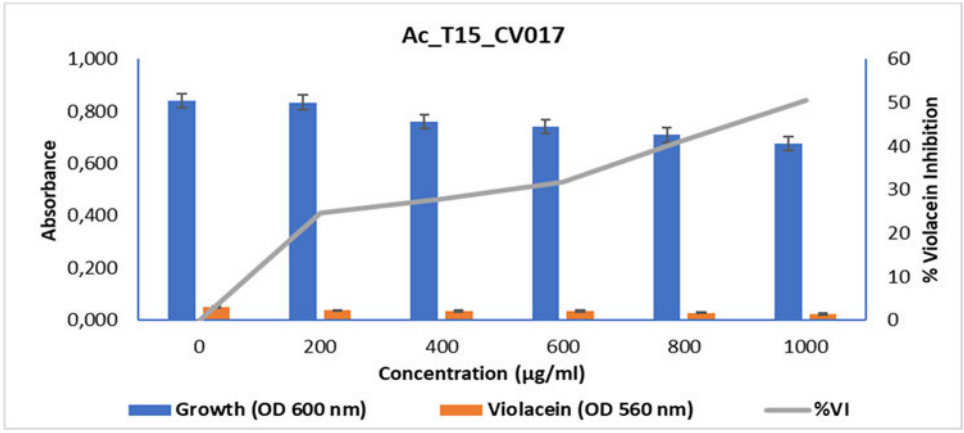

Ac_M40_ATTC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

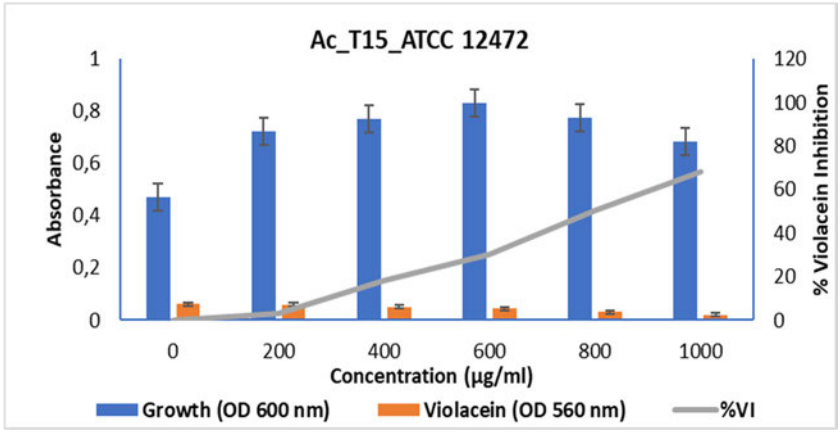


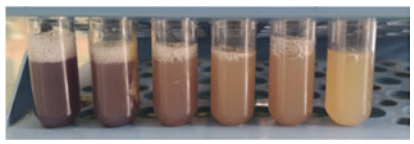

Ac_S14_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI

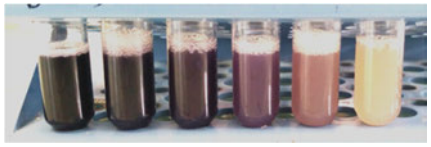

Ac_S14_ATCC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI




Ac_T15_CV017
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI


Ac_T15_ATCC 12472
Absorbance
% Violacein Inhibition
Concentration (µg/ml)
Growth (OD 600 nm)
Violacein (OD 560 nm)
%VI





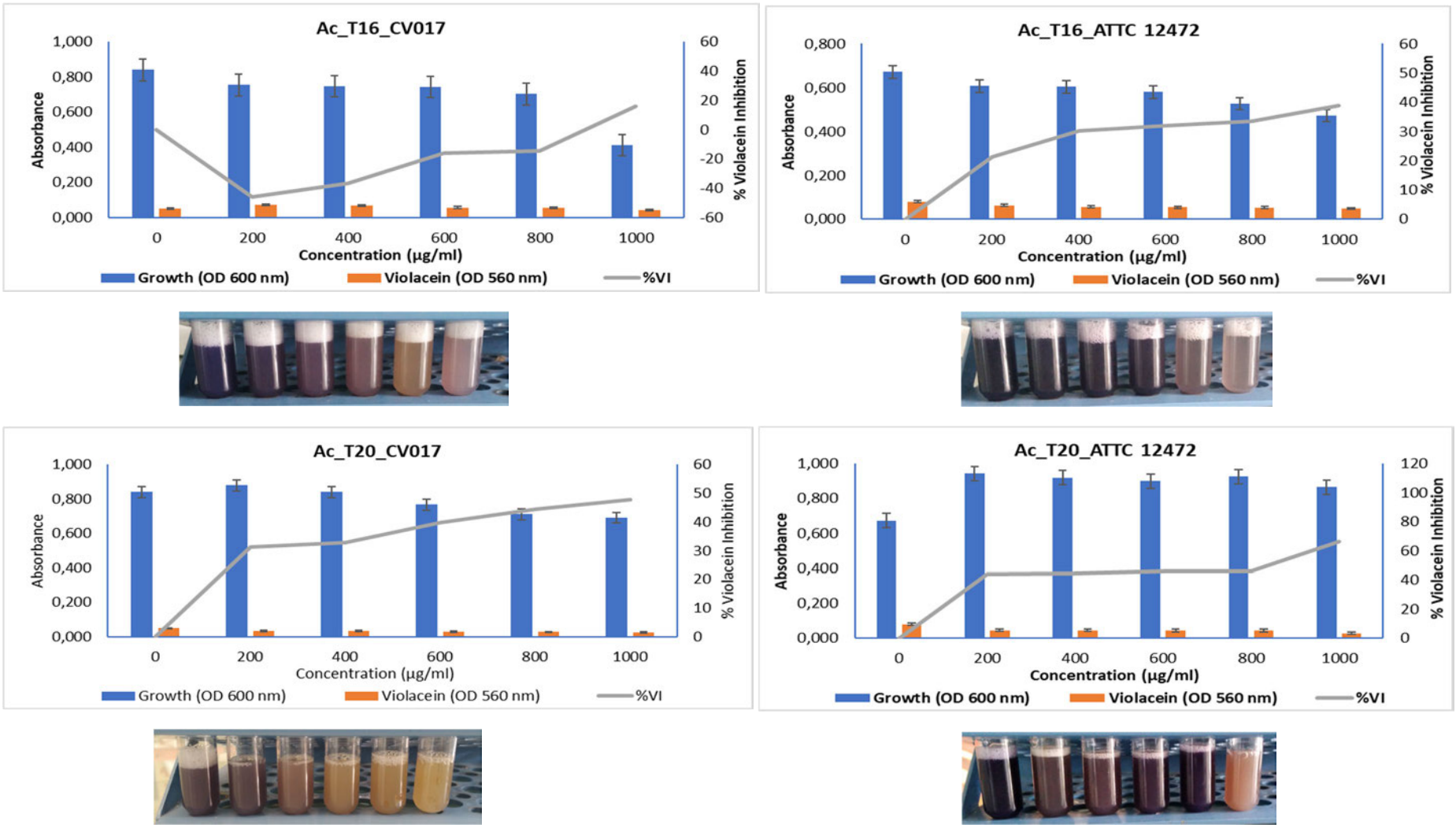
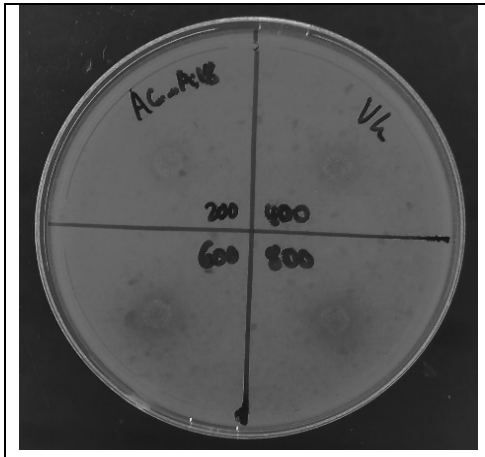
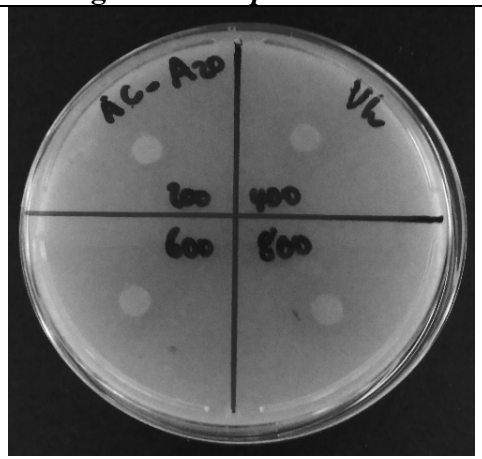


Figure S4.4: Quantitative analysis of violacein inhibitory effects of 24 *Acropora* CAB extracts against *Chromobacterium subtsugae* CV017 and *Chromobacterium violaceum* ATCC 12472. Bacterial growth measured at $OD_{600\ nm}$ and violacein production at $OD_{560\ nm}$ are indicated as bar graphs, while the percentage violacein inhibition (%VI) is indicated by the line graph. Data represent the mean of two independent experiments performed in triplicate**.**

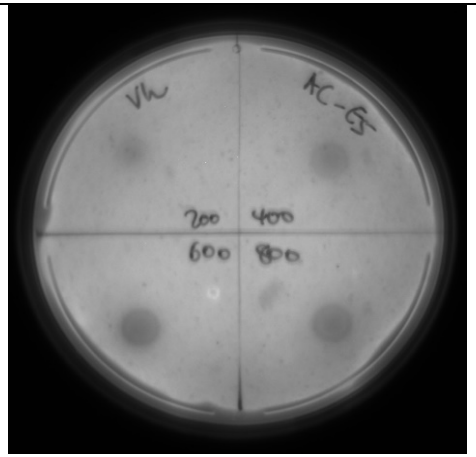
4.6.4 Autoinducer-2 inhibition screening of 24 *Acropora* CAB extracts.



