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Unveiling the bioactive potential of marine bacteria

Desvendando o potencial bioativo de bactérias marinhas

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Microbiologia, realizada sob a orientação científica da Doutora Olga Maria Oliveira da Silva Lage, Professora Associada do Departamento de Biologia da Universidade do Porto e do Doutor Artur Jorge da Costa Peixoto Alves, Professor Auxiliar com Agregação do Departamento de Biologia da Universidade de Aveiro.

Science makes people reach selflessly for truth and objectivity; it teaches people to accept reality, with wonder and admiration, not to mention the deep awe and joy that the natural order of things brings to the true scientist. – Lise Meitner

o júri

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palavras-chave

Compostos bioativos, Atividade antimicrobiana, Antibióticos, OSMAC, PKS-I, NRPS, Conjugação Triparental.

resumo

Para além de um metabolismo primário, os organismos vivos possuem um metabolismo secundário que permite a produção de metabolitos secundários que, normalmente, não são fundamentais para o crescimento e sobrevivência do organismo. Estes metabolitos permitem uma melhor adaptação ao ambiente envolvente, uma vez que atuam como mecanismos de defesa e podem ser bioativos contra vários agentes patogénicos, podendo ser utilizados como medicamentos para os eliminar eficazmente.

Inicialmente, a procura por novos compostos naturais pela comunidade científica era focada em ambientes terrestres. No entanto, mais recentemente, cada vez mais atenção é dada aos oceanos.

Uma vez que tem existido um abuso geral na utilização de antibióticos, o número de bactérias resistentes a antibióticos tem vindo a aumentar rapidamente. Por isso, é essencial investir na descoberta de novos compostos que podem ser usados como alternativas aos antibióticos tradicionais.

Este trabalho teve como principal objetivo a análise do potencial antimicrobiano de uma coleção de bactérias marinhas através de ensaios moleculares e de despiste.

Com o objetivo de aumentar a produção de metabolitos secundários, neste trabalho, uma transformação do planctomycete *Roseimaritima ulvae* UC8^{T} foi realizada utilizando a técnica de conjugação triparental. Esta bactéria foi escolhida uma vez que *Planctomycetes* mostraram serem promissores a nível de potencial bioativo. No entanto, esta estirpe exibiu a produção de algum tipo de composto bioativo capaz de inibir o crescimento de *Escherichia coli* ATCC 25922 e que, possivelmente, afetou o crescimento da *E. coli* DH5α dadora e ajudante envolvida no processo de transformação.

Em paralelo, um ensaio molecular para a amplificação de sintases de policétidos tipo I (PKS-I) e sintetases de péptidos não ribossomais foi realizado com 329 estirpes recentemente isoladas e pertencentes ao Laboratório de Ecofisiologia Microbiana da Universidade do Porto. Destas estirpes 36% deram origem a amplicões para PKS-I e 24% para NRPS. As restantes não amplificaram para nenhum destes genes. Posteriormente, uma seleção foi realizada baseada na amplificação destes genes e as estirpes promissoras foram escolhidas para serem testadas contra *E. coli* ATCC 25922 e *Staphylococcus aureus* ATCC 29213.

Para além disso, uma abordagem de uma estirpe muitos compostos (OSMAC) foi realizada com as estirpes que mostraram ser bioativas no ensaio preliminar em 5 meios de cultura diferentes (1:10 M607, M607, M600, MA e CGY).

Das bactérias selecionadas apenas 16 estirpes foram consideradas bioativas e principalmente contra *E. coli* ATCC 25922, ao contrário de *Streptomyces flavoviridis* PMIC_1A8B que foi altamente bioativa contra *S. aureus* ATCC 29213. A maior parte das estirpes bioativas pertencem ao filo *Actinobacteria* exceto *Arenibacter aquaticus* PMIC_1E11B.2, *Aquimarina algiphila* PMO90_19.1 e uma nova espécie PMO138_17 relacionada com *Methylotenera mobilis*.

No ensaio OSMAC, os valores mais altos de bioatividade foram obtidos nos extratos de estirpes crescidas em 1:10 M607, o meio de cultura menos rico em nutrientes. Para além disso, também foram obtidas bioatividades altas para o meio de cultura mais rico em nutrientes CGY.

Em conclusão, a abordagem OSMAC mostrou a importância da composição do meio de cultura para a produção de compostos bioativos.

keywords

Bioactive compounds, Antimicrobial activity, Antibiotics, OSMAC, PKS-I, NRPS, Triparental Mating.

abstract

Besides a primary metabolism, living organisms possess a secondary metabolism which allows for the production of secondary metabolites that, normally, are not fundamental to the organism's growth and survival. These metabolites allow for a better adaptability to their environment as they act as defence mechanisms, can be bioactive against several pathogenic agents and can be used as drugs to efficiently eliminate them.

At first, the search for new natural compounds by the scientific community was based on terrestrial environments. However, more recently, more and more attention has been paid to the oceans.

Since there has been a general abuse in the use of antibiotics, the numbers of antibiotic resistant bacteria have been rising rapidly. Therefore, it is essential to invest in the discovery new compounds that can be used as alternatives to the traditional antibiotics.

The main aim of this work was the analysis of the antimicrobial potential of a collection of marine bacteria by molecular and screening assays.

Aiming at obtaining a higher yield of secondary metabolites, in this work a transformation of the planctomycete *Roseimaritima ulvae* UC8^T was performed using the triparental mating technique. This bacterium was chosen since *Planctomycetes* have shown promising bioactive potential. However, this strain exhibited the production of some kind of bioactive compounds that inhibited the growth of *Escherichia coli* ATCC 25922 and possibly affected the growth of the donor and helper *E. coli* DH5α involved in the transformation process.

In parallel, a molecular screening for the amplification of polyketide synthases type I (PKS-I) and non-ribosomal peptide synthetases (NRPS) was performed in 329 newly isolated strains which belong to Laboratório de Ecofisiologia Microbiana da Universidade do Porto (LEMUP) collection. From these strains 36% generated amplicons for PKS-I and 24% for NRPS. The rest did not amplify for either one of these genes. Then, a selection was made based on the amplification for these genes and the promising strains were chosen to be tested against *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213. Furthermore, a one strain many compounds (OSMAC) approach was performed to the strains that showed to be bioactive in the preliminary antimicrobial assay in 5 different culture media (1:10 M607, M607, M600, MA and CGY).

From all the bacteria that were selected only 16 strains were considered bioactive, and mostly against *E. coli* ATCC 25922, unlike *Streptomyces flavoviridis* PMIC_1A8B which was highly bioactive against *S. aureus* ATCC 29213. Most of the bioactive strains belong to the *Actinobacteria* phylum except for *Arenibacter aquaticus* PMIC_1E11B.2, *Aquimarina algiphila* PMO90_19.1 and the new species PMO138_17 closely affiliated with *Methylotenera mobilis*.

In the OSMAC assay, the highest values of bioactivity were obtained in extracts from strains grown in 1:10 M607, the lowest nutrient rich culture medium. Also, high activities were displayed in the more nutrient rich CGY culture medium.

In conclusion, the OSMAC approach showed the importance of the culture medium composition for the production of bioactive molecules.

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List of abbreviations

®	Registered trademark
A.U.	Arbitrary units
bp	Base pairs
CDC	Centers for Disease Control and Prevention
CGY medium	Casitone-Glycerol-Yeast extract medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamine tetraacetic acid
et al.	*et alii* - "and others"
Fig. /Figs.	Figure/Figures
FtsZ	Filamenting temperature-sensitive mutant Z protein
HCl-Tris	Tris-Hydrochloride
HGT	Horizontal Gene Transfer
kb	Kilo base
LB medium	Luria Broth medium
LEMUP	Laboratório de Ecofisiologia Microbiana da Universidade do Porto
MA medium	Marine Agar medium
NA/NB medium	Nutrient agar/ Nutrient broth medium
NAG	N-acetylglucosamine
NRPS	Non-Ribosomal Peptide Synthetases
ºC	Degree Celsius

OD	Optical Density
OSMAC	One Strain Many Compounds
PBS buffer	Phosphate Buffered Saline buffer
PCP	Peptidyl Carrier Protein
PCR	Polymerase Chain Reaction
pH	Potential of Hydrogen
PKS/PKS-I	Polyketide Synthases/ Type I Polyketide Synthases
rpm	Rotations per minute
sp.	Specie
^{T}	Type strain
T_0	Initial time
TAE	Tris-Acetate EDTA
T_f	Final time
™	Trademark
Tris	Tris(hydroxymethyl)aminomethane
WHO	World Health Organization

1. Introduction

1.1. Bioactive Compounds

Living organisms have a primary metabolism, which mediates absolutely vital reactions and pathways fundamental for survival. Furthermore, the secondary metabolism allows for the production of secondary metabolites which, normally, are not fundamental to the organism's growth or reproduction (Petersen et al., 2018). The production of these metabolites generally results in better adaptability to their environment and they act as defence mechanisms (Colegate & Molyneux, 2008). Secondary metabolites are not always produced, and there is a need for particular conditions to be met in order for the organisms to produce them (Dewick, 2009). These molecules can be bioactive against pathogenies, turning them into great opportunities for new drug development since they possess a large number of medical applications that have been explored along the centuries (Blunt et al., 2018).

The history of compounds extracted from natural sources began with salicylic acid, a compound present in willow trees that was used by Sumerians to treat inflammatory rheumatic diseases (Montinari et al., 2019). Other tribes and populations also used extracts from this plant to treat diseases and dimmish pain in several cases.

At first, the search for new natural compounds by the scientific community was based on terrestrial environments rather than the oceans (Haefner, 2003). The oceans have a vast biological diversity comprehending up to 10^{30} bacterial and archaeal cells (Salazar & Sunagawa, 2017). Only in the second half of the 1950s, more consideration was given towards the idea of extracting natural products from the oceans. The first natural products from the sea, vidarabine and cytarabine, extracted from sponges, possess anticancer activities and have passed the clinical trials, being used for many years (Bergmann & Feeneyz, 1951). Natural compounds can also be found in organisms like tunicates and algae (Molinski et al., 2008). For example, didemnin B, with cytotoxic and antiviral activities, was isolated from the tunicate *Tridemnum solidum* (Rinehart et al., 1981). However, didemnin B presented too much toxicity to be used as a medicine.

The rich biological diversity in the marine environment leads to a strong competition between species where the natural products are important weapons (Younis et al., 2016).

Furthermore, since life on Earth started in the water and only later on moved to the terrestrial environment, oceans have undergone the vastest period of evolution. This means that oceans possess the greatest amount of time to create complex biotic interactions that stimulate the production of natural products (Romano et al., 2016). The major problem with the approach to find new marine natural products is the quantity of product obtained, since these compounds are only produced in trace amounts specially by organism like sponges (Varijakzhan et al., 2021). Nevertheless, this can be avoided by using fermentation in large scales, in the case of bacteria or algae, even though it might prove to be challenging, and sometimes, the upscale does not work as intended (Jiménez, 2018).

1.2. Bioactive compounds from bacteria

The main bacterial phyla producing bioactive natural compounds are primarily *Actinobacteria* and *Myxobacteria* (Diez et al., 2012; Takahashi & Omura, 2003) but also *Firmicutes*, *Proteobacteria*, *Bacteroidetes* and *Planctomycetes* (Stincone & Brandelli, 2020). *Actinobacteria* are responsible for the production of the most promising compounds, namely salinosporamide A, which is a compound that is in phase 3 of the clinical trials and is a very potent proteosome inhibitor. Salinosporamide A has antitumor activity against several cancers, namely myeloma (Takacsová et al., 2016). The *Streptomyces* genus, in particular, possesses a great bioactive potential, containing species that have antibacterial, anticancer and antifungal activities. For example, *Streptomyces kanamyceticus* produces the antibiotic kanamycin, *Streptomyces venezuelae* produces the antibiotic chloramphenicol, *Streptomyces griseus* produces streptomycin and *Streptomyces nodosus* produces amphotericin B, a landmark in antifungals (Solanki et al., 2008). There is also, *Streptomyces antibioticus* which produces vidarabine, a compound with antiviral activity, capable of curing systemic infections, being effective against herpes virus, since it stops the viral DNA replication (Hong et al., 1986). While most *Actinobacteria* have been isolated from the soil, the marine *Actinobacteria* are still underexplored. More and more marine *Actinobacteria* are known by their bioactive potential since they produce terpenes, peptides, polyketides, quinones and other compounds which hold activities such as antimicrobial and anticancer (Solanki et al., 2008).