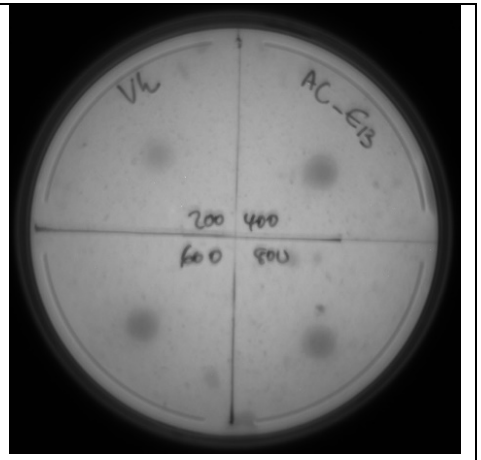
Ac_A18_*V. harveyi*



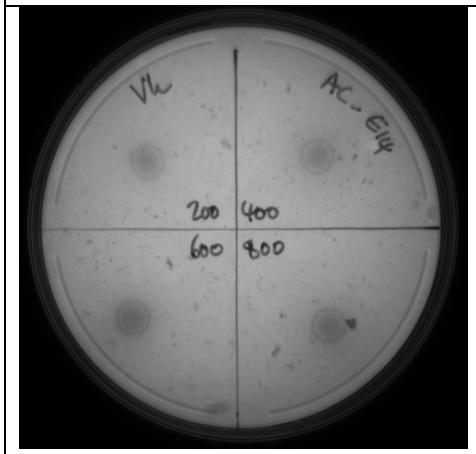
Ac_A20_*V. harveyi*



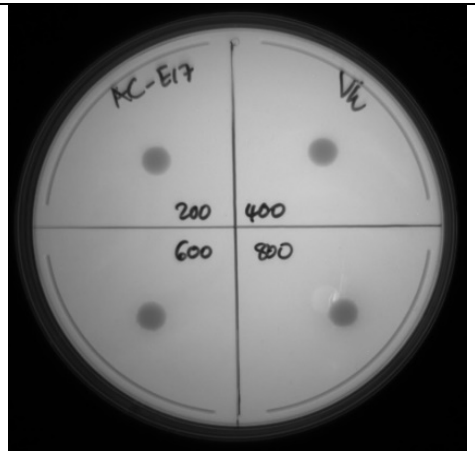
Ac_E5_*V. harveyi*



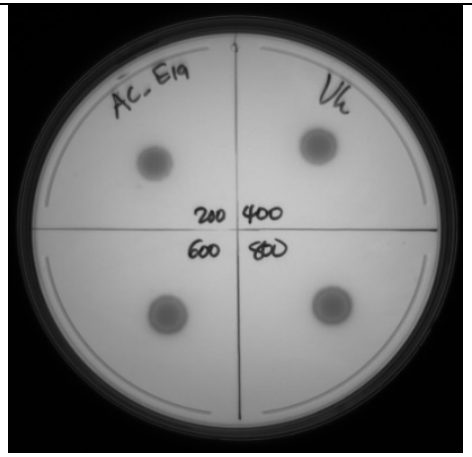
Ac_E13_*V. harveyi*



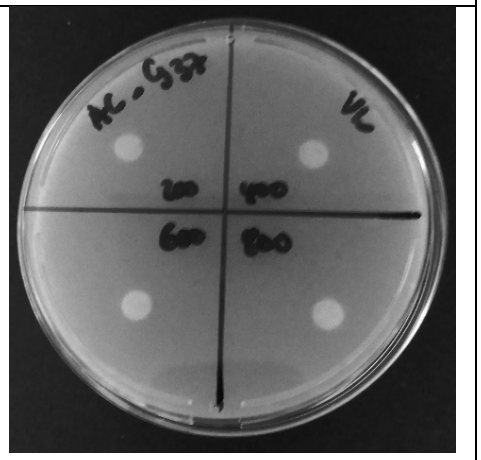
Ac_E14_*V. harveyi*



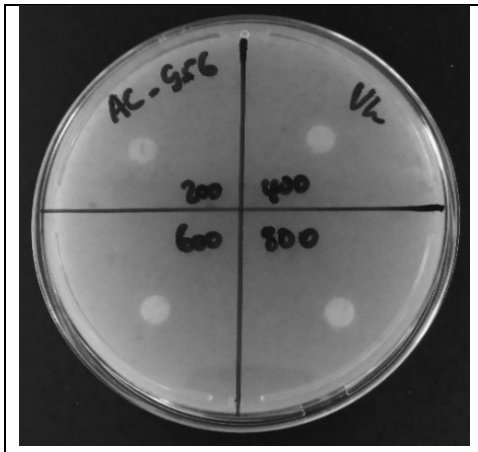
Ac_E17_*V. harveyi*



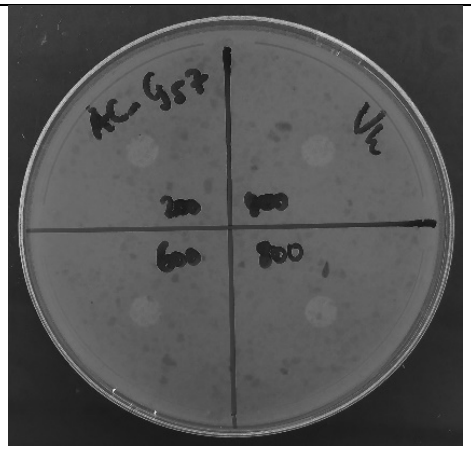
Ac_E19_*V. harveyi*



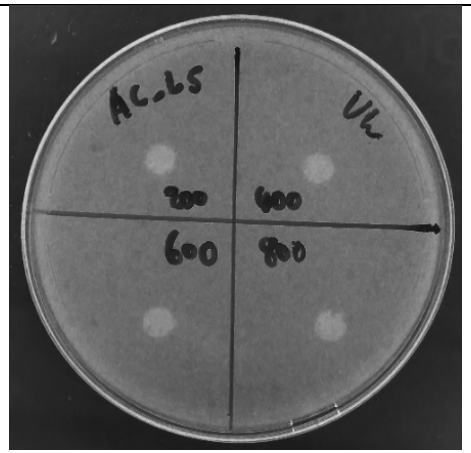
Ac_G37_*V. harveyi*



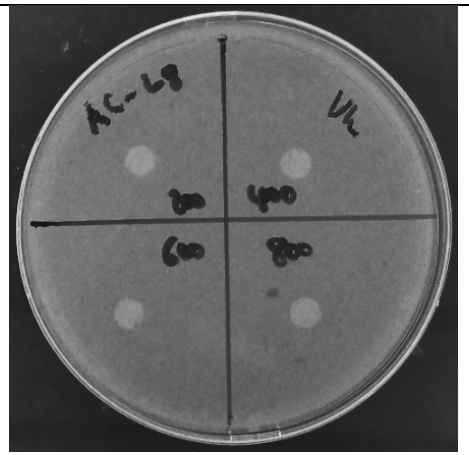
Ac_G56_*V. harveyi*



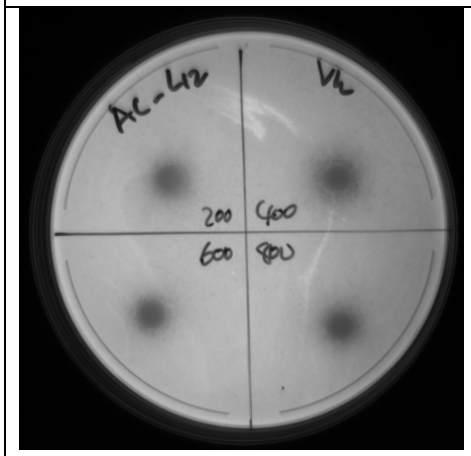
Ac_G57_*V. harveyi*



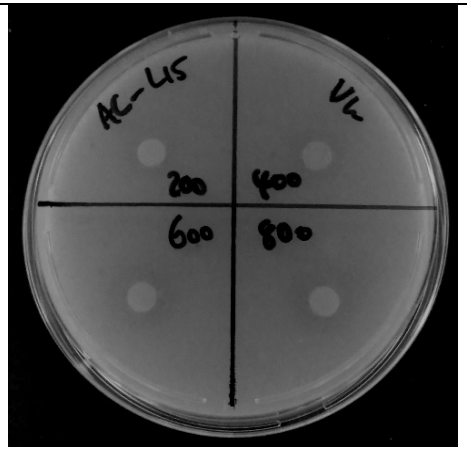
Ac_L5_*V. harveyi*



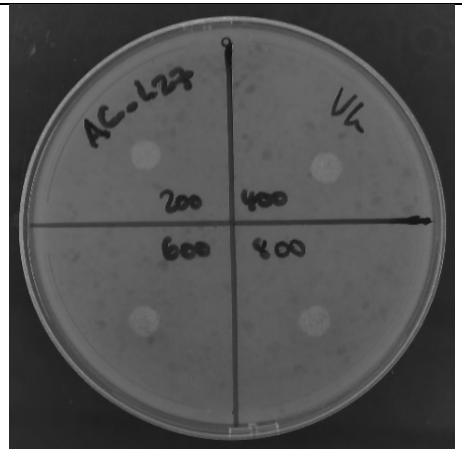
Ac_L8_*V. harveyi*



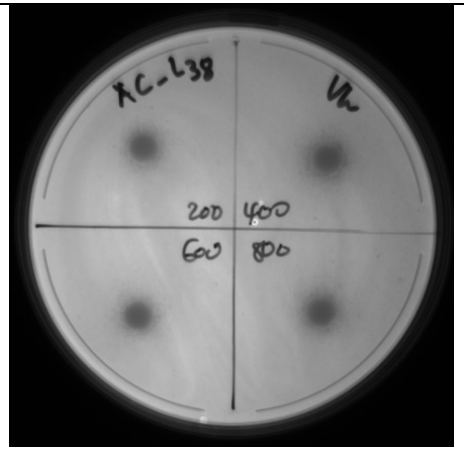
Ac_L12_*V. harveyi*



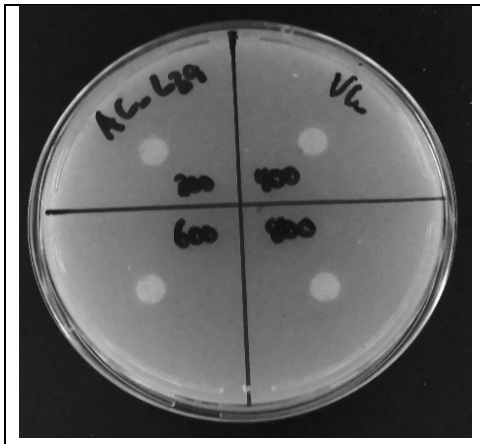
Ac_L15_*V. harveyi*



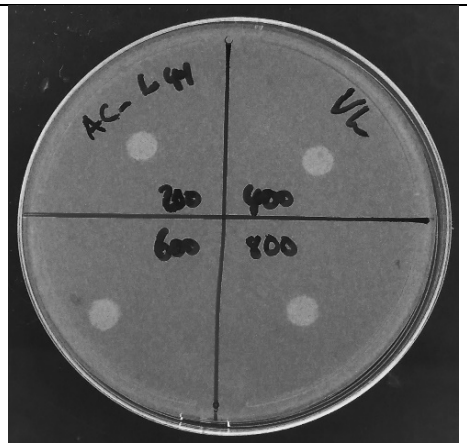
Ac_L27_*V. harveyi*



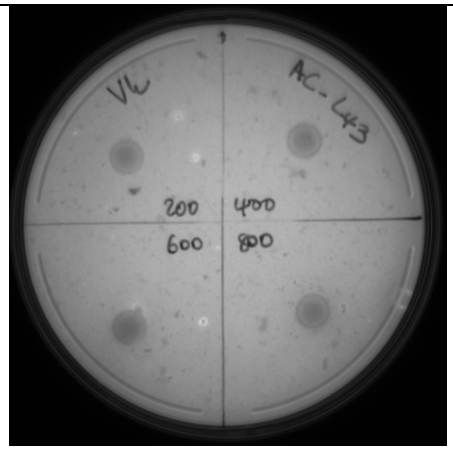
Ac_L38_*V. harveyi*