Also, *Myxobacteria* show a great bioactive potential. However, not many compounds produced by *Myxobacteria* have been approved. Still, the first compound extracted from a myxobacterium was haliangicin, which was extracted from *Haliangium luteum* isolated from a macroalgal sample in Japan (Fudou et al., 2001). This compound hinders the respiratory chain of filamentous fungi. Also extracted from a bacterium of the same genus, haliamide, is a cytotoxic compound against tumour cells and it is a hybrid between Polyketide Synthases (PKS) and Non-Ribosomal Peptide Synthetases (NRPS) (Sun et al., 2016), enzymes involved in the production of bioactive compounds. Almost all the marine bioactive *Myxobacteria* are from the genus *Enhygromyxa*. The earliest compound found from this genus was salimabromide, a natural product that has effect against *Arthrobacter* sp. (Felder et al., 2013).

Regarding other phyla, the *Firmicutes Enterococcus faecalis* strain #118_3 has shown to have a strong activity against *Trypanosoma cruzi* causing total growth inhibition (Santos et al., 2020). Also recently, *Bacillus rugosus* was discovered to be a producer of a diketopiperazine (3,6-diisobutylpiperazine-2,5-dione) (Bhattacharya et al., 2020), which had a strong antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* (Bhattacharya et al., 2019). Furthermore, a compound with antibacterial activity, bacicyclin, was discovered in the bacterium *Bacillus* sp. (Wiese et al., 2018). This compound is a cyclic hexapeptide that can affect the growth of *S. aureus* and *E. faecalis*.

In the phylum *Proteobacteria*, there are some compounds that have been synthetized from marine strains, namely solonamides produced by *Photobacterium* sp. which have been bioactive against methicillin resistant *S. aureus* (Nielsen et al., 2014). More examples of bioactive *Proteobacteria* are presented in Table 1.

The *Bacteroidetes Pontibacter korlensis* produces pontifactin, a lipopeptide biosurfactant. This species showed to be bioactive against *Streptococcus mutans*, *Micrococcus luteus*, *Salmonella typhi* and *Klebsiella oxytoca* (Balan et al., 2016).

Planctomycetes also possess promising potential, since, recently, *Stieleria maiorica* has shown to produce stieleriacines (Kallscheuer et al., 2020). These compounds are moderately bioactive against *Bacillus subtilis*, *Micrococcus luteus*, *S. aureus* and *Mucor hiemalis.* Furthermore, it has been proven that planctomycetal strains possess activity against *E. coli* in antimicrobial assays (Jeske et al., 2016) as well as against *Candida albicans* (Graça et al., 2016). Likewise, *Planctomycetes* also possess anticancer activity,

since the several *Planctomycetes* were capable of stimulating apoptosis in prostate and kidney cancer cells, as well as diminishing their growth (Calisto et al., 2019). Besides, *Planctomycetes* also display antialgae activity by producing the 3,5-dibromo-p-anisic acid, which was the first bioactive compound to be discovered in this group (Panter et al., 2019).

Table 1 – Bioactive metabolites produced by marine *Proteobacteria* (Adapted from Anjum, 2021).

Affiliation	Metabolites	Bioactivity	References
Vibrio sp.	Moiramide	Antibacterial activity	(Pohlmann et al., 2005; Slightom & Buchan, 2009)
	Kahalalides		
	Andrimid		
Photobacterium sp.	Solonamides	Antibacterial activity	(Mansson et al., 2011; Oku et al., 2004)
	Ngercheumicins		
	Unnarmicins		
Myxococcus fulvus	Myxothiazols	Antibacterial activity	(Irschik et al., 1983)
	Myxovalargins		
	Althiomycin	Antifungal activity	(Yamaguchi et al., 1957)

1.3. The importance of antimicrobials

Since their discovery, antibiotics have shown their great potential to overcome some of humanity's greatest hurdles against bacteria and are partly responsible for the extension of our average life span (Ventola, 2015). As an example, their use in certain surgeries, such as organ transplantation and insertion of medical devices, greatly improves patients' outcomes (Rossolini et al., 2014).

The first chemically pure natural antibiotic was first discovered by accident by the bacteriologist Alexander Fleming, after noticing that one of his cultures was contaminated by fungi which had the power to influence the growth of the bacteria nearby (Swann, 1983). This contamination was in fact *Penicillium notatum* producing penicillin. This molecule was capable of binding to the active site of an enzyme called transpeptidase avoiding this enzyme from forming links between peptidoglycan strands, an essential step for the formation of peptidoglycan, which is a very important component to the bacterial cell wall (Lobanovska & Pilla, 2017).

However, the mishandling of antibiotics induced the appearance of antibiotic resistant bacteria which number is increasing rapidly, a fact predicted by Fleming himself (Norrby et al., 2005). An example of abuse in the use of antibiotics is in livestock production. The population increase implies a greater need of meat production for which is necessary to invest in more intensive production systems that rely mostly on antibiotics. Around the world, 73% of the antibiotics are used to empower this industry (Boeckel et al., 2019).

The rise in antimicrobial resistance in bacteria is leading also to an increase in mortality in patients. For example, according to the World Health Organization (WHO), the mortality in patients infected with *E. coli* which is resistant to 3rd generation cephalosporin has doubled. The same scenario is true for methicillin resistant *S. aureus*, for which its infections have increased their mortality ratio (WHO, 2018). Some Gram negative bacteria present a huge public health risk (Brown & Wright, 2016) and, according to WHO the most problematic bacteria under this group are the carbapenem-resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacteriaceae*, including *E. coli* and *Klebsiella pneumoniae* which are responsible for a large amount of the bacterial infections caused to humans (Willyard, 2017). The fight against these bacteria is hindered by the decrease in effective antibiotics and also by the lack of interest in the discovery of new antibiotics by the pharmaceutical industry since the amount of profit per treatment is very reduced, due to the large costs associated with drug development (Norrby et al., 2005).

Antibiotic resistance in bacteria happens due to several mechanisms, which can either be intrinsic or extrinsic (Murray et al., 2007). Acquired resistance results from transformation, transduction or conjugation, and can involve plasmids, integrons, transposons and bacteriophages (Murray et al., 2007). Therefore, a mechanism that contributes to mutation and consequently, antibiotic resistance, is horizontal gene transfer (HGT), as it also corresponds to the most common cause for evolution in bacteria (Giedraitienė et al., 2011).

Regarding the mechanisms of inactivation of antibiotics, one consists in the production of enzymes that take action by complementing the molecules with chemical motifs that destroy the drug (Munita et al., 2016). Another mechanism, especially in Gram negative bacteria, is the decrease of the permeability of the membrane to some compounds. Some of the drugs affected by this mechanism include β-lactams, tetracyclines and several

fluoroquinolones (Pagès et al., 2008). Bacteria can also change the efflux through efflux pumps. Due to these pumps, it is possible for them to inhibit the action of many antibiotics. It is also possible to change the target of the antibiotics, avoiding their engagement (Munita et al., 2016).

Antifungal resistance is also rising (Mille-Lindblom et al., 2006). Infections by these microorganisms seem to be increasing rapidly, and there appears to be a greater number of unique fungi causing infection (Lockhart & Guarner, 2019). According to The Centre for Disease Control and Prevention (CDC), 7% of *Candida* isolated from the bloodstream is already resistant to fluconazole (Toda et al., 2019). *Candida auris*, an emerging fungus, also shows resistance to amphotericin B (33%) and fluconazole (90%) (Lockhart et al., 2017). *Aspergillus* infections are not as common as *Candida* infections, since they normally infect immunosuppressed patients. However, there has been a growth in its numbers (Pfaller, 2012). Therefore, it is essential to invest in the discovery new compounds that can be used as alternatives to the traditional antifungals, allowing to battle this rising resistance and fight efficiently fungi (Interagency Coordination Group on Antimicrobial Resistance, 2019).

To better battle these superbugs, it is necessary to expand our knowledge, namely prioritize the sequencing and study of several bacteria with the potential to produce bioactive compounds (Spízek et al., 2010). Furthermore, to search for these new compounds there are several approaches, such as searching for new drugs using metagenomics in environmental DNA and genome sequencing to identify gene clusters responsible for the synthesis of these compounds (Spízek et al., 2010). This last approach is getting easier and easier to achieve since with technology advancements it was possible to achieve genome sequencing in a much faster, cheaper and reliable way (Ansorge, 2009). Thanks to new bioactive compounds it is possible to reduce the multi drug resistance crisis. (Redondo-Blanco et al., 2017).

1.4. Non-Ribosomal Peptide Synthetases and Polyketide Synthases

Non-ribosomal peptide synthetases (NRPS) possess several modules which make them large enzymes that are responsible for the production of non-ribosomal peptides which are obtained from the secondary metabolism of the cell (Walsh, 2008). Much like the NRPS,

polyketide synthases (PKS) also function as modules. NRPS and PKS represent the most common gene clusters responsible for the production of new bioactive molecules. This way, the presence of these enzymes is normally associated with the discovery of new natural compounds (Donadio et al., 2007). Since they are modular enzymes, NRPS and PKS are a product of the elongation of acyl-S-proteins (Fischbach & Walsh, 2006), after an initiation module and respective elongation, the terminal module allows for the release of the acyl chain from the thioester link (Walsh, 2008).

PKS can be found in three types. Type I PKS is divided in two groups: iterative or modular. In iterative type I PKS there are enzymes which are used more than once to condense carbon blocks into the palmitate fatty acid. One example of this type of PKS can be observed in the fatty acid synthase in humans (Keatinge-Clay, 2012). As for modular type I PKS as the name indicates, they possess modules that can condense blocks of carbon into a polyketide chain. One of these enzymes is the erythromycin PKS (Keatinge-Clay, 2012). Furthermore, type I PKS can be found in several microorganisms such as *Actinomycetes*, *Myxobacteria*, *Cyanobacteria* and fungi (Bulkley et al., 2010). Plus, *Planctomycetes* have been screened for PKS and it has been found that they do possess type I PKS (Graça et al., 2016). As for type II PKS, they have heterodimers and are exclusive to bacteria (Shen, 2000). Type III is a homodimer with a ketosynthase domain, unlike type I and type II. This domain then performs the functions that would be performed by the several domains in the modules of type I and type II PKS. Type III PKS can be found in plants, fungi and several bacteria (Shimizu et al., 2016).

NRPS carry peptide and amino-acid intermediates to catalytic domains to perform the initiation step, then the elongation and afterwards, termination, leading to peptide release (Marahiel et al., 1997). For the initiation, there is amino-acid adenylation and the intermediate that is formed is then bound to peptidyl carrier protein (PCP) domain, which is similar to the one in fatty acids synthesis. Next, it is the elongation step, in which there is the formation of bonds to elongate the peptide chain through the action of the condensation domain. And finally, in the termination step, there is the release of the long and completed peptide from the PCP domain (Figure 1) (Miller & Gulick, 2016).

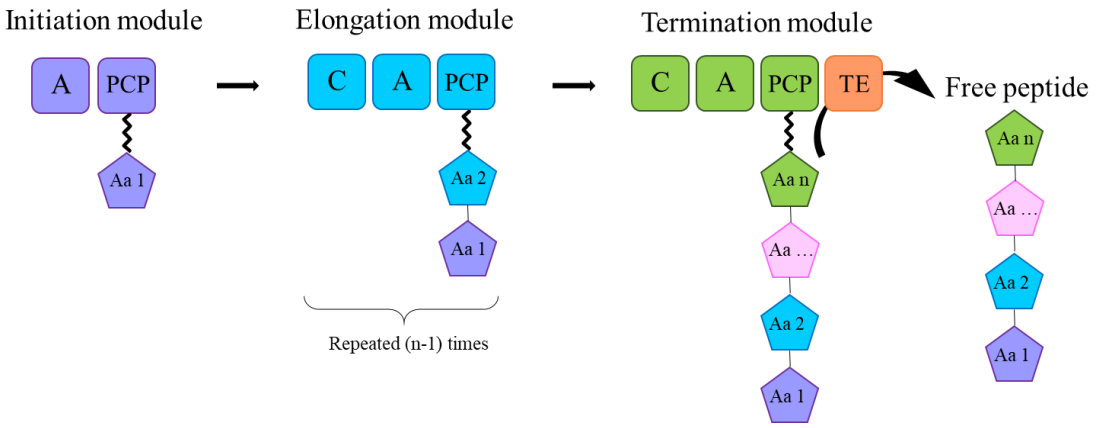

Figure 1 - NRPS synthesis schematic. Aa: amino-acid; A: adenylation domain; C: condensation domain; PCP: peptidyl carrier protein and TE: thioesterase domain (Adapted from Desriac et al., 2013).

1.5. One Strain Many Compounds (OSMAC) approach

The One Strain Many Compounds approach was developed by Bode et al., (2002). The idea behind this approach was to enable the research field to compete with the pharmaceutical industry as well as the big biotechnology companies that possess a much larger amount of funds, since this methodology involved the detailed study of few organisms and a shift in the cultivation conditions, which made this work a lot cheaper. The hypothesis formed was that a single microorganism could produce several compounds. However, those compounds were only produced when the different growth parameters were changed (Bode et al., 2002). This way, thanks to the OSMAC approach, Bode et al., (2002) were able to discover that *Aspergilus ochraceus* produced several compounds, when, initially it was thought to produce only one compound. With the change of nutrients, temperature, co-cultivation and other changes in physical and chemical parameters, it is possible to uncover the hidden potential of microorganisms, namely the ones that are not compatible with genetic manipulation. And it is also possible to use the OSMAC approach without having any previous knowledge about the biosynthetic gene clusters involved (Reen et al., 2015; Romano, 2018).