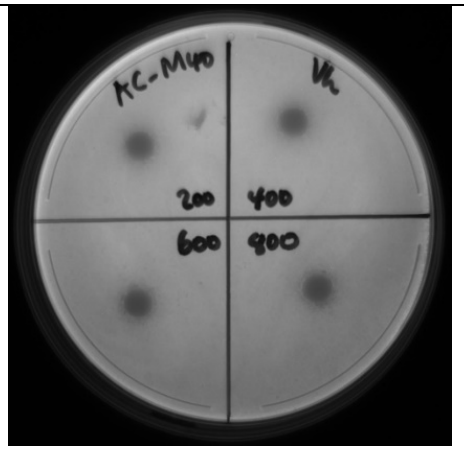
Ac_L39_*V. harveyi*



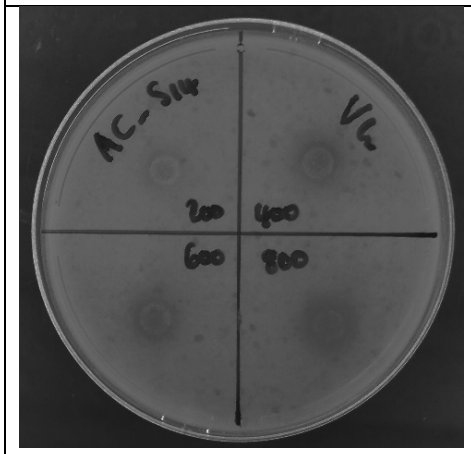
Ac_L41_*V. harveyi*



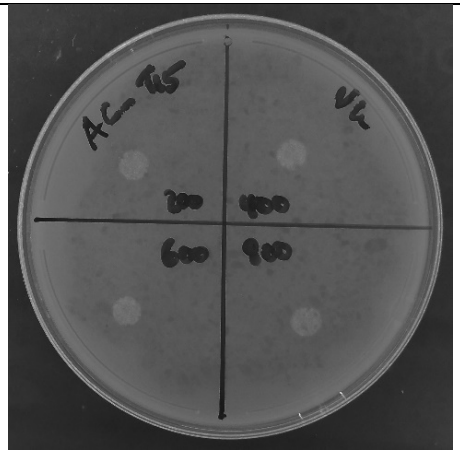
Ac_L43_*V. harveyi*



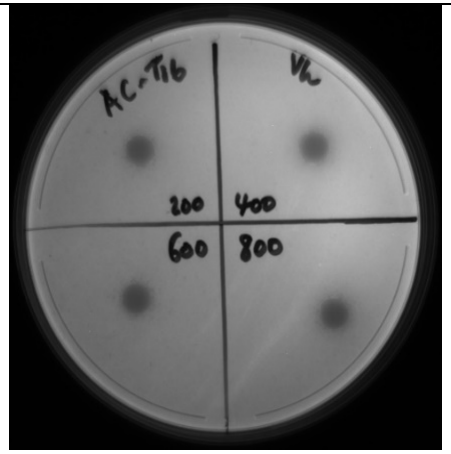
Ac_M40_*V. harveyi*



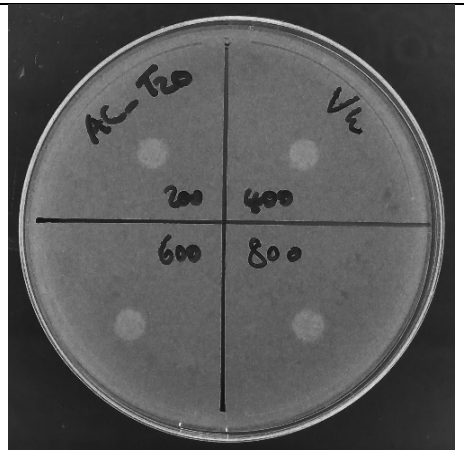
Ac_S14_*V. harveyi*



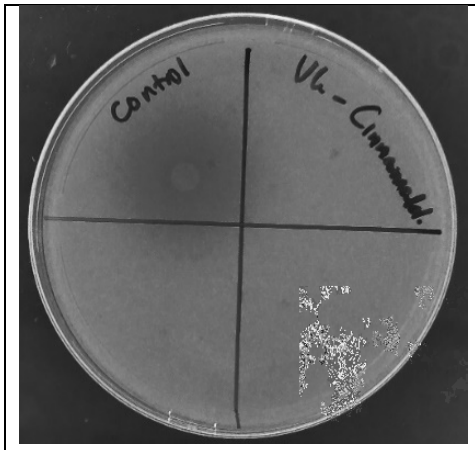
Ac_T15_*V. harveyi*



Ac_T16_*V. harveyi*



Ac_T20_*V. harveyi*



Control_*V. harveyi*

Figure S4.5: Autoinducer-2 inhibition of 24 *Acropora* CAB extracts against bioluminescent marine strain *Vibrio harveyi* BB120. AI-2 inhibition was identified by the appearance of dark zones, lacking bioluminescence around the discs. Clear zones around the discs were indicative of killing activity (Ac_A18 - Ac_T20).

Table S4.1: Quantitative analysis of percentage biofilm reducing potential (%BFR) and percentage growth inhibition (%GI) of *Acropora* CAB extracts against initial adhesion and mature biofilms of *Pseudomonas aeruginosa* ATCC 27853.