It has been proven that changes in the ratio between carbon and nitrogen, as well as salinity have influence over the production of bioactive compounds (Pan et al., 2019). The carbon source is important since it is the energy source provided to the microorganisms and it is also vital for the formation of the bioactive metabolites because it grants carbon bases

necessary for their structure. The nitrogen source is necessary for the production of proteins and nucleic acids involved in the synthesis of secondary metabolites (Singh et al., 2017). Differences in the nitrogen and carbon sources cause differences in the degradation of the medium components that are providing these sources to the microorganisms, which in turn, trigger changes in the pH of the culture medium. These changes are unique from medium to medium and result in the production of diverse secondary metabolites when the microorganisms are exposed to different culture media (Ma et al., 2009). Salinity is key to determine the seawater chemistry. Salinity is variable and so, the expression of secondary metabolites has evolved to adapt to these conditions. It has been proven that the addition of sea salts, as well as differences in salinity, influence the production of such compounds (Overy et al., 2017; Wang et al., 2011). Furthermore, salinity impacts the osmotic pressure. In order to maintain regular growth, it is necessary to possess the right amount of salinity, since high osmotic pressure causes the dehydration of the cells and, consequently, has effect in the biochemical reactions that occur in the cells (Wang et al., 2011).

Overall, different cultivation parameters that mimic the conditions that the microorganisms find in their natural environment might be the trigger since natural conditions are often variable (Pan et al., 2019). The key is to figure out which are the right conditions for the production of bioactive compounds. For example, Breinlinger et al., (2021) was able to identify that the cause for the death of waterfowl and raptors in the south eastern United States from avian vacuolar myelinopathy was the production of a neurotoxin by the cyanobacteria *Aetokthonos hydrillicola*, which is an epiphytic organism, living in the surface of the invasive *Hydrilla verticillata*. However, the neurotoxin responsible for the death of the birds was only produced when in the presence of bromide. Since bromide was accumulated by the invasive plant that lived in association with this bacterium and the culture media used in the laboratory was not supplemented with bromide, under regular conditions the neurotoxin would not be produced. The compound produced by the cyanobacterium possessed bromine in its structure. Brominated compounds are produced by marine microorganisms such as *Actinobacteria* (Faulkner, 2001; Gribble, 2000) and are often associated with a strong antimicrobial activity (Gribble, 2015).

1.6. *Planctomycetes*

1.6.1. *Planctomycetes* cell biology

Planctomycetes is a phylum which belongs to the *Planctomycetes – Verrucomicrobia – Chlamydia* (PVC) superphylum (Devos, 2014). When *Planctomycetes* were discovered, they were thought to be eukaryotes, since they were mistaken as fungi (Dedysh et al., 2020; Gimesi, 1924). Only afterwards, in 1972 were they considered to be bacteria (Hirsch, 1972). It was later speculated that they could be the missing link between bacteria and eukaryotes (Devos et al., 2004; Fuerst & Sagulenko, 2011). *Planctomycetes* were thought to possess several eukaryotic features such as endocytosis (Boedeker et al., 2017; Jermy, 2010; Lonhienne et al., 2010; Wiegand et al., 2018). Recently, it was discovered that a *Planctomycetes*-related bacterium can actually perform phagocytosis and engulf other bacterial cells, just like eukaryotes do for their nutrition (Shiratori et al., 2019).

Originally, *Planctomycetes* were not neither aligned with Gram negative or Gram positive bacteria (Fuerst & Webb, 1991). Therefore, instead of the classical peptidoglycan cell wall, *Planctomycetes* were thought to possess a proteic cell wall (König et al., 1984). According to this theory, the cell cytoplasm was divided between paryphoplasm and pirellulosome (Lindsay et al., 2001), with the pirellulosome containing the cell's DNA compacted into a nucleoid at least in one species (Fuerst & Sagulenko, 2011). However, it has been proven that, in fact, *Planctomycetes* do possess peptidoglycan (Jeske et al., 2015). Peptidoglycan is a striking characteristic of bacterial cell wall as it allows them to maintain a certain level of osmotic pressure and avoid the collapse of the cell, as well as maintain their shape (Lovering et al., 2012). Another aspect was the concept of compartmentalization which has shifted and the intracytoplasmic membrane is now considered to be the cytoplasmatic membrane and the former cytoplasmatic membrane is considered to be the outer membrane. This way, it is possible to place *Planctomycetes* into the Gram negative bacterial group, with the unique trait of having a larger periplasmatic space (Wiegand et al., 2018).

The planctomycetal cells still harbour many mysteries (Wiegand et al., 2018) as, for example, they do not possess FtsZ, a tubulin analogue, which is essential in bacteria for the formation of the septum that allows for the development of the binary fission process (Bernander & Ettema, 2010). *Planctomycetes* have been reported to display two types of

division: polar and lateral budding in *Planctomycetia* (Wiegand et al., 2018) and binary fission by *Phycisphaerae* (Fukunaga et al., 2009) and anammox planctomycetes, which are capable of oxidizing ammonium anaerobically (Wagner & Horn, 2006). These have great importance in the nitrogen cycle and perform an imperative role in the oceanic oxygen minimum zones (Fuerst, 2017).

Planctomycetes have intricated and long-life cycles, just like *Actinobacteria* and *Myxobacteria*, known producers of bioactive compounds. *Planctomycetes* is a group that has shown great potential in this area, as they have shown to present NRPS and PKS, which indicate the production of bioactive compounds (Calisto et al., 2019; Donadio et al., 2007; Graça et al., 2016).

1.6.2. *Planctomycetes* ecology

Planctomycetes can be found in a wide variety of habitats. Initially, it was even thought that the majority of *Planctomycetes* inhabited aquatic environments, both marine and freshwater, especially associated to marine snow (Lage & Bondoso, 2014) and sediments (Bondoso et al., 2011). Yet, it has been reported that *Planctomycetes* can be found in abundance in soils (Buckley et al., 2006) and can even be found in subarctic environments (Dedysh & Ivanova, 2019). *Planctomycetes* have also been reported to be in association with other organisms, such as sponges (Izumi et al., 2013) and several macroalgae (Lage & Bondoso, 2011). In fact, compounds secreted by algae can be utilized by *Planctomycetes* for growth but also to produce bioactive compounds, which can help them thrive in those complex and competitive environments (Graça et al., 2016; Jeske et al., 2013).

1.7. Bacterial genetic manipulation

Marine bacteria represent a huge amount of opportunity in the biotechnology field, as they can be used for several pharmaceutical applications, such as antimicrobial, antiparasitic or anticancer drugs (Xiong et al., 2013). However, due to problems in isolation or in cultivation sometimes it becomes very difficult to use the hidden potential of these microorganisms, as there is a lack of genetic tools for them (Joint et al., 2010; Prakash et

al., 2013). Therefore, it is very important to reconsider this paradigm and start to apply innovative methods for genetic manipulation of marine bacteria (Joint et al., 2010).

Bacterial cells can assimilate DNA from exogenous sources spontaneously through cell to cell contact by conjugation (Chen et al., 2012), transduction by bacteriophage contact (Lang et al., 2012) or by transformation. These mechanisms that occur naturally in bacterial cells can be used to genetically manipulate them. For the manipulation it is necessary to have plasmids. Plasmids are made up from extrachromosomal DNA and are treated as shuttle vectors that are then utilized in methods based on conjugation (Wang et al., 2007).

1.7.1. Triparental mating

Triparental mating is a method based in conjugation that happens naturally in the bacterial cells. For this to happen it is required the processing of DNA and its transference to the recipient cell. Therefore, a single strand of DNA is formed by cleaving the DNA that is to be transferred with a relaxase that binds to the origin of transfer (Garcillán-Barcia et al., 2009). The transportation of the target DNA is performed by a type IV secretion apparatus (Grohmann et al., 2003). Relaxase carries a great role in this process since it emits signals for the recognition of the substrate (Chen et al., 2012).

The triparental mating method uses three bacterial strains, a donor, a helper and a recipient. The donor possesses a plasmid that carries the target gene and a selection marker (Wise et al., 2006). In the classical route, the helper plasmid which has mobility will move into the donor cell and help mobilize the donor plasmid, which is not mobile, into the recipient cell. In the recipient cell there will be recombination between the donor plasmid and the genomic DNA (Timmery et al., 2009).

1.7.2. Genetic manipulation in *Planctomycetes*

As planctomycetal cells present a unique cell conformation and have been an underexplored group it was just recently explored the possibility of engineering their genomes (Wiegand et al., 2018). One of the hurdles that made it impossible until now is their great resistance capacity to several antibiotics, such as beta-lactams and

aminoglycosides. This way, it is difficult to use them in techniques that involve antibiotics as selection markers (Wiegand et al., 2018).

However, Jogler et al., (2011) was capable of performing genetic manipulation in *Planctopirus limnophila*, one of the fastest growing *Planctomycetes*. Later, electroporation and transposon mutagenesis was described for this group of bacteria, with the purpose of expressing fluorescent proteins and to analyse the cell compartmentalization through gene deletion (Boedeker et al., 2017). Electroporation was also used later for exploring the presence of microcompartments gene clusters (Erbilgin et al., 2014). Lastly, more recently triparental mating has been applied to freshwater and marine *Planctomycetes* (Rivas-Marín et al., 2016). The same authors used triparental mating to assess the presence and the effects of the lack of sterols in *Gemmata obscuriglobus* (Rivas-Marín et al., 2019).

1.8. Objectives

The objective of this study consisted in evaluating the bioactive potential of several marine bacterial strains by molecular approaches and antimicrobial screenings. The marine bacteria used are newly isolated bacteria from the "Laboratório de Ecofisiologia Microbiana da Universidade do Porto" (LEMUP) collection. These bacteria belong to diverse phyla including *Actinobacteria*, *Planctomycetes*, *Firmicutes*, *Proteobacteria* and *Bacteroidetes*. An OSMAC approach was also applied to the bacteria that showed to be bioactive in an initial screening for optimization of the compound production.

This work also focuses on the attempt to genetically manipulate the planctomycete *Roseimaritima ulvae* strain UC8^{T} through the triparental mating technique in order to overexpress biosynthetic genes, and therefore, increase the production of bioactive compounds. As it was said above, *Planctomycetes* are still an underexplored group. However, planctomycetal growth is very slow, which becomes a hurdle when talking about scale up studies since it is necessary to obtain a great quantity of biomass for the antimicrobial assays. These bacteria are also hard to handle in genetic transformation assays so, in this study, the triparental mating technique was used since it has already been described for *Planctomycetes*.

2. Materials and Methods

2.1. Biological material and culture conditions

For this study, several marine bacterial strains belonging to the collection of LEMUP were chosen as biological material. These strains include members of diverse phyla: *Actinobacteria*, *Planctomycetes*, *Proteobacteria*, *Firmicutes* and *Bacteroidetes*. Moreover, they were previously isolated by our group from macroalgae, sand sediments and mussels collected in the Portuguese north coast at Memória Beach, in Leça da Palmeira (41° 13'N, 8° 43' W). Some examples of these bacteria are shown in Figure 2.

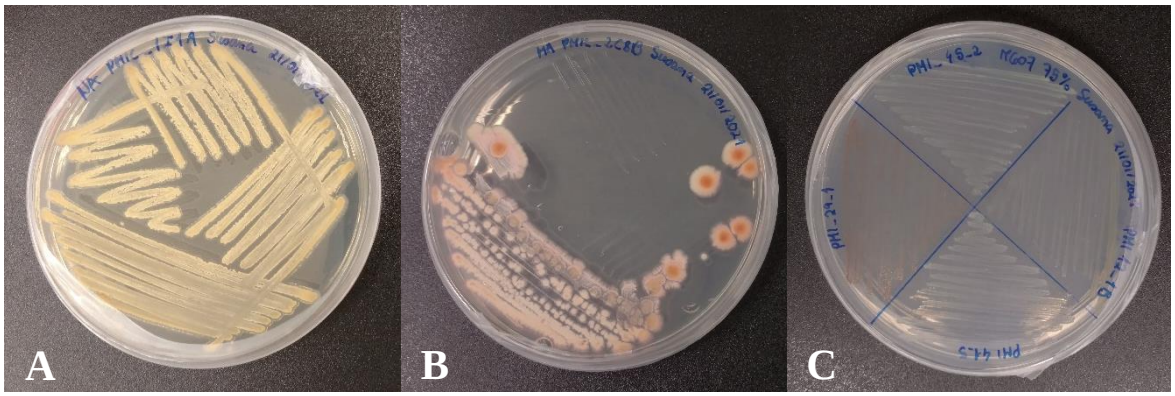

Figure 2– Examples of marine bacteria from LEMUP's culture collection that were utilized in this thesis. A) *Streptomyces hydrogenans* strain PMIC_1I1A; B) *Streptomyces ardesiacus* strain PMIC_2C8B and C) From left to right: *Sphingopyxis ummariensis* strain PMI45_2, *Sphingorhabdus* sp. strain PMI12_1B, *Ochrobactrum* sp. strain PMI41_5 and *Erythrobacter* sp. strain PMI29_1.