Extract	*Pseudomonas aeruginosa* ATCC 27853															
	Initial								Mature							
	200 µg/ml		400 µg/ml		600 µg/ml		800 µg/ml		200 µg/ml		400 µg/ml		600 µg/ml		800 µg/ml	
	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR
Ac_A18_*Streptomyces globisporus*	14,99	8,71	17,97	11,35	20,41	11,21	23,17	13,41	22,28	7,12	20,34	7,86	20,37	10,13	22,18	10,41
Ac_A20_*Bacillus altitudinis*	51,93	6,76	53,00	9,47	53,20	12,00	56,49	13,39	39,58	5,87	39,73	7,40	38,54	8,18	36,84	12,98
Ac_E5_*Bacillus altitudinis*	6,23	57,91	7,33	59,19	7,62	64,42	9,16	67,84	15,98	12,39	13,16	17,03	11,60	20,01	10,33	22,02
Ac_E13_*Labrenzia marina*	2,32	54,49	4,04	57,43	5,14	65,33	5,67	66,94	16,64	7,59	9,96	8,54	7,23	8,65	12,77	10,77
Ac_E14_*Labrenzia alexandrii*	3,53	32,79	5,98	31,44	6,68	29,50	7,36	28,65	15,98	23,18	18,27	24,55	17,68	30,54	18,12	30,57
Ac_E17_*Bacillus aerophilus*	14,70	55,22	20,66	38,87	21,22	31,86	25,87	32,46	17,93	28,30	21,05	37,33	20,85	45,86	21,17	44,95
Ac_E19_*Phaeobacter gallaeciensis*	52,53	32,15	61,59	55,57	63,88	58,19	63,97	60,23	23,91	20,99	23,51	20,99	23,64	35,90	29,72	48,63
Ac_G37_*Bacillus velezensis*	4,29	24,12	4,80	27,70	4,68	29,80	5,27	30,18	4,65	25,02	5,65	26,93	7,15	29,66	7,87	33,88
Ac_G56_*Erythrobacter nanhaisediminis*	15,20	13,11	16,07	12,35	18,00	8,35	20,00	6,76	21,26	9,66	16,53	13,26	15,93	15,18	11,65	15,92
Ac_G57_*Erythrobacter nanhaisediminis*	8,64	11,24	11,66	12,89	11,69	12,74	14,92	12,17	21,73	12,65	14,18	13,53	12,24	14,86	12,36	20,61
Ac_L5_*Phaeobacter piscinae*	18,31	9,94	19,63	18,97	29,17	30,14	45,80	44,34	18,81	17,74	20,77	18,46	21,22	20,29	31,35	21,81
Ac_L8_*Bacillus altitudinis*	17,69	38,08	17,42	47,92	19,44	50,78	32,07	80,85	27,36	50,06	27,63	65,46	28,55	71,38	29,83	76,27
Ac_L12_*Phaeobacter gallaeciensis*	-1,22	93,32	-1,01	52,58	-0,42	33,31	26,54	29,16	19,92	33,72	21,18	43,33	20,80	43,52	21,57	50,01
Ac_L15_*Bacillus aerophilus*	-0,30	26,83	47,46	28,85	48,26	31,37	50,29	31,55	42,53	29,61	44,63	34,59	47,21	33,93	49,82	36,82
Ac_L27_*Bacillus altitudinis*	-14,28	46,93	-10,68	48,23	-7,94	59,78	-5,93	59,86	6,74	49,99	8,32	50,49	8,48	45,54	9,24	34,96
Ac_L38_*Ruegeria atlantica*	7,15	22,44	8,90	24,72	9,71	25,55	11,80	31,22	24,19	35,01	26,85	34,09	27,99	27,44	29,61	25,38
Ac_L39_*Sporosarcina pasteurii*	8,24	20,50	9,72	21,79	22,59	23,95	25,55	28,86	20,09	30,11	22,42	26,39	23,29	26,14	24,68	24,94
Ac_L41_*Streptomyces pratensis*	5,17	25,51	7,46	26,50	11,50	25,88	15,34	29,88	18,55	31,01	19,89	29,34	20,06	28,90	21,51	29,42
Ac_L43_*Microbulbifer variabilis*	4,53	24,04	4,55	25,92	5,17	31,47	4,49	39,56	1,39	22,41	1,39	22,41	12,90	27,74	14,67	31,21
Ac_M40_*Metabacillus indicus*	5,47	25,21	1,68	32,34	3,55	35,96	2,53	40,05	24,63	24,65	24,94	24,11	25,21	24,56	27,07	25,30
Ac_S14_*Streptomyces violascens*	17,12	38,16	17,51	37,96	21,51	43,19	23,99	60,71	24,17	42,14	26,06	50,03	29,10	55,72	35,15	55,48
Ac_T15_*Bacillus pumilus*	0,14	32,70	4,53	37,00	4,57	39,66	5,28	39,93	10,38	20,13	11,95	21,98	13,17	23,44	21,35	26,21
Ac_T16_*Bacillus pumilus*	8,05	28,34	11,09	29,44	11,97	31,66	17,11	34,01	15,86	14,74	16,19	15,53	16,15	19,91	17,79	22,63
Ac_T20_*Bacillus pumilus*	15,50	28,59	15,63	31,26	16,38	32,81	19,19	35,26	24,05	28,51	25,03	31,16	25,12	37,07	25,45	39,27
Total extracts displaying ≥ 50% BFR and < 40% GI	4		3		4		5		1		3		2		3	
Total extracts displaying ≥ 50% BFR and ≥ 40% GI	0		1		1		1		0		0		0		0	

Table S4.2: Quantitative analysis of percentage biofilm reducing potential (%BFR) and percentage growth inhibition (%GI) of *Acropora* CAB extracts against initial adhesion and mature biofilms of *Staphylococcus aureus* ATCC 43300.

Extract	*Staphylococcus aureus* ATCC 43300															
	Initial								Mature							
	200 µg/ml		400 µg/ml		600 µg/ml		800 µg/ml		200 µg/ml		400 µg/ml		600 µg/ml		800 µg/ml	
	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR
Ac_A18_*Streptomyces globisporus*	29,19	13,83	31,21	13,43	31,64	8,10	32,88	4,25	25,94	19,09	27,02	29,76	28,05	34,33	28,15	41,24
Ac_A20_*Bacillus altitudinis*	53,54	5,93	60,01	10,16	61,23	11,81	61,74	13,74	36,26	25,21	36,93	28,53	37,19	30,87	37,76	37,87
Ac_E5_*Bacillus altitudinis*	20,08	33,60	20,91	39,04	21,40	41,07	24,02	57,19	18,62	48,11	19,08	58,19	21,76	53,22	23,14	55,72
Ac_E13_*Labrenzia marina*	17,26	30,66	17,66	39,74	18,35	52,88	20,59	55,22	17,68	19,27	18,51	32,45	20,74	38,19	22,36	38,39
Ac_E14_*Labrenzia alexandrii*	25,25	7,27	26,01	6,62	26,98	4,09	30,00	1,31	13,97	1,83	16,81	20,54	19,07	38,84	19,26	42,94
Ac_E17_*Bacillus aerophilus*	28,12	8,28	29,24	15,40	29,67	14,94	32,56	18,09	26,33	5,01	27,87	39,03	29,47	47,25	30,48	46,77
Ac_E19_*Phaeobacter gallaeciensis*	28,16	18,68	39,28	35,77	52,78	39,79	54,50	47,30	30,23	25,61	31,13	35,54	33,15	36,90	36,40	39,96
Ac_G37_*Bacillus velezensis*	20,83	11,59	26,18	13,51	26,25	24,05	27,89	30,69	10,47	6,72	11,28	9,77	12,97	10,12	14,04	11,44
Ac_G56_*Erythrobacter nanhaisediminis*	32,55	8,56	32,33	8,03	33,33	8,20	33,53	8,30	16,32	15,39	19,84	18,68	21,82	21,78	25,93	22,86
Ac_G57_*Erythrobacter nanhaisediminis*	26,44	6,18	30,68	8,14	31,91	9,46	32,29	11,09	18,69	15,13	20,73	17,09	23,22	22,74	25,12	27,14
Ac_L5_*Phaeobacter piscinae*	29,55	16,50	31,36	16,66	36,92	17,58	37,32	21,84	26,69	31,50	27,50	46,51	28,85	52,42	34,84	56,57
Ac_L8_*Bacillus altitudinis*	23,83	30,89	30,10	31,86	34,13	35,26	34,46	35,39	21,00	51,77	29,76	60,78	32,47	67,83	35,13	66,60
Ac_L12_*Phaeobacter gallaeciensis*	10,86	12,13	10,06	12,53	16,54	14,12	17,07	22,23	19,29	83,99	18,99	89,33	17,95	92,26	12,79	108,74
Ac_L15_*Bacillus aerophilus*	24,55	29,25	31,11	43,56	31,68	47,46	32,55	53,89	42,03	64,09	45,92	70,20	46,39	76,20	49,27	77,32
Ac_L27_*Bacillus altitudinis*	-0,80	27,65	0,00	29,89	-0,99	33,39	-0,54	33,74	0,00	68,48	4,73	72,07	7,31	86,54	8,25	87,09
Ac_L38_*Ruegeria atlantica*	20,58	11,57	24,71	10,52	25,38	10,46	25,93	10,23	19,53	45,32	19,15	55,37	23,61	58,78	24,39	60,23
Ac_L39_*Sporosarcina pasteurii*	18,48	10,93	21,52	12,85	25,50	12,16	25,53	15,10	22,18	54,86	18,61	56,16	18,84	62,73	18,81	66,12
Ac_L41_*Streptomyces pratensis*	13,38	17,26	20,08	10,23	20,20	7,77	20,46	5,79	15,05	64,94	17,87	70,37	21,29	73,90	21,64	72,49
Ac_L43_*Microbulbifer variabilis*	1,59	15,66	3,86	7,53	8,61	2,43	16,49	-0,17	17,90	53,63	14,23	57,59	14,39	64,97	11,41	76,56
Ac_M40_*Metabacillus indicus*	20,50	13,76	22,76	7,20	22,19	6,23	25,12	5,35	25,57	60,05	25,72	60,95	27,19	61,51	27,62	77,51
Ac_S14_*Streptomyces violascens*	24,08	11,31	25,08	12,52	32,22	13,18	32,81	14,28	23,38	88,81	23,44	88,23	24,22	97,19	31,15	98,91
Ac_T15_*Bacillus pumilus*	13,08	10,40	13,83	8,44	15,49	5,30	23,60	3,42	16,13	52,36	16,96	54,68	17,11	57,12	18,32	70,76
Ac_T16_*Bacillus pumilus*	10,93	9,57	14,27	6,56	15,59	6,34	17,18	5,28	11,04	69,72	16,54	68,44	16,71	72,10	16,73	84,52
Ac_T20_*Bacillus pumilus*	17,05	13,85	18,73	11,36	19,49	7,42	21,35	5,23	17,83	37,75	17,94	37,30	19,32	42,39	23,34	52,81
Total extracts displaying ≥ 50% BFR and < 40% GI	0		0		1		3		10		12		13		14	
Total extracts displaying ≥ 50% BFR and ≥ 40% GI	0		0		0		0		0		0		0		0	

Table S4.3: Quantitative analysis of percentage biofilm reducing potential (%BFR) and percentage growth inhibition (%GI) of *Acrop* CAB extracts against initial adhesion and mature biofilms of *Shewanella putrefaciens* ATCC 8071.