Bacteria were maintained either in M607 (modified M13) medium (Lage & Bondoso, 2011) or in Marine Agar (MA) medium and M607 (Table 2) supplemented with 10 mL of a 5% N-acetylglucosamine (NAG) solution per liter, and incubated at 25 ºC, in the darkness. For the OSMAC approach, five culture media were experimented: M607, 1:10 M607, M600, CGY, and MA (Table 2). Two more nutrient-poor media were chosen (M607 and 1:10 M607) and three more nutrient-rich media (M600, CGY and MA) were chosen to perform an OSMAC approach. The M607, M600 and MA were chosen since these strains were isolated in these media. CGY was chosen because it has different carbon sources.

Table 2 – Composition of the culture media used in this study. The culture media utilized in the OSMAC approach were prepared using 75% of seawater in order to equalize the amount of seawater in all media.

Reagents	M607 (75%)	M600 (75%)	CGY (75%)	1:10 M607 (75%)	MA (75%)	NA	LB	M600 (25%)
Peptone	0.25 g	1 g	---	0.025 g	5 g	5 g	---	1 g
Yeast extract	0.25 g	1 g	1 g	0.025 g	1 g	3 g	5 g	1 g
Agar	16 g	16 g	16 g	16 g	16 g	16 g	16 g	16 g
Tryptone	---	---	---	---	---	---	10 g	---
Casitone	---	---	5 g	---	---	---	---	---
Glycerol	---	---	5 mL	---	---	---	---	---
Filtred sea water	750 mL	750 mL	750 mL	750 mL	750 mL	---	---	220 mL
Deionized water	160 mL	160 mL	250 mL	169 mL	250 mL	1000 mL	1000 mL	660 mL
0.1 M HCl -Tris pH = 7.5	50 mL	50 mL	---	50 mL	---	---	---	50 mL
Glucose solution[1] (2.5%)	10 mL	40 mL	---	1mL	---	---	---	40 mL
Vitamins solution[2]	10 mL	10 mL	---	10 mL	---	---	---	10 mL
Hutner's Basal Salts solution[3]	20 mL	20 mL	---	20 mL	---	---	---	20 mL

Glucose, Vitamin and Hutner's Basal Salt's solutions were added after the medium autoclavation and sterilized through a 0.22 μm pore filter.

[2] 2 mg/L Biotin, 2 mg/L Folic Acid, 10 mg/L Pyridoxine-HCl, 5 mg/L Riboflavine, 5 mg/L Thiamine-HCl.2H_2O, 5 mg/L Nicotinamide, 5 mg/L D-Ca-pantothenate, 0.2 mg/L Vitamin B12, 5 mg/L p-Aminobenzoic acid.

[3] 99 mg/L $FeSO_4.7H_2O$, 12.67 mg/L $NaMoO_4.2H_2O$, 3.34 g/L $CaCl_2.2H_2O$, 29.70 g/L $MgSO_4.7H_2O$, 50 mL/L "44" Metals and 10.0 g/L Nitrilotriacetic acid.

"44" Metals: 12% EDTA, 52.63% $ZnSO_4.7H_2O$, 24% $FeSO_4.7H_2O$; 7.4% $MnSO_4.H_2O$, 1.64% $CuSO_4.5H_2O$; 1.19% $Co(NO_3)_2.6H_2O$, 0.85% $Na_2B_4O_710H_2O$.

The planctomycete *R. ulvae* UC8^T, previously isolated by our group from *Ulva* sp. in Carreço (41º44'N, 8º52'W) (Lage & Bondoso, 2011) was used for the triparental mating experiments. *R. ulvae* UC8^T was cultivated in M600 with 25% seawater (Table 2) instead of the regular 90% and was incubated at 25 ºC, 250 rpm, in the darkness.

E. coli DH5α, used in the triparental mating method, was cultivated in Luria broth (LB culture medium) (Table 2), which was supplemented with erythromycin (250 mg/L) and

ampicillin (100 mg/L) when carrying the donor plasmids (pOL003, pOL004 and pOL006) or kanamycin (25 mg/L) for the helper plasmid (pKR2013).

Regarding the antimicrobial assays, *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 were chosen as biological targets. These were maintained in Nutrient Agar (NA), at 37 ºC (Table 2).

2.2. Triparental Mating

In order to obtain a transformed planctomycete that could overexpress biosynthetic genes related to bioactivity, a triparental mating assay was performed. This technique is performed using a helper, a donor, both *E. coli* DH5α and a receptor, which was *R. ulvae* UC8^{T}.

In brief, the helper cell's role consists in allowing the transmission of the donor cell's plasmid to the recipient cell, since the donor does not possess mobility to move by itself to the receptor. Through the contact of the *tra* genes, contained in the helper plasmid, with genes in the donor plasmid it is possible to allow for mobilization. The helper cell is kanamycin resistant while the donor cell possesses a plasmid that has a selection marker, which is an erythromycin resistance gene (*ermC*) (Godinho, 2018). Previous studies by Godinho et al., (2019) showed that *R. ulvae* UC8^{T} is susceptible to erythromycin, making this a perfect marker to select for transformed cells. Moreover, the donor plasmid allows the recombination of the target genes with the receptor genome, since it holds a sequence that matches that of the receptor's genome. Furthermore, the donor cell is ampicillin and erythromycin resistant. These two antibiotics correspond to selection markers for the donor, used for the introduction of the plasmid inside the cells. Lastly, the receptor receives the donor plasmid and there is recombination with the homologous sequence from the donor plasmid (Fig. 3). The receptor is sensitive to erythromycin, which is a selection marker for the transformed cells. Likewise, there are two selection markers for the helper and the donor, ampicillin and kanamycin to which these strains are resistant and were used for the introduction of the plasmid inside the cells.

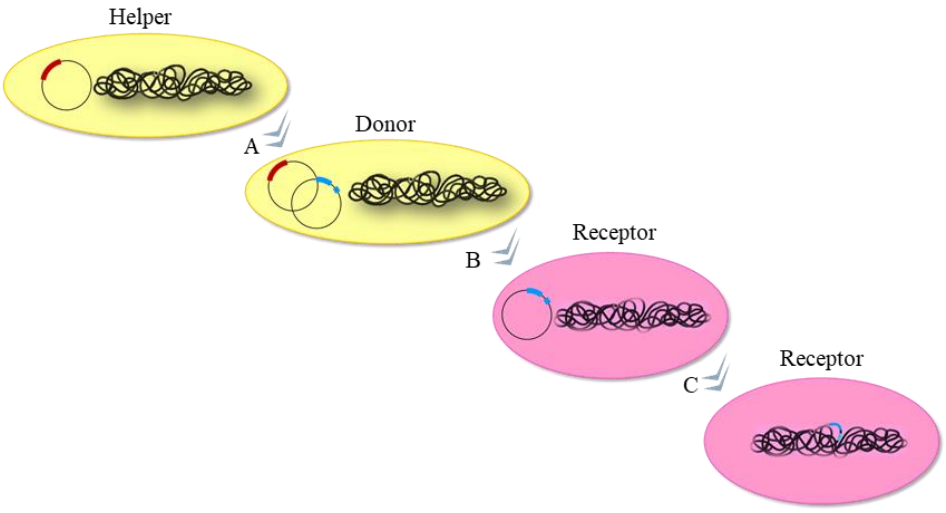

Figure 3- Triparental mating process schematic: A) Helper plasmid mobilizes itself to the donor cell; B) Helper plasmid allows for the transmission of the donor plasmid to the receptor cell; C) There is recombination between the donor plasmid and the genomic DNA of the receptor cell and the target genes are knocked-out in the receptor genome.

In this technique, three strains of *E. coli* DH5α were used: a helper, a donor with an empty plasmid and a donor with the full plasmid. The helper was *E. coli* DH5α pKR2013 and the two donor cells utilized were *E. coli* DH5α pOL003 with the empty vector that does not possess the recombinant sequence and pOL004 with the full vector that possesses the recombinant sequence (Table 3).

Table 3 – List of plasmids used in this study.

Plasmid	Genotype	References
pRK2013 (Helper)	*ColE1* origin, *Tra*$^{+}$, Km^{R}, *Mob*$^{+}$	(Figurski & Helinski., 1979)
pOL003 (Empty donor)	pBKS with *ColE1* origin, Erm^{R}, *Mob*$^{+}$, Amp^{R}	(Godinho, 2018)
pOL004 (Full Donor)	pBKS with *ColE1* origin, Erm^{R}, *Mob*$^{+}$, *bla*, Amp^{R}	(Godinho, 2018)
pOL006 (Donor)	pBKS with *ColE1* origin, Erm^{R}, *Mob*$^{+}$, big protein gene, Amp^{R}	(Godinho, 2018)

Erm^{R} – erythromycin resistant; Amp^{R} – ampicillin resistant; Km^{R} – Kanamycin resistant; *ColE1* - colicin E1; *Tra*$^{+}$ - transfer gene; *Mob*$^{+}$ - Mobility; *bla* – betalactamase.

The experiment involves the execution of 3 patches, with different conditions, which consist of drops that were spread in the petri dishes. The first patch was composed by the receptor, the donor cells with the full plasmid (pOL004) and the helper cells (pKR2013).

This was the main patch, since patch 2 and patch 3 were controls and served to validate the results in patch 1. In patch 2 there was the receptor, the donor cells with the empty plasmid (pOL003) and the helper cells (pKR2013). This condition served as a control to assess if the plasmid was non-replicative. If the plasmid is, in fact, replicative, then it can replicate inside the cell and maintain itself in its lineage. However, in the desired condition this would not happen, and the daughter cells would not incorporate the plasmid and therefore, would not possess the antibiotic resistance associated with the plasmid. Finally, in patch 3, it was tested the receptor with the helper cells (pKR2013) with Phosphate Buffered Saline (PBS) buffer replacing the donor cells. This condition is considered the negative control.

The protocol used for triparental mating was adapted from Rivas-Marín et al. (2016), with some minor modifications, including different helper and donor strains and differences in the washing steps to remove the antibiotics. Initially, *R. ulvae* UC8^{T} was incubated at 25 ºC, in M600 culture medium with 25% seawater, containing ampicillin (100 mg/L) and cycloheximide (50 mg/L), for 7 days, at 250 rpm. On the day prior to the mating assay, the donor and helper *E. coli* strains were cultivated in LB, at 37 ºC and 250 rpm, containing either kanamycin (25 mg/L) for the helper strain or ampicillin and erythromycin (250 mg/L) for the donor strain. Afterwards, the receptor culture was diluted to 0.1 A.U. at OD_{600nm} and when the receptor reached 0.35 A.U. OD_{600nm}, the helper and the donors were diluted to 0.1 A.U. OD_{600nm}. When all the OD_{600nm} were between 0.35 A.U. and 0.4 A.U., 15 mL of the receptor culture were centrifuged and the cell pellet resuspended in 1mL of PBS buffer. Then it was centrifuged again and the cell pellet was resuspended in 600 µL of PBS. Next, 200 µL of the helper strain and 200 µL of the donor strain were mixed with 200 µL of PBS, they were centrifuged again and washed with 400 µL PBS. After centrifugation, they were resuspended in 400 µL of PBS and the receptor was added. Later, this mixture was then centrifuged and resuspended in 100 µL of PBS and plated in 25% seawater M600. After 24 h, the patch was resuspended in 1 mL of PBS and serial dilutions until 10^{6} were prepared. Then, 100 µL of the serial dilutions were plated in 25% seawater M600 selective and viable media. The selective medium was supplemented with ampicillin, streptomycin, cycloheximide and erythromycin, while the viable medium was supplemented with ampicillin, streptomycin and cycloheximide. Ampicillin inhibits the growth of the helper strain, streptomycin inhibits the donor strain growth, cycloheximide inhibits the growth of

fungi and erythromycin, as it was mentioned above, constitutes the selection marker for the receptor. After 12 days, the growth in each patch was evaluated.

2.3. Molecular Analyses

329 marine bacteria from the LEMUP collection were evaluated regarding their bioactive potential by searching for the presence of two different putative biosynthetic genes, PKS-I and NRPS and in Table 4 are the primers used for the search of these genes. Genomic DNA was extracted using the protocol from Omega E.Z.N.A. Bacterial DNA Isolation Kit, and Polymerase Chain Reaction (PCR) was applied according to the protocol described by Graça et al., (2013) (Table 5).

Table 4 – PKS-I and NRPS primers used for PCR.

Primer	Sequence 5'- 3'	Target genes	References
MDPQQRf	RTRGAYCCNCAGCAICG	PKS - I	(Kim et al., 2005)
HGTGTr	VGTNCCNGTGCCRTG		
MTf	CCNCGDATYTTNACYTG	NRPS	(Tambadou et al., 2014)
MTr	GCNGGYGGYGCNTAYGTNCC		

The PCR was performed in a MyCycler™ Thermo Cycler (Bio-Rad) thermocycler and the amplification products detected in a 1.2% agarose gel stained with Green Safe (NZYTech®), and submerged in 1x Tris(hydroxymethyl)aminomethane (Tris) Acetate EDTA (TAE) buffer. The ladder used for the validation of the results was Generuler 1kb Plus DNA Ladder (Thermo Scientific®). The results were viewed in a GenoPlex Transilluminator (VWR®).

Table 5 – Quantities of reagents per PCR reaction.

Reagents	1 reaction quantities
NZY Taq 2x Green Master Mix	12.5 µL
MDPQQRf / MTf (10 mM)	2 µL
HGTGTr / MTr (10 mM)	2 µL
DNA	2 µL
Milli Q water	6.5 µL

2.4. Extraction procedures for bioactivity assays

2.4.1. Extraction from *R. ulvae* UC8^{T}

To evaluate the bioactive potential of *R. ulvae* UC8^{T}, a liquid extraction protocol using methanol was performed. Liquid cultures were used in order to obtain more biomass.

Firstly, a 10 mL pre-inoculum of UC8^{T} was prepared in M600 medium with 25% seawater and incubated for 7 days under 200 rpm. It was then upscaled to a 250 mL culture which was incubated for another 7 days at 200 rpm. The whole culture was centrifuged at 13 000 rpm during 10 minutes in an Eppendorf ^{TM} Centrifuge 5810 R. The cell pellet was collected, stored at - 80 ºC and freeze-dried in a BenchTop Pro with Omnitronics^{TM} freeze drier (VirTis SP Scientific®). Subsequently, the pellet was rinsed with methanol to disrupt the cell's membrane and later, the methanol was dried in a Buchi® R-100 rotary evaporator at 25 ºC. The extracted compounds obtained were then collected by adding 1.5 mL of dichloromethane and transferred to a previously weighted vial (Fig. 4A). Subsequently, the extract was left to dry overnight and the weight of the extracted product residue determined. The extract was solubilized in 20% dimethyl sulfoxide (DMSO) mixture (Fig. 4B) at the final concentration of 10 mg/mL.

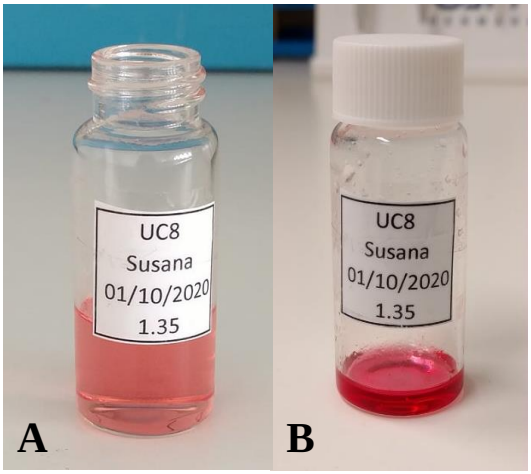

Figure 4 – *R. ulvae* UC8^{T} extract: A) Dissolved in dichloromethane before the final drying and B) in a 20% DMSO solution.

2.4.2. Solid culture extraction using ethyl acetate

The promising selected strains that possessed PKS-I or NRPS potential genes were cultivated in 25 mL agar plates of M607 medium. The cultures were then incubated at 25 ºC during 2 weeks in the dark (Fig. 5A). Afterwards, the culture medium was removed from the petri dishes and 50 mL of ethyl acetate were added. This mixture was left to steep overnight (Fig. 5B). The day after, the mixture was collected and the flasks rinsed two times with 25 mL of ethyl acetate. Later, it was transferred to a Buchi R-100 rotary evaporator (Fig. 5C), at 25 ºC to dry. The solid residues were collected and dissolved in 500 µL of a 20% DMSO solution.

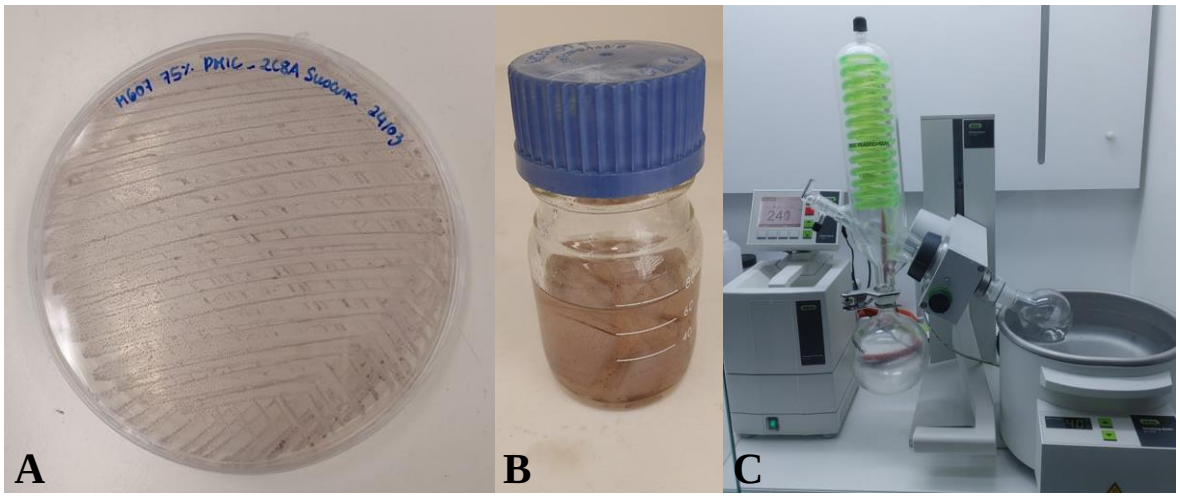

Figure 5 – A) Bacterial growth of *Streptomyces ardesiacus* strain PMIC_2C8A after two weeks incubation at 25 ºC in darkness, B) Agar cubes of the same strain left to steep overnight and C) Rotary evaporator used to dry the extracts.

Afterwards, strains that showed to be bioactive against *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 were tested using an OSMAC approach and were cultivated in different culture media. The protocol utilized for extraction was similar to the one described above for the strains that amplified for PKS-I and/ or NRPS.

2.5. Antimicrobial assays

2.5.1. Antimicrobial assays of marine bacterial extracts

The antimicrobial activity of the extracts obtained from the 329 marine bacterial strains was evaluated against *E. coli* ATCC 25922 and *S. aureus* ATCC 29213.

For the antimicrobial assays, 10 mL pre-inocula of *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 were incubated at 37 ºC and 250 rpm overnight. The day after, several conditions were tested in a 96 well plate and based on predetermined growth curves of both targets, the OD_{600nm} was measured and the following equations applied for standardization of the *E. coli* ATCC 25922 (1) and *S. aureus* ATCC 29213 (2) cultures for a final concentration of 5×10^5 cells:

$$x = 4 \times 10^9 \times OD_{600nm} - 5 \times 10^7 \textbf{ (1)}$$

$$x = 5 \times 10^9 \times OD_{600nm} - 2 \times 10^7 \textbf{ (2)}$$

The conditions applied to the wells were: 90 µL of the target strains with 10 µL of ampicillin (40 mg/mL) or streptomycin (10 mg/mL), for *S. aureus* ATCC 29213 and *E. coli* ATCC 25922, respectively, or with 10 µL extract, DMSO or distilled water and 100 µL of culture medium.

Afterwards, the OD_{600nm} was measured in a Thermo Scientific® Multiskan Go 96 well plate reader and then the plate was incubated at 37 ºC for 24 h and subsequently the OD_{600nm} was read again. The growth of the culture present in each well was then calculated throught the following formula:

$$Growth\ \% = 100 \times \frac{(T_{f\,S} - T_{0\,S}) - (T_{f\,B} - T_{0\,B})}{(T_{f\,G} - T_{0\,G}) - (T_{f\,B} - T_{0\,B})} \textbf{ (3)}$$

Where, T_f represents the final OD_{600nm} and T_0 represents the initial OD_{600nm}. B represents the blank (culture media control), G the DMSO growth control and S the sample that is being analysed.

2.5.2. *R. ulvae* UC8^T extract antimicrobial assays

The antimicrobial capacity of *R. ulvae* UC8^T was evaluated against the various *E. coli* used in the triparental mating assay. For the antimicrobial assays, 10 mL pre-inocula of helper *E. coli* (pKR2013) and donor *E. coli* strains (pOL003, pOL004 and pOL006) with either kanamycin, for the helper, or ampicillin and erythromycin, for the donors, were incubated at 37 ºC and 250 rpm, overnight. The day after, several conditions were tested in a 96 well plate. In a Thoma cell counting chamber, the concentration of cells in the culture

was measured and an adequate dilution was made in order to have 5×10^5 cells in each well. Then, 90 μL of the different *E. coli* target strains (helper and donor) were plated on the wells of the 96 well plate with either 10 μL of streptomycin (10 mg/mL), or 10 μL of ampicillin (40 mg/mL) (for donors) or 10 μL of kanamycin (25 mg/L) (for helper) or 10 μL of a 20% DMSO solution or 10 μL of *R. ulvae* UC8^T extract. Plus, there were wells with only the target strains and wells with only culture media (100 μL). Subsequently, the process is similar to the one described for the marine bacterial extracts' antimicrobial assays.

2.6. Statistical analyses

In order to evaluate if the results from the antimicrobial assays were statistically significant, normality tests were performed. The data samples that were considered to have a normal distribution by the Shapiro-Wilk test ($p > 0.05$) were analysed using the one sample t-test and were considered significant if $p < 0.05$. Data samples that did not possess a normal distribution were analysed using the Wilcoxon signed rank test and were considered significant if $p < 0.05$. The software used for the statistical analyses as well for the boxplots was IBM® SPSS® Statistics 25.

3. Results and Discussion

3.1. Triparental Mating

In this study, a triparental mating assay was performed in order to obtain a mutant planctomycete that could produce a higher amount of the bioactive compounds' mass. Firstly, it was necessary to validate the technique, and therefore, the planctomycete *R. ulvae* UC8^T was used. This technique was previously used by Godinho and collaborators (2018) for the same strain and using a beta lactamase gene as target. The results obtained were not satisfactory because the transformation was not successful. However, it was demonstrated by Rivas-Marín et al., (2020); Rivas-Marín et al., (2019) that the triparental mating can be adapted to marine as well as freshwater *Planctomycetes*. In order to minimize the potential effects of salinity in the triparental mating assay, *R. ulvae* strain UC8^T was cultivated in M607 medium using only 25% of the regular amount of seawater, since it was the minimum percentage of salinity that this strain could handle. This strain was used since it has already shown to possess a hybrid NRPS-PKS and PKS-I genes (Graça et al., 2016). This indicates that *R. ulvae* UC8^T has bioactive potential, since these genes are most commonly associated with the production of bioactive secondary metabolites (Donadio et al., 2007). The results obtained for the triparental mating using *R. ulvae* UC8^T are described in Table 6.

Table 6 – *R. ulvae* UC8^T Triparental Mating results.

Patch	Plasmids	Viable media		Selective media	
		Antibiotics	Colonies	Antibiotics	Colonies
1	pRK2013 + pOL004	Ampicillin + Streptomycin + Cycloheximide	UC8^T	Ampicillin + Streptomycin + Cycloheximide + Erythromycin	UC8^T / *E. coli*
2	pRK2013 + pOL003		UC8^T		UC8^T / *E. coli*
3	pRK2013		UC8^T		UC8^T

Patch 1 represents the condition where the genetic transformation should occur. Here, *R. ulvae* UC8^T colonies should grow in both selective and viable media, if the transformation occurred. In this study, it was observed that *R. ulvae* UC8^T colonies grew in both selective

and viable media, which means the transformation may have occurred. However, as *E. coli* colonies also grew (probably because the antibiotics did not work as intended due to a high amount of *E. coli* DH5α biomass), no final conclusion about the transformation can be taken.

If the plasmid shows to be replicative inside the planctomycete, then, in patch 2 growth of *R. ulvae* UC8^T in the viable and in the selective media should occur. However, if the plasmid is not replicative, then it should only grow in the viable media. In this experiment, *R. ulvae* UC8^T grew in both the viable and the selective media, which might indicate that the plasmid is replicative inside the planctomycete. Once again, non planctomycetal colonies appeared, which could also be due to antibiotics not working as intended (Table 6). In both patches, the *E. coli* strains should not have grown because streptomycin and ampicillin are antibiotics to which the donor and helper *E. coli*, respectively, are susceptible.