Extract	*Shewanella putrefaciens* ATCC 8071															
	Initial								Mature							
	200 µg/ml		400 µg/ml		600 µg/ml		800 µg/ml		200 µg/ml		400 µg/ml		600 µg/ml		800 µg/ml	
	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR
Ac_A18_*Streptomyces globisporus*	18,23	9,72	19,02	11,01	19,12	11,26	20,57	13,07	14,93	19,23	19,89	20,51	21,44	24,66	22,43	29,24
Ac_A20_*Bacillus altitudinis*	28,95	12,91	37,43	12,54	42,98	20,07	50,64	26,14	33,48	22,62	35,89	24,45	37,08	19,78	37,80	18,83
Ac_E5_*Bacillus altitudinis*	6,80	18,72	11,33	20,37	15,55	20,80	20,78	22,46	8,37	20,42	10,58	22,11	11,42	23,05	13,59	29,39
Ac_E13_*Labrenzia marina*	0,53	14,37	7,19	15,05	7,12	17,20	8,28	19,06	8,84	19,40	10,28	19,09	14,82	19,73	17,05	19,37
Ac_E14_*Labrenzia alexandrii*	7,73	9,48	10,31	5,12	15,01	2,54	15,50	2,21	10,22	24,45	11,18	26,13	12,56	27,32	14,15	29,71
Ac_E17_*Bacillus aerophilus*	15,52	8,64	18,54	17,00	20,69	32,47	23,13	33,53	18,21	20,83	18,47	34,96	19,22	34,06	19,46	34,23
Ac_E19_*Phaeobacter gallaeciensis*	13,21	23,18	26,80	32,32	29,90	44,11	32,83	45,92	17,15	27,32	19,50	32,25	20,20	32,42	21,13	34,77
Ac_G37_*Bacillus velezensis*	19,25	2,46	19,27	4,55	23,36	26,74	30,66	34,51	16,77	20,26	18,06	22,42	18,67	23,22	19,29	24,65
Ac_G56_*Erythrobacter nanhaisediminis*	17,01	9,09	18,04	11,72	19,66	13,19	22,68	15,02	21,85	13,17	21,84	12,71	23,78	17,93	24,48	19,24
Ac_G57_*Erythrobacter nanhaisediminis*	13,44	8,50	21,90	15,26	22,38	20,31	22,35	23,89	17,57	12,78	19,96	17,02	24,19	19,29	25,86	20,96
Ac_L5_*Phaeobacter piscinae*	17,04	19,22	23,98	29,46	26,76	30,96	28,75	31,82	20,13	15,17	24,60	18,80	25,48	23,07	26,04	23,69
Ac_L8_*Bacillus altitudinis*	22,49	-2,09	27,18	2,22	43,86	5,73	51,16	6,12	15,74	40,27	16,74	45,32	20,92	44,81	22,39	51,84
Ac_L12_*Phaeobacter gallaeciensis*	18,72	10,38	19,16	13,42	19,95	18,13	26,41	25,26	8,97	40,08	9,40	43,18	9,42	43,23	6,63	47,28
Ac_L15_*Bacillus aerophilus*	24,11	7,95	25,53	12,11	28,15	46,88	52,55	47,88	24,98	47,25	35,33	50,66	37,90	51,87	40,94	60,66
Ac_L27_*Bacillus altitudinis*	19,36	4,40	20,61	7,10	22,44	13,19	26,71	16,15	-3,83	37,98	-2,31	38,55	1,43	44,35	1,53	44,36
Ac_L38_*Ruegeria atlantica*	14,41	1,04	15,64	7,78	18,51	8,37	24,86	9,57	13,93	35,22	22,10	44,74	23,22	44,76	24,50	47,23
Ac_L39_*Sporosarcina pasteurii*	19,11	6,56	20,36	7,62	53,08	9,42	55,84	12,97	16,15	39,20	20,44	39,25	21,30	41,14	22,19	43,88
Ac_L41_*Streptomyces pratensis*	12,29	2,79	12,33	5,36	27,09	6,75	28,42	6,79	15,57	35,11	21,41	37,17	24,07	38,12	14,31	42,77
Ac_L43_*Microbulbifer variabilis*	26,27	3,50	40,15	5,55	40,36	6,61	49,11	6,44	9,16	35,84	11,64	35,73	10,45	36,84	10,79	45,54
Ac_M40_*Metabacillus indicus*	39,85	2,14	40,67	6,29	45,03	6,67	45,44	10,66	12,57	39,46	16,61	43,03	17,42	43,81	14,36	47,81
Ac_S14_*Streptomyces violascens*	16,35	9,63	18,22	32,36	18,32	32,55	18,62	35,23	8,35	58,53	16,84	63,93	16,93	77,99	26,77	92,64
Ac_T15_*Bacillus pumilus*	17,40	3,10	29,76	11,13	47,88	11,33	50,67	17,59	8,44	45,11	16,82	45,94	16,25	46,41	16,74	50,29
Ac_T16_*Bacillus pumilus*	16,41	6,07	29,59	16,74	42,76	23,64	53,72	23,02	4,69	41,08	9,95	43,59	16,12	44,29	17,97	47,12
Ac_T20_*Bacillus pumilus*	46,11	12,78	52,80	18,49	60,33	19,41	60,42	19,81	14,06	49,18	14,03	60,16	32,81	74,18	34,55	75,19
Total extracts displaying ≥ 50% BFR and < 40% GI	0		0		0		0		1		3		3		4	
Total extracts displaying ≥ 50% BFR and ≥ 40% GI	0		0		0		0		0		0		0		0	

Table S4.4: Quantitative analysis of percentage biofilm reducing potential (%BFR) and percentage growth inhibition (%GI) of *Acropora* CAB extracts against initial adhesion and mature biofilms of *Vibrio coralliilyticus* ATCC_BAA 450.

Extract	*Vibrio coralliilyticus* ATCC BAA 450															
	Initial								Mature							
	200 µg/ml		400 µg/ml		600 µg/ml		800 µg/ml		200 µg/ml		400 µg/ml		600 µg/ml		800 µg/ml	
	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR
Ac_A18 *Streptomyces globisporus*	82,60	76,03	83,25	77,63	83,65	81,73	84,70	83,19	22,55	11,64	46,22	16,56	66,78	19,80	68,16	20,21
Ac_A20 *Bacillus altitudinis*	53,03	73,16	62,85	79,14	68,86	82,70	70,80	85,08	66,84	14,06	69,60	77,92	74,15	94,33	74,98	114,38
Ac_E5 *Bacillus altitudinis*	84,31	77,19	85,15	79,63	87,44	78,30	89,23	81,84	13,44	16,02	23,84	58,34	46,58	75,01	68,31	95,81
Ac_E13 *Labrenzia marina*	73,53	81,26	84,23	81,98	84,32	82,71	89,06	82,71	28,71	19,28	35,27	40,08	40,46	51,06	47,47	78,58
Ac_E14 *Labrenzia alexandrii*	79,79	75,90	79,67	75,33	82,74	80,66	82,93	85,02	43,58	27,70	45,94	47,34	50,90	52,31	75,17	125,06
Ac_E17 *Bacillus aerophilus*	79,89	74,19	80,23	78,81	81,11	79,81	81,05	81,79	54,81	32,59	55,59	53,85	60,75	55,99	64,17	87,04
Ac_E19 *Phaeobacter gallaeciensis*	68,06	71,77	78,48	75,16	78,48	79,79	83,10	80,09	45,41	34,11	64,37	40,21	82,31	111,73	84,17	111,98
Ac_G37 *Bacillus velezensis*	80,71	82,11	82,71	86,00	82,78	88,30	85,61	87,31	45,04	31,15	46,01	44,46	50,76	49,53	60,09	50,22
Ac_G56 *Erythrobacter nanhaisediminis*	83,82	86,94	83,96	88,77	84,29	90,09	86,79	91,86	31,49	19,48	33,63	29,08	48,18	32,74	86,49	58,32
Ac_G57 *Erythrobacter nanhaisediminis*	87,18	92,16	87,45	95,28	88,13	95,33	88,72	95,33	36,31	15,40	49,89	38,42	73,19	75,69	83,06	56,81
Ac_L5 *Phaeobacter piscinae*	52,22	82,17	59,94	89,10	72,71	93,28	79,98	95,20	39,76	25,01	47,55	28,84	46,28	30,97	47,38	54,27
Ac_L8 *Bacillus altitudinis*	71,36	91,65	73,04	91,74	81,65	95,21	88,00	95,13	48,40	18,24	58,04	26,56	64,53	36,69	79,74	78,27
Ac_L12 *Phaeobacter gallaeciensis*	-85,40	-4,84	-84,12	13,41	-72,16	19,08	-47,81	24,78	17,12	-28,48	36,18	-30,98	45,86	56,79	59,84	66,35
Ac_L15 *Bacillus aerophilus*	34,47	78,10	51,12	83,62	52,15	85,94	55,18	89,40	71,55	-42,84	77,19	-36,64	78,32	-33,46	79,76	-28,95
Ac_L27 *Bacillus altitudinis*	-108,47	37,72	-108,06	48,67	-95,83	63,37	79,68	79,59	33,69	-23,94	36,06	77,72	39,75	98,17	79,60	98,35
Ac_L38 *Ruegeria atlantica*	-80,38	-0,17	-98,43	16,75	-56,00	82,51	-54,41	83,94	32,72	-23,44	68,86	52,75	82,39	90,09	82,98	131,47
Ac_L39 *Sporosarcina pasteurii*	-73,31	-9,10	-61,83	55,53	-41,11	72,25	-30,63	73,64	10,82	-14,01	21,04	-11,40	34,62	3,39	44,99	12,08
Ac_L41 *Streptomyces pratensis*	-84,79	-6,16	-81,34	55,65	-58,07	68,21	11,58	70,00	26,74	-8,42	61,51	12,91	64,39	81,87	74,41	83,89
Ac_L43 *Microbulbifer variabilis*	-94,13	0,57	-89,60	55,83	-36,01	72,47	65,91	83,83	20,81	22,99	54,71	71,18	57,54	88,57	73,53	97,04
Ac_M40 *Metabacillus indicus*	-101,22	-23,87	-101,50	21,49	-88,75	63,49	-87,48	65,83	11,34	8,40	60,98	88,97	70,70	99,68	71,65	101,03
Ac_S14 *Streptomyces violascens*	-68,16	-21,70	-44,30	-19,13	-19,77	-14,82	-18,79	-12,45	44,12	15,70	48,75	21,19	64,36	23,12	67,28	23,75
Ac_T15 *Bacillus pumilus*	-92,46	14,91	-78,68	61,96	-62,00	78,71	-59,95	80,39	36,74	28,62	66,82	43,21	71,15	57,44	71,19	71,59
Ac_T16 *Bacillus pumilus*	-86,32	-17,58	-84,41	52,91	8,41	81,13	74,99	87,27	28,83	33,98	72,63	68,43	78,51	82,82	78,89	108,25
Ac_T20 *Bacillus pumilus*	-65,94	-18,30	-40,31	76,36	57,76	75,29	61,00	77,82	61,00	33,32	65,05	53,95	67,81	57,57	71,23	67,37
Total extracts displaying ≥ 50% BFR and < 40% GI	1		0		2		2		0		2		0		0	
Total extracts displaying ≥ 50% BFR and ≥ 40% GI	12		18		19		20		0		7		12		18	