Finally, in the negative control (patch 3), a few *R. ulvae* UC8^T colonies were observed. This should not have happened since the donor is not present and the culture medium contains erythromycin to which *R. ulvae* UC8^T is susceptible.

The results obtained may be indicative of occurrence of transformation but as other colonies appeared and *R. ulvae* UC8^T also grew in the patch 3 it is not possible to determine if transformation really happened.

The triparental mating technique has already shown results with *Planctomycetes*, since the freshwater planctomycete *Gemmata obscuriglobus* has been transformed to prove that the genes that synthesize sterol are essential for its growth (Rivas-Marín et al., 2019). Besides, *Planctopirus limnophila* was also transformed by Rivas-Marín et al., (2020) with the purpose of uncovering the importance of the FtsZ protein in cellular growth. Furthermore, triparental mating has been successfully used to transform rhizosphere associated bacteria such as *Agrobacterium* (Gürel, 2001). More recently, it was discovered that, when transformed with triparental mating, *Pseudomonas putida* was able to show antimicrobial activity with the insertion of an amino acid synthase in its genome (Lee et al., 2019).

3.2. *R. ulvae* UC8^T extract antimicrobial assays

Since the results of the triparental mating assay were not satisfactory, the hypothesis of *R. ulvae* UC8^T being bioactive against the other bacteria involved in the transformation process was considered. Therefore, an antimicrobial assay using a *R. ulvae* UC8^T extract against the helper and the donor *E. coli* as targets was performed, obtaining 97.22% growth inhibition for *E. coli* DH5α pOL003; 98.37% for pOL004; 93.79% for pOL006 and 97.84% for the helper. However, the results were not conclusive since the DMSO control showed bioactivity (97.66% for *E. coli* DH5α pOL003; 99.83% for pOL004; 99.32% for pOL006 and 63.97% for the helper), which should not have happened because DMSO is the solvent used to dissolve the extracts. This way, it is not possible to conclude if the bioactivity shown was due to the solvent or the bacterial secondary metabolites themselves. DMSO is an amphiphilic solvent that is often used to dissolve extracts since it does not affect the drug binding (Singh et al., 2017). *E. coli* has shown to withstand a certain concentration of DMSO (Dyrda et al., 2019) in spite of being affected by this solvent at higher concentrations (Ansel et al., 1969). The antimicrobial activity that the DMSO displayed might have been due to problems with the growth of the *E. coli* strains. Since the antimicrobial assay was not standardized for these *E. coli* strains, there might have been some variability in the cell counting which might have originated dilution errors.

Despite the non-conclusive results obtained in this antimicrobial assay against the triparental mating *E. coli* strains, later it was observed that *R. ulvae* UC8^T was bioactive against *E. coli* ATCC 25922 with an average mean of 54.89% antimicrobial activity (Fig. 12 and Supplementary Table 2). These results were obtained in an antimicrobial assay where the DMSO solvent control was not bioactive and *E. coli* ATCC 25922 was able to grow normally. We can hypothesize that the triparental mating assay was not successful due to the secondary metabolites that were being produced by *R. ulvae* UC8^T and that were affecting the capability of the donor and helper *E. coli* strains to perform the bacterial transformation. As it was mentioned above, *R. ulvae* UC8^T possesses PKS-I and a hybrid NRPS-PKS-I, which are indicative of its bioactive potential. According to Graça et al. (2016) and Calisto et al., (2019), *R. ulvae* UC8^T has also the potential to produce anticancer and antifungal secondary metabolites. Besides, presenting mild bioactivity against *E. coli* ATCC 25922 (54.89%) it also presented some kind of effect in *S. aureus* ATCC 29213 (48.26%

bioactivity) (Figs. 12 and 13 and Supplementary Table 2). A 1:10 dilution of the initial extract was performed in order to assess if the bioactivity would be maintained. Although, it was not considered bioactive in this study, the dilution still presented some kind of effect on both *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 cells. This experiment was also applied to *R. ulvae* UC8^T in order to observe if in a scenario where the DMSO control had no negative effects in the target bacteria's cells and the targets grew normally the bioactivity that was shown initially would be maintained.

The OSMAC approach was also performed on *R. ulvae* UC8^T. In this assay, *R. ulvae* UC8^T was not able to grow in CGY and MA, even when supplemented with the vitamins, glucose and hutner's basal salts solution used to supplement the M600 and M607 culture media. Both MA and CGY, possess higher concentration of nitrogen when compared to M600 and M607. However, it was previously reported by Pollet et al., (2014) that planctomycetal communities found in lakes are not affected by a greater afflux of nitrogen. Despite that, *R. ulvae* UC8^T was mildly bioactive against *E. coli* ATCC 25922 in M607 and 1:10 M607 culture media with 41.95% and 26.42% growth inhibition respectively. It was also mildly bioactive against *S. aureus* ATCC 29213 in the 1:10 M607 culture medium with 30.01% growth inhibition.

3.3. PKS-I and NRPS amplification

To assess the bioactive potential of the newly isolated 329 bacterial strains of the laboratory collection, a PKS-I and NRPS gene amplification was performed since these genes are the most common ones involved in the production of bioactive compounds (Donadio et al., 2007).

Of the screened bacteria, 163 belong to the phylum *Proteobacteria* and 110 to *Actinobacteria.* Less represented phyla were *Bacteroidetes*, *Firmicutes* and *Planctomycetes*, which together represent only 17% of the total bacteria used for this study. Of these, the most abundant were the *Firmicutes*, with 26 strains, followed by *Bacteroidetes*, with a representation of 19 strains and 10 strains of *Planctomycetes* (Fig. 6).

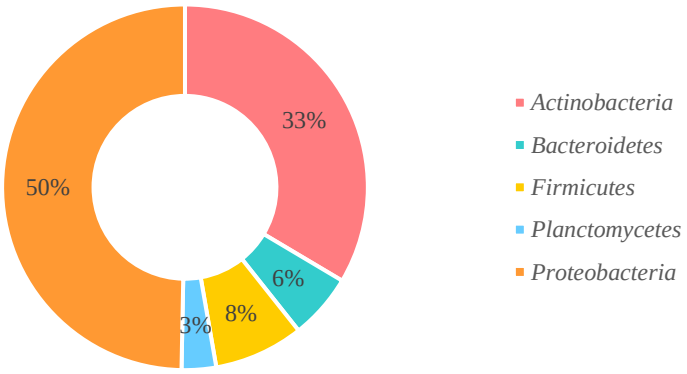

Figure 6 – Representation of the different phyla in the 329 bacterial strains used in this study for the PKS-I and NRPS screening. Most strains belong to the phyla *Proteobacteria* and *Actinobacteria*.

Figures 7 and 8 are examples of the results obtained for the PKS-I (amplicon size of 700 base pairs (bp)) and the NRPS (amplicon size of 1000 bp). The results of the amplification of PKS-I and NRPS genes are given in Supplementary Table 1. From the 329 bacterial strains screened, 80 strains possessed NRPS, 121 possessed PKS-I and 28 strains amplified for both.

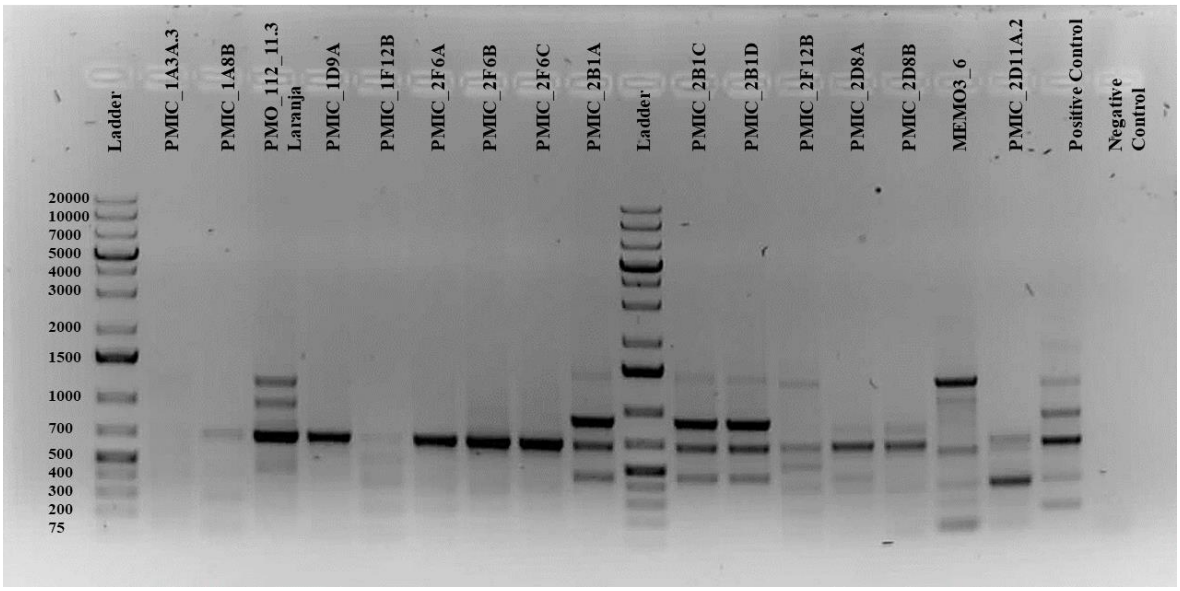

Figure 7 – Example of one of the PKS-I electrophoresis agarose gel. The strains that possess PKS-I had an expected fragment amplified at 700 bp. The affiliation of the isolates is given in Supplementary Table 1.

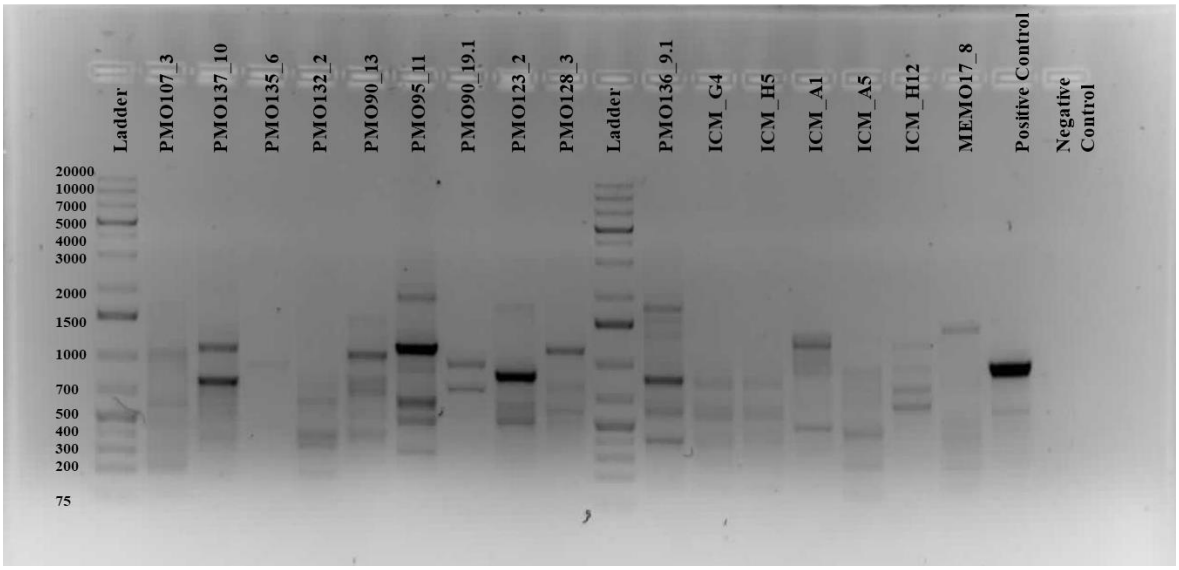

Figure 8 – Example of one of the NRPS electrophoresis agarose gel. The strains that possess NRPS had an expected fragment amplified at 1000 bp. The affiliation of the isolates is given in Supplementary Table 1.

Regarding all isolates that were screened in this study, the phylum that presented the higher percentage of PKS-I amplification (60%) was the *Planctomycetes*. Six out of the 10 isolates studied showed amplification for PKS-I gene but none amplified for NRPS gene. Five of the *Planctomycetes* were affiliated with *Rhodopirellula baltica* SH1^{T}. According to the literature, the genome of this strain has shown to possess two NRPS genes and two PKS genes and also a hybrid NRPS-PKS gene (Donadio et al., 2007; Glöckner et al., 2003). Our results confirmed the presence of PKS-I genes but not NRPS genes in the studied strains.

Regarding *Actinobacteria*, the isolates were mostly affiliated with *Streptomyces* sp., which did not display amplification for NRPS, except for PMIC_1C12A (*Streptomyces albidoflavus* DSM 40455), PMIC_2C8B (*Streptomyces ardesiacus* NRRL B-1773) and PMIC_2D11C (*Streptomyces hydrogenans* NBRC 13475). Despite that, it has been shown that several *Streptomyces* strains possess NRPS (Komaki et al., 2018). As for PKS, out of 58 *Streptomyces*, 21 amplified for PKS. *Streptomyces* possess several polyketides such as rapamycin or oleandomycin, produced by *Streptomyces hygroscopicus* and *Streptomyces antibioticus* respectively (Dutta et al., 2014; Rodríguez et al., 2001).

According to Anjum, (2021), the *Proteobacteria* bioactive potential derives essentially from non-ribosomal pathways. In this study, however, the number of isolates with NRPS (35.58%) is similar to the number of isolates with PKS (32.51%). The isolates belong to a high number of genera with emphasis in the *Pseudoalteromonas* genus. This genus is

mostly explored in terms of the production of alkaloids (Offret et al., 2016), and more recently it has been possible to explore its potential regarding PKS and NRPS, which also include other classes of chemical compounds, thanks to the genome mining technology (Graça et al., 2015; Machado et al., 2015; Ross et al., 2015).

In the *Firmicutes*, only one isolate amplified for PKS, PMIC_1E1B.1 (*Bacillus aryabhattai* B8W22) and seven amplified for NRPS. This data adds up with the literature, since *Firmicutes* are mostly known by the production of NRPS instead of PKS (Lukoseviciute et al., 2021). *Bacillus* is the most common genus between the isolates studied. *Bacillus* are known for producing surfactin, bacilysin and bacillomycin (Mariappan et al., 2012; Roongsawang et al., 2011; Wang et al., 2010). Surfactin inhibits fibrin clot formation and presents antimicrobial, antiviral and anticancer activity (Rodrigues et al., 2006), while bacilysin is an antimicrobial non-ribosomal peptide (Mariappan et al., 2012) and bacillomycin is an antifungal (Moyne et al., 2001).

In the phylum *Bacteroidetes*, 26.92% of the isolates amplified for NRPS while only 3.85% amplified for PKS. The phylum *Bacteroidetes* is represented in the isolates mainly by the *Aquimarina* genus. It has been proven that *Aquimarina* possess PKS (Esteves et al., 2013) and more recently, genome mining allowed for the detection of NRPS in several *Aquimarina* genomes (Hudson et al., 2019; Keller-Costa et al., 2016; Ranson et al., 2018). Furthermore, some isolates from this phylum belong to the *Arenibacter* genus which is still very underexplored when it comes to their bioactive potential (Romanenko et al., 2020).

3.4. Antimicrobial assays

3.4.1. Initial screening with M607 75% seawater culture medium

From the 329 bacterial strains screened, 80 strains possessed NRPS (24%) and 121 possessed PKS-I (36%) and 28 strains amplified for both. Based on the analysis of the PKS and NRPS genes, the most promising strains were selected, and 116 extracts were prepared. Of these extracts, only 16 strains were considered bioactive against *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 (Fig. 9). Therefore, only a small percentage of the strains (5%) that were screened actually showed to be bioactive against these targets in an initial screening using M607 culture medium with 75% seawater. These strains were analysed using and OSMAC approach and their phenotypical characteristics are shown in Table 7.

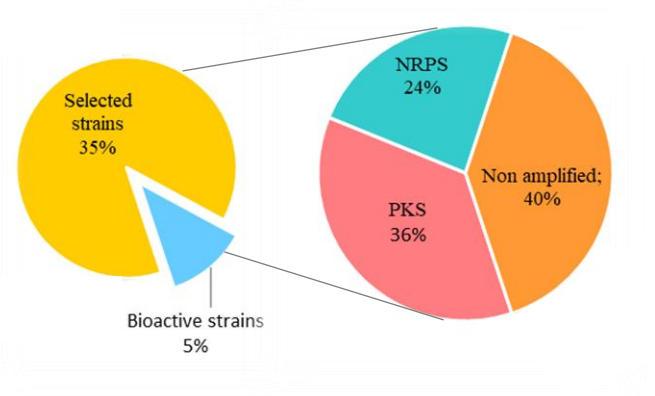

Figure 9 – PKS-I and NRPS amplification. 36% of the strains amplified for PKS and 24% for NRPS. The rest did not amplify for either one of these genes. From the selection made based on the strains bioactive potential only 5% showed to be bioactive.

Table 7 – Bioactive strains from the initial assay using 75% seawater M607 that were used for the OSMAC approach and their phenotypical characteristics.

Strain designation	Taxon	Phenotype
PMIC_2A12B.1	99.77% *Streptomyces albogriseolus* NRRL B-1305	Grayish white aerial mycelium; brown substrate mycelium; smooth spore surface and long or oval spores
PMIC_1A10B	99.82% *Nocardia nova* NBRC 15556	Filamentous bacilli; “cotton candy” aerial mycelium and white substrate mycelium
PMIC_1F12B	100% *Streptomyces setonii* NRRL ISP-5322	Grey aerial mycelium; grey-yellow substrate mycelium; smooth spore surface and spores organized in straight chains
PMIC_2H2C.2	100% *Nocardiopsis alba* DSM 43377	White aerial mycelium; cream substrate mycelium; long branched filaments
PMIC_1E10C	99.69% *Rhodococcus coprophilus* NBRC 100603	Non filamentous coccoid cells; light orange/ white colonies
PMIC_2C12	99.69% *Streptomyces albidoflavus* DSM 40455	White aerial mycelium; dark yellow substrate mycelium
PMIC_1F6A.3	99.76% *Nocardiopsis alba* DSM 43377	White aerial mycelium; cream substrate mycelium; long branched filaments
PMIC_1I1A	100% *Streptomyces hydrogenans* NBRC 13475	Yellow aerial mycelium; dark yellow substrate mycelium
PMIC_1D9B	99.75% *Streptomyces griseoflavus* LMG 19344	Dark brown aerial mycelium; dark brown substrate mycelium

PMIC_2A11B.1	100% *Nocardiopsis alba* DSM 43377	White aerial mycelium; cream substrate mycelium; long branched filaments
PMIC_1A8B	99.43% *Streptomyces flavoviridis* NBRC 12772	Grey aerial mycelium; green- grey substrate mycelium
PMIC_1E11B.2	99.59% *Arenibacter aquaticus* GUO666	Non flagellated and non-sporulating rod shaped cells; light yellow colonies
PMIC_1E12B	100% *Arthrobacter gandavensis* R 5812	Rod or coccoid cells; smooth bright yellow colonies
PMIC_1A11B.2	100% *Nocardiopsis alba* DSM 43377	White aerial mycelium; cream substrate mycelium; long branched filaments

For the extraction process ethyl acetate was used as a solvent, which is capable of extracting non-polar compounds. Furthermore, it presents the advantage of being immiscible in water. This represents a massive benefit for the extraction process, since it allows for a better separation between phases, avoiding the necessity of lyophilizing the cultures (Siek, 1978). The extracts that were made are part of a small-scale study, in order to select the most promising strains and perform scale up assays.

From the 16 bioactive strains (Supplementary Table 2), the most represented bacterial group were the *Actinobacteria*. From all the currently known drugs that are derived from microbial bioactive compounds, 45% are produced by *Streptomyces* (Azman et al., 2017; Berdi, 2005). This information also matches up with this study's results since from the 13 actinobacterial strains that showed bioactivity, 6 were *Streptomyces*.

For the antimicrobial assay performed against *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 using extracts made in 75% seawater M607 medium, the bioactive strains and the respective controls are represented in Figs. 10 and 11 in the first assay, Figs. 12 and 13 for the second assay and Figs. 14 and 15 for the third assay. In this study, only strains that possessed an average growth inhibition equal or higher to 50% were considered bioactive. The average activity and the activity values of the 3 replicates as well as the information regarding the non-bioactive strains are shown in Supplementary Table 2.

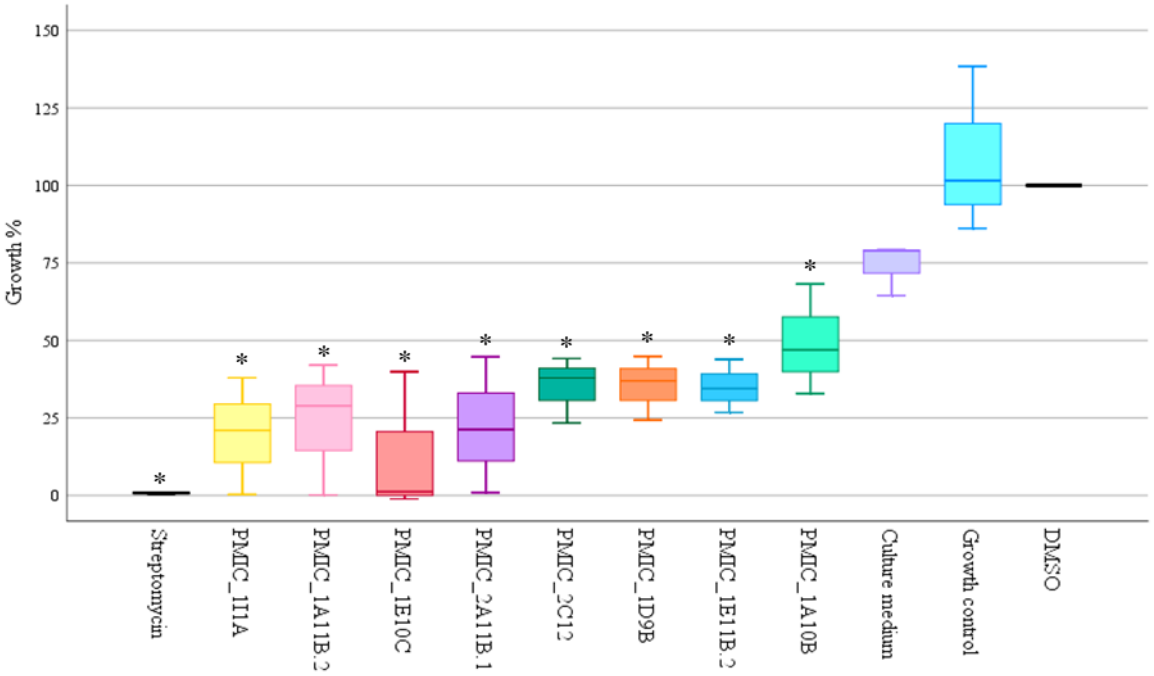

Figure 10 – Growth of *E. coli* ATCC 25922. Bioactive strains from the first assay as well as the streptomycin control, the culture medium extract control, the growth control and the DMSO control. The asterisks correspond to statistically significant conditions ($p < 0.05$).

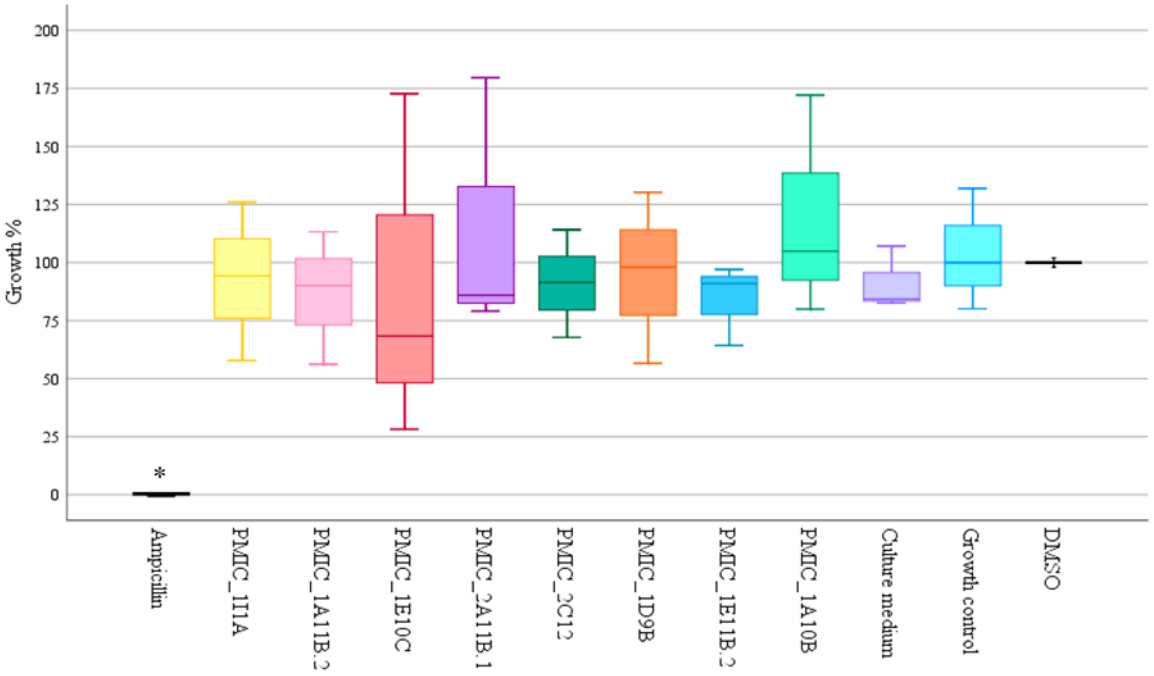

Figure 11 – Growth of *S. aureus* ATCC 29213 when in contact with the same bioactive strains' extracts reported in Fig. 10 as well as the ampicillin control, the culture medium extract control, the growth control and the DMSO control. No bioactivity was observed for these strains. The asterisks correspond to statistically significant conditions ($p < 0.05$).