Table S4.5: Quantitative analysis of percentage biofilm reducing potential (%BFR) and percentage growth inhibition (%GI) of *Acropor*a CAB extracts against initial adhesion and mature biofilms of *Vibrio parahaemolyticus* ATCC 17802.

Extract	*Vibrio parahaemolyticus* ATCC 17802															
	Initial								Mature							
	200 µg/ml		400 µg/ml		600 µg/ml		800 µg/ml		200 µg/ml		400 µg/ml		600 µg/ml		800 µg/ml	
	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR
Ac_A18_*Streptomyces globisporus*	25,60	7,69	25,80	9,56	26,20	18,10	28,56	27,99	21,52	14,21	21,74	17,96	25,08	18,61	25,42	23,85
Ac_A20_*Bacillus altitudinis*	24,41	7,40	27,83	11,79	35,39	10,35	52,41	19,46	30,05	18,57	33,14	20,98	34,06	22,03	37,19	24,62
Ac_E5_*Bacillus altitudinis*	15,77	-4,30	18,52	11,34	19,26	26,29	24,39	79,74	8,27	13,81	17,45	28,42	18,83	28,74	20,93	28,44
Ac_E13_*Labrenzia marina*	12,01	33,75	15,40	36,87	16,22	58,57	16,99	117,58	5,28	19,29	13,63	27,16	13,56	2,31	14,59	39,78
Ac_E14_*Labrenzia alexandrii*	16,66	12,37	19,17	28,58	26,06	32,07	27,84	34,28	21,14	10,41	22,33	12,34	22,60	20,10	27,33	20,86
Ac_E17_*Bacillus aerophilus*	22,02	36,04	23,30	42,55	25,55	44,75	25,98	59,74	19,80	32,80	24,89	32,42	25,82	29,09	25,77	21,45
Ac_E19_*Phaeobacter gallaeciensis*	37,82	55,22	76,15	69,46	77,40	75,68	81,17	73,36	25,23	14,19	28,02	19,49	32,87	25,07	33,40	28,87
Ac_G37_*Bacillus velezensis*	11,32	42,07	12,61	48,96	14,97	54,21	21,07	59,83	9,83	6,51	13,48	4,98	14,04	12,66	15,02	25,41
Ac_G56_*Erythrobacter nanhaisediminis*	20,24	28,47	27,40	41,76	33,86	53,22	35,23	53,78	22,60	7,68	22,80	8,49	24,10	7,84	25,40	9,15
Ac_G57_*Erythrobacter nanhaisediminis*	23,88	31,10	28,69	70,30	31,99	92,59	33,22	112,62	20,24	14,25	24,02	16,37	28,08	19,01	28,09	18,92
Ac_L5_*Phaeobacter piscinae*	24,10	44,25	25,78	50,78	37,76	52,33	51,47	54,88	27,62	19,64	31,58	17,46	33,74	28,22	35,19	32,91
Ac_L8_*Bacillus altitudinis*	18,16	16,82	23,17	18,97	23,51	77,21	31,85	110,53	21,11	20,62	24,22	21,92	25,03	22,70	30,69	28,28
Ac_L12_*Phaeobacter gallaeciensis*	10,79	2,35	12,77	3,26	12,61	3,88	15,94	11,46	10,19	22,11	16,44	23,63	17,64	24,22	19,87	25,71
Ac_L15_*Bacillus aerophilus*	0,57	15,18	1,48	25,74	0,16	27,67	-1,90	27,30	34,02	22,89	35,12	31,07	35,99	41,50	38,12	42,30
Ac_L27_*Bacillus altitudinis*	21,50	1,51	30,73	12,99	32,44	13,12	44,84	17,75	2,10	18,48	3,38	23,69	6,18	27,47	9,67	38,85
Ac_L38_*Ruegeria atlantica*	14,15	4,30	8,54	10,82	8,26	15,09	7,41	19,34	24,40	12,33	21,88	12,66	16,54	12,69	13,96	14,83
Ac_L39_*Sporosarcina pasteurii*	9,68	4,44	15,13	10,12	17,52	13,24	19,25	23,92	20,47	16,79	24,10	18,51	25,12	18,55	26,47	19,28
Ac_L41_*Streptomyces pratensis*	6,97	6,96	8,96	9,93	9,62	14,38	15,45	16,54	17,0	9,36	20,1	13,51	22,1	15,19	24,6	15,41
Ac_L43_*Microbulbifer variabilis*	15,07	10,45	14,77	11,98	17,37	12,82	20,19	17,21	6,05	15,78	10,27	16,62	12,02	17,23	13,15	21,02
Ac_M40_*Metabacillus indicus*	13,41	12,20	15,66	12,25	19,20	16,54	25,34	18,13	21,16	16,71	20,25	20,93	20,35	22,66	23,47	28,33
Ac_S14_*Streptomyces violascens*	12,61	16,82	15,68	27,55	19,73	43,99	22,75	71,08	23,10	25,64	23,53	28,45	30,75	34,44	35,37	45,98
Ac_T15_*Bacillus pumilus*	31,05	12,04	52,35	19,87	55,51	28,30	56,33	31,09	18,10	22,03	15,08	28,17	12,63	27,68	8,17	30,95
Ac_T16_*Bacillus pumilus*	17,21	13,40	21,90	16,11	24,02	18,69	28,83	42,35	15,16	30,47	11,88	33,90	11,85	34,57	9,35	34,85
Ac_T20_*Bacillus pumilus*	16,31	4,35	16,93	5,08	17,08	5,58	17,85	6,03	19,29	28,43	23,14	28,58	29,82	29,37	41,83	31,20
Total extracts displaying ≥ 50% BFR and < 40% GI	1		2		6		8		0		0		0		0	
Total extracts displaying ≥ 50% BFR and ≥ 40% GI	0		1		1		2		0		0		0		0	

Table S4.6: Quantitative analysis of percentage biofilm reducing potential (%BFR) and percentage growth inhibition (%GI) of *Acropora* CAB extracts against initial adhesion and mature biofilms of *Vibrio shilonii* ATCC_BAA 91.

Extract	*Vibrio shilonii* ATCC_BAA 91															
	Initial								Mature							
	200 µg/ml		400 µg/ml		600 µg/ml		800 µg/ml		200 µg/ml		400 µg/ml		600 µg/ml		800 µg/ml	
	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR	% GI	%BFR
Ac_A18_*Streptomyces globisporus*	41,04	52,40	60,87	60,61	61,56	65,87	73,55	69,18	17,74	48,41	59,96	66,89	60,61	68,63	64,26	79,06
Ac_A20_*Bacillus altitudinis*	58,43	53,34	59,61	71,04	70,00	76,50	79,51	79,12	25,73	42,70	26,05	48,47	63,30	49,36	73,61	54,14
Ac_E5_*Bacillus altitudinis*	67,71	60,80	82,89	63,33	84,47	72,07	84,69	74,46	51,56	40,65	62,14	60,05	67,76	65,27	68,60	80,66
Ac_E13_*Labrenzia marina*	44,60	46,09	74,23	36,96	78,99	78,68	79,47	89,42	18,45	33,82	24,27	42,14	57,54	82,74	62,35	88,55
Ac_E14_*Labrenzia alexandrii*	28,86	35,79	78,64	43,07	79,26	45,89	79,37	49,88	40,19	56,88	58,05	66,56	59,41	76,35	62,72	89,01
Ac_E17_*Bacillus aerophilus*	51,62	28,69	75,02	36,94	77,00	44,18	77,69	45,61	19,32	51,38	43,18	60,52	50,40	71,32	53,71	74,19
Ac_E19_*Phaeobacter gallaeciensis*	45,04	36,57	58,99	46,09	74,02	50,14	76,31	51,13	35,99	71,67	37,57	72,79	42,10	73,69	52,17	77,48
Ac_G37_*Bacillus velezensis*	24,30	53,37	56,19	59,03	60,23	69,22	72,97	74,92	35,31	42,00	41,08	43,75	60,21	89,53	61,14	90,38
Ac_G56_*Erythrobacter nanhaisediminis*	44,96	54,66	69,93	56,81	80,40	60,26	83,73	60,83	65,29	83,66	71,40	92,96	73,93	94,95	74,09	96,27
Ac_G57_*Erythrobacter nanhaisediminis*	51,55	29,17	79,52	37,25	81,01	31,29	86,32	52,28	73,92	78,30	74,37	82,25	77,32	86,11	78,25	87,12
Ac_L5_*Phaeobacter piscinae*	68,63	54,90	69,29	54,10	70,62	60,38	73,99	65,86	66,28	90,53	57,88	93,32	52,74	93,66	48,79	95,63
Ac_L8_*Bacillus altitudinis*	37,71	17,88	76,40	18,98	77,32	29,76	80,82	30,37	59,86	75,78	64,93	86,54	65,32	88,81	66,50	91,03
Ac_L12_*Phaeobacter gallaeciensis*	20,72	-1,36	68,70	55,30	72,02	56,29	73,67	57,64	37,37	54,26	64,09	74,21	64,87	75,20	66,54	78,22
Ac_L15_*Bacillus aerophilus*	64,25	18,05	79,07	51,26	78,37	62,26	80,49	62,30	77,55	37,97	69,45	64,82	66,80	65,11	62,40	66,21
Ac_L27_*Bacillus altitudinis*	31,70	58,42	62,55	65,13	67,34	65,46	74,30	65,83	34,41	70,94	39,29	73,03	61,13	73,75	70,36	76,23
Ac_L38_*Ruegeria atlantica*	58,69	61,81	65,18	63,08	66,28	64,26	76,20	70,81	63,38	76,58	69,79	76,34	72,06	76,56	73,21	80,58
Ac_L39_*Sporosarcina pasteurii*	25,08	16,35	63,38	42,51	72,61	49,39	77,96	59,90	49,82	44,28	52,77	81,20	60,36	82,66	64,53	85,94
Ac_L41_*Streptomyces pratensis*	22,56	-28,51	45,03	48,95	57,71	51,61	63,84	59,48	38,93	83,58	42,59	85,62	47,27	86,84	50,09	89,69
Ac_L43_*Microbulbifer variabilis*	26,18	39,32	56,43	60,65	62,14	67,31	68,84	69,31	30,23	51,32	37,34	79,97	61,08	81,38	65,89	88,48
Ac_M40_*Metabacillus indicus*	22,08	-56,25	58,97	61,62	61,64	60,97	65,57	62,45	21,10	46,04	23,39	46,48	23,67	47,18	25,16	47,18
Ac_S14_*Streptomyces violascens*	38,04	5,30	63,39	21,27	70,44	32,43	71,22	41,34	43,87	48,25	57,08	68,91	63,85	77,29	64,48	81,03
Ac_T15_*Bacillus pumilus*	26,97	29,73	71,30	39,57	72,48	56,88	77,13	60,30	32,15	48,49	62,59	61,30	67,86	81,42	70,31	82,20
Ac_T16_*Bacillus pumilus*	23,21	11,60	31,82	16,47	63,74	18,71	72,99	66,24	45,20	46,36	58,72	76,65	70,26	82,01	71,80	89,17
Ac_T20_*Bacillus pumilus*	5,37	35,23	21,79	54,74	63,65	55,47	65,38	63,60	17,72	45,63	64,40	83,20	66,29	88,67	67,71	90,19
Total extracts displaying ≥ 50% BFR and < 40% GI	2		1		0		0		6		2		0		0	
Total extracts displaying ≥ 50% BFR and ≥ 40% GI	4		12		17		20		5		15		20		22	

CHAPTER 5

5.1 Discussion and conclusion

The increase in antibiotic resistance in both environmental and clinical settings has become a major problem globally (ElAhwany *et al*., 2015). The marine environment has become a significant source in the search of novel secondary metabolites to combat this challenge (Modolon *et al.,* 2020). Coral-associated bacteria (CAB), however, have become a new trend in the search for novel environmental and pharmaceutical compounds which can be used for drug development and to combat animal and human pathogens (Atencio *et al.,* 2018). The diversity of CAB is less known compared to other invertebrates; however, previous studies have reported a diversity of cultivable bacterial isolates to be associated with soft and hard corals (ElAhwany *et al.,* 2015; Sarmiento-Vizcaíno *et al.,* 2017; Zhang *et al.,* 2013). These bacterial isolates are suggested to produce a wide range of secondary metabolites that exhibit various biological activities, mainly antibacterial, antiviral, anti-quorum sensing, antitumor and/or anti-biofilm agents to protect their host (Pereira *et al*., 2017; Fang *et al.,* 2021). This study reported here focused on a preliminary isolation of *Pocillopora* and *Acropora* -associated bacteria using eight selective media. Results demonstrated that the bacterial isolates obtained were media-dependent, as more bacteria were obtained from Luria-Bertani media for the *Acropora* species while more bacteria were obtained from Marine agar isolation with the *Pocillopora* species. This is not surprising as Nithyanand *et al.* (2011) reported members of the actinobacteria species from *Acropora* coral to be media-dependant as they demonstrated greater culture yields on low nutrient media. Gram stains were also carried out to establish cellular morphology and to identify Gram-positive and Gram-negative isolates from these two coral species. The results of this study demonstrated rod-like Gram-positive bacteria to be the most dominant of both *Acropora* and *Pocillopora* species bacterial isolates. This is in line with Kalender (2016) who reported Gram-positive bacteria to be dominant in corals.

Several studies have been carried out to screen CAB for production of antimicrobial compounds (Chen *et al*., 2012; Yang *et al*., 2013). Primary screening for antimicrobial activity of both *Acropora* and *Pocillopora* bacterial isolates against multidrug-resistant *Pseudomonas aeruginosa* ATCC 27853 and methicillin-resistant *Staphylococcus aureus* ATCC 43300 suggested greater activity against *S. aureus* compared to *P. aeruginosa*. Sang *et al.* (2019) and Hettiarachchi *et al*. (2017) suggested that this may be due to the enhanced activation of cryptic genes responsible for producing metabolites. Primary screening of quorum sensing (QS)

inhibition of bacterial isolates against *Chromobacterium violaceum* ATCC 12472 and *Chromobacterium subtsugae* CV017 demonstrated that less than 50% of the isolates demonstrated QS inhibition against both strains. This is in line with Busseti *et al.* (2015) who reported 34.61% of their CAB isolates strongly inhibited QS. However, El-Kurdi (2021) demonstrated a greater yield of 66.2% of QS inhibitory CAB. This suggests that CAB may be a potential source of QS inhibition compounds. Screening of autoinducer-2 inhibition demonstrated that both *Pocillopora* and *Acropora* bacterial isolates interfered with the production of bioluminescence with minimal-to-no killing being observed. This is in line with Teasdale *et al.* (2011) who observed that some marine bacterial isolates were able to interfere with *Vibrio harveyi* bioluminescence by production of secondary metabolites.

Previous studies on CAB have identified a diversity of bacteria using molecular identification based on 16S rRNA sequencing (Mahmoud *et al.,* 2016; Sarmiento-Vizcaíno *et al*., 2017). In the present study, molecular identification based on 16S rRNA of *Pocillopora* and *Acropora* bacterial isolates identified a diverse set of bacterial isolates that represent from different genera. Five bioactive bacterial genera were identified from the *Pocillopora* species including: *Bacillus, Halomonas, Phaeobacter, Staphylococcus* and *Streptomyces.* Nine bioactive genera members were identified from the *Acropora* species. including *Bacillus, Erythrobacter, Labrenzia, Metabacillus, Microbulbifer, Phaeobacter, Ruegeria, Sporosarcina* and *Streptomyces.* These results demonstrated that *Bacillus, Phaeobacter* and *Streptomyces* are commonly associated with corals and agrees with previous studies (Li *et al.,* 2014; Sarmiento-Vizcaíno *et al*., 2017). Members of the genus *Bacillus* are known to exhibit faster growth rates and doubling time, which is why they are isolated in large numbers in both marine and terrestrial environments (ElAhwany *et al.,* 2015). However, in the marine environment *Bacillus* species are known to produce secondary metabolites with antimicrobial properties, and thus demonstrate a potential in the discovery of new antimicrobial compounds (ElAhwany *et al.,* 2015). Other dominant culturable bacteria belonged to the genus *Streptomyces*, and different bioactive compounds have been studied including sarcophytolide from *Streptomyces glaucum* which demonstrated antimicrobial properties (ElAhwany *et al.,* 2015). Antimicrobial compounds obtained from *Streptomyces* species have been extensively studied, however, less is reported about their QS and biofilm inhibitory compounds (Tan *et al*., 2016). Other bacterial extracts from *Erythrobacter, Labrenzia, Metabacillus, Microbulbifer, Ruegeria,* and *Sporosarcina* species demonstrated antimicrobial activity in this study, although other bioactivities were also observed to different extents.

Several studies have demonstrated CAB to inhibit the colonization and growth of both clinical and environmental pathogens. This study aimed to assess the antimicrobial activity of selected bacterial extracts from *Acropora* and *Pocillopora* bacterial isolates. Against clinical pathogens *P. aeruginosa* and *S. aureus*, *Pocillopora* bacterial extracts were not effective against *P. aeruginosa,* with only *Phaeobacter gallaeciensis* P_M18 extract demonstrating activity against *S. aureus*. This is in line with Bourne *et al.* (2016) who reported the antimicrobial potential of *Phaeobacter* species due to production of tropodithietic acid. It has been also reported that this genus demonstrated antagonistic characteristics against a wide range of bacteria including clinical pathogens such as *S. aureus* (Egan and Gardiner, 2016; Fang *et al.,* 2019). Extracts from the *Acropora* -associated bacteria were not very effective against both clinical indicators, except for two *Bacillus* extracts which demonstrated intermediate and strong activity against both indicators. This is in line with Ramachandra *et al*. (2014) who reported *Bacillus* species to produce a wide range of antimicrobial compounds such as bacteriocins with the ability to deal with the global multidrug resistance problems. They also reported a broad-spectrum antimicrobial activity of *Bacillus* species against various clinical indicators including *S. aureus*.

Antimicrobial activity by the *Pocillopora* bacterial extracts against marine indicators *Shewanella putrefaciens*, *Vibrio alginolyticus*, *Vibrio coralliilyticus, Vibrio parahaemolyticus* and *Vibrio shilonii* demonstrated that *Phaeobacter* and *Bacillus* species were more effective against these organisms. The *Phaeobacter* sp. strain P_M18 extract was more effective compared to the extracts from *Bacillus* species as it yielded activity against multiple indicators. This is in line with D'Alvise *et al.* (2013) who reported *P. gallaeciensis* to inhibit a wide range of bacteria especially *Vibrio* species. For the *Acropora* bacterial extracts, the *Bacillus* extracts demonstrated intermediate to strong activity against *Vibrio* species with *Erythrobacter* extracts demonstrating activity against *V. alginolyticus*. Several studies have demonstrated *Bacillus* species to inhibit *Vibrio* species that are pathogens (Raina *et al.,* 2016). Vizcaino *et al.* (2010) demonstrated *Erythrobacter* species to effectively inhibit several *Vibrio* species . These results suggest that members of the genus *Bacillus* demonstrate broad spectrum of antimicrobial activity and could be of great potential in producing novel antimicrobial agents. More studies are, however, required to evaluate and explore their potential.

The *Pocillopora* bacterial extracts demonstrated QS inhibition activity against both *C. subtsugae* CV017 and *C. violaceum* ATCC 12472, with more extracts demonstrating QS inhibitory activity against *C. violaceum* ATCC 12472. *Bacillus* extracts exhibited a broad-spectrum QS inhibition activity with activity against both biosensors, followed by the *P. gallaeciensis* P_M18 extract. This is in line with Ma *et al.* (2018) who reported *Bacillus* species to be among the known QS inhibitors. Their study further reported *Bacillus* species to be among the 15% (30) bacterial isolates that demonstrated significant anti-QS activity by reducing the purple pigment of *C. violaceum* ATCC 12472. For the *Acropora* bacterial extracts, five extracts demonstrated QS inhibition activity against both biosensors, while three demonstrated activity against individual biosensors. More QS inhibition was observed against short chain acyl homoserine lactone (AHL)-producing *C. subtsuage* CV017 compared to long chain AHL-producing *C. violaceum* ATCC 12472. *Bacillus, Streptomyces* and *Erythrobacter* extracts demonstrated more QS inhibitory activity compared to extracts from other identified genera. *Bacillus* extracts generally demonstrated broad spectrum activity against both biosensors. This is in line with Thenmozhi *et al.* (2009) who reported *Bacillus* species to have demonstrated QS inhibitory activity against both *C. violaceum* ATCC 12472 and CV026. Reina *et al*. (2019) also reported CAB to inhibit virulence factors of *P. aeruginosa* PAO1 such as the production of pyoverdine and reduced swarming.

Interference with autoinducer-2 (AI-2) QS is a promising approach to combat bacterial infections in both clinical and environmental settings. Greater than 60% of the *Pocillopora* bacterial extracts interfered with *V. harveyi* bioluminescence, with 50% of the *Bacillus* species demonstrating inhibition of AI-2 and AHLs, with killing observed for some extracts. This is in line with Teasdale *et al.* (2011) who reported *Bacillus* species to inhibit *V. harveyi*. Their study further reported that this may be due to *Bacillus* species are known for their production of secondary metabolites with the ability to degrade AHL signals and may be responsible for some of the bioluminescence inhibition (Teasdale *et al.,* 2011). For the *Acropora* bacterial extracts, 30% demonstrated *V. harveyi* inhibition, with no killing being observed with any of the extracts.

Biofilm formation by pathogens is a promising target for therapeutic intervention and has received significant attention recently, leading to the discovery of biofilm inhibitors for many of the commonly known bacterial pathogens (Ma *et al*., 2018; Wang *et al*., 2018). In this study, the *Pocillopora* bacterial extracts were found to be ineffective against initial adhesion of clinical pathogens *P. aeruginosa* and *S. aureus*, with only a single extract demonstrating

bactericidal activity. Against mature biofilms, extracts demonstrated ≥50% biofilm reduction (BFR) activity against *S. aureus*, with no activity against *P. aeruginosa* mature biofilms. *Bacillus* extracts demonstrated better BFR compared to extracts from other genera. This is in line with Modolon *et al.* (2020) who reported that CAB were strong agents for controlling clinical pathogens. The present study demonstrated that *Bacillus* species showed statistically significant anti-biofilm activity against *S. aureus.* The current study also has demonstrated that *Streptomyces* extracts had anti-biofilm activity against *S. aureus*. This is in line with Bakkiyaraj and Pandian (2010) who reported actinomycetes to have inhibited biofilm formation of *S. aureus.* Against marine indicators, the extracts demonstrated better initial adhesion inhibition compared to mature biofilm reduction, with some extracts demonstrating bactericidal activity. This may be due to the extracellular polymeric substance layer being easy to penetrate at initial adhesion stages compared to complex mature biofilms (Kaur *et al.,* 2019). *Bacillus* and *Phaeobacter* extracts demonstrated better activity against both initial adhesion and mature biofilms. This is in line with Prado *et al.* (2009) who reported that *Phaeobacter* species to antagonistic characteristics against various pathogens especially *Vibrio* species. However, *Bacillus* species showed greater biofilm inhibition against *V. coralliilyticus*. This is in line with Nithya and Pandian (2010) who reported *Bacillus* species to have inhibited initial and mature biofilm of marine *Vibrio* species Song *et al.* (2018) reported extracts from CAB isolated from *Pocillopora damicornis* to inhibit biofilm formation against a wide range of *Vibrio* species The present study is in line with these studies as *Bacillus* species have been shown to produce bioactive compounds with anti-biofilm properties against both clinical and marine pathogens. Results from this study also demonstrate that CAB may be important sources for anti-biofilm agents.

The *Acropora* bacterial extracts displayed greater inhibition of initial adhesion of *P. aeruginosa, V. coralliilyticus* and *V. parahaemolyticus*, but better mature biofilm reduction for *S. aureus, S. putrefaciens* and *V. shilonii. Bacillus* and *Phaeobacter* extracts exhibited better BFR against methicillin-resistant *S. aureus* and multi-drug resistant *P. aeruginosa* for both initial and mature biofilms at varying concentrations. Against marine pathogens, only *Bacillus* extracts exhibited better BFR for both initial adhesion and mature biofilm. Furthermore, *Bacillus* extracts demonstrated broad spectrum activity against *Vibrio* species. This is in line with El-Kurdi *et al*. (2020) who reported CAB including those of the genus *Bacillus* to have inhibited biofilm formation against clinical and marine pathogens *P. aeruginosa* and *V. alginolyticus*. Gowrishankar *et al.* (2012) also reported that *Bacillus* species. reduced

exopolysaccharide production of *S. aureus*. This suggests *Bacillus* species to be a promising source of anti-biofilm compounds against clinical and marine pathogens.

In conclusion, microorganisms are a rich source of novel secondary metabolites to combat environmental stress and the production of these compounds may also be because of symbiotic interactions among the host symbionts (Pereira *et al*., 2017). This study identified CAB as a novel source of bioactive compounds for developing antimicrobial, anti-QS and anti-biofilm compounds against marine pathogens and to a lesser extent, clinical pathogens. Bacteria belonging to the genera *Phaeobacter* and *Streptomyces* which had broad-spectrum antimicrobial activity and *Bacillus* species which demonstrated both anti-QS and anti-biofilm activities have been identified. More studies still need to be carried out on CAB with the ability to inhibit QS virulence production factors by pathogens. This will help identify more antimicrobial and anti-QS compounds as the discovery of these compounds is still at an infancy stage. In this study, many CAB with the ability to produce QS inhibitory compounds were identified. This suggests that these bacteria may also be able to prevent biofilm formation caused by biofouling bacteria. These QS inhibition compounds could be applied in human therapy or as natural antifouling agents. They are a promising source of pharmaceutical and environmental applications. Future studies will focus on isolating individual compounds from CAB exhibiting QS inhibition potential and discover their exact role in the inhibitory mechanism. Additionally, cocktails of "probiotic" bacteria with differing bioactivities could be composed and assessed for their potential in dealing with marine challenges such as coral bleaching or biofouling.

5.2 References

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