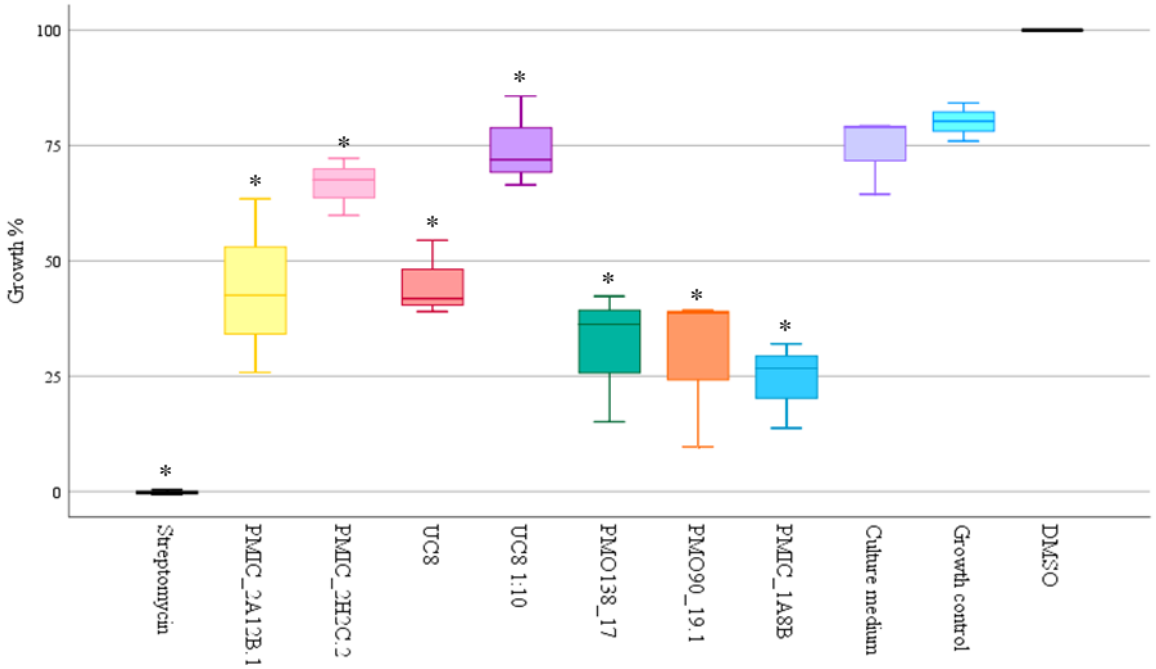

Figure 12 - Growth of *E. coli* ATCC 25922. Bioactive strains from the second assay as well as the streptomycin control, the culture medium extract control, the growth control and the DMSO control. The asterisks correspond to statistically significant conditions ($p < 0.05$).

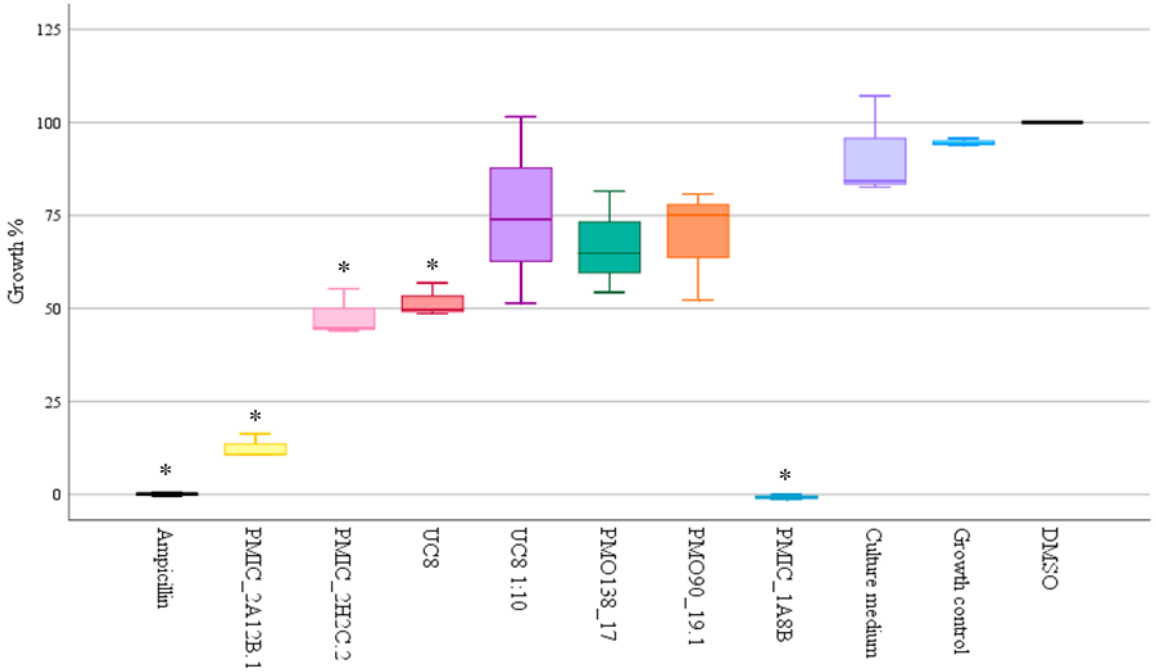

Figure 13 - Growth of *S. aureus* ATCC 29213 when in contact with the same bioactive strains' extracts reported in Fig. 12 as well as the ampicillin control, the culture medium extract control, the growth control and the DMSO control. The asterisks correspond to statistically significant conditions ($p < 0.05$).

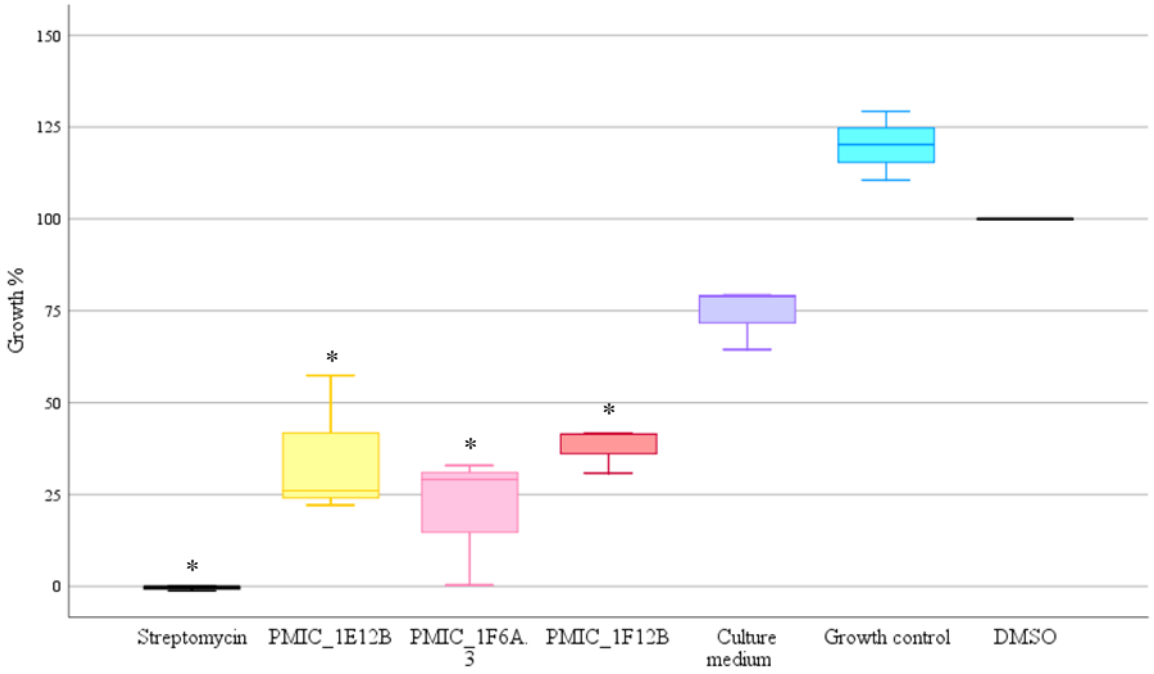

Figure 14 - Growth of *E. coli* ATCC 25922. Bioactive strains from the third assay as well as the streptomycin control, the culture medium extract control, the growth control and the DMSO control. The asterisks correspond to statistically significant conditions ($p < 0.05$).

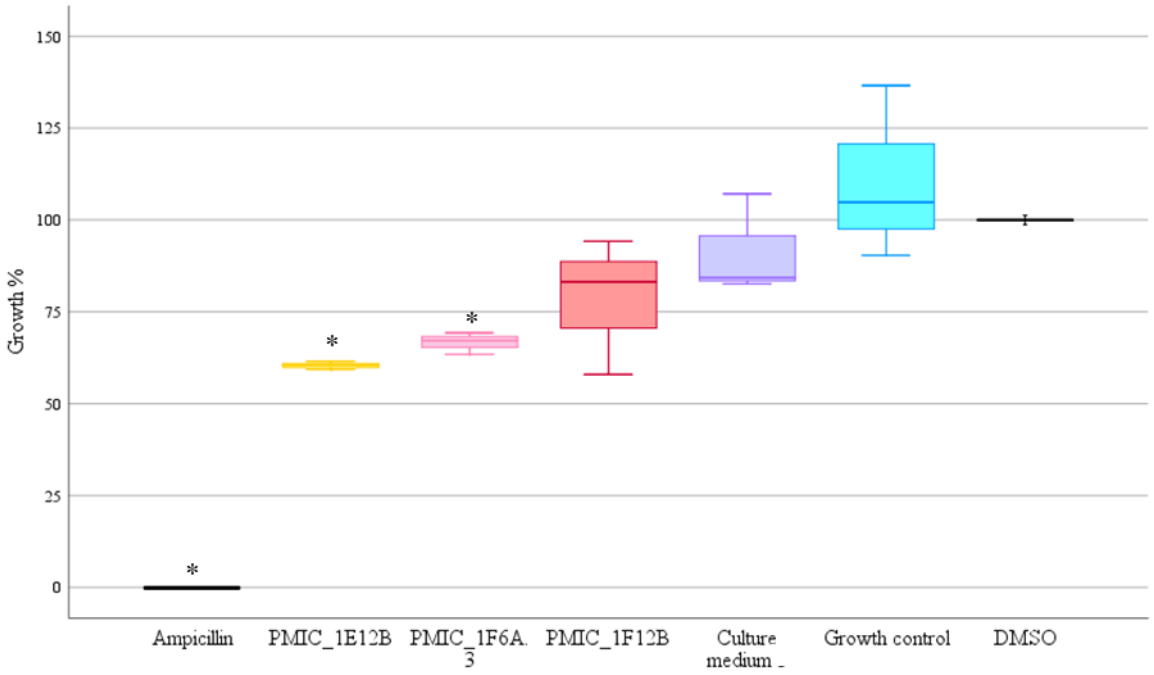

Figure 15 - Growth of *S. aureus* ATCC when in contact with the same bioactive strains' extracts reported in Fig. 14 from the same strains reported in Fig. 14 as well as the ampicillin control, the culture medium extract

control, the growth control and the DMSO control. The asterisks correspond to statistically significant conditions ($p < 0.05$).

Strains PMIC_1I1A, PMIC_2C12, PMIC_1D9B, PMIC_2A12B.1, PMIC_1F12B and PMIC_1A8B that showed bioactivity are all affiliated with the *Streptomyces* genus.

The actinomycete *Streptomyces hydrogenans* PMIC_1I1A has shown to be bioactive only against *E. coli* ATCC 25922 (average bioactivity of 80.24%). In the literature, it has been reported that *S. hydrogenans* produces the antibiotic actinomycin D (Kulkarni et al., 2017). Actinomycin D is a non-ribosomal peptide (Vardanyan & Hruby, 2016), this way it would make sense that PMIC_1I1A would have amplified for NRPS in the molecular analysis that was performed prior to the antimicrobial assays, however, that did not occur (Supplementary Table 1). Furthermore, actinomycin D, was considered the first antibiotic that also possessed anticancer activity, and it has been used in the clinic since 1954 in the treatment of tumours such as sarcomas, choriocarcinoma and testicular cancer (Mauger & Lackner, 2005). More recently it has been discovered that *S. hydrogenans* also has the potential to produce a new antifungal compound called 10-(2,2-dimethyl-cyclohexyl)-6,9-dihydroxy-4,9-dimethyl-dec-2-enoic acid methyl ester, or compound SH2, for short. Compound SH2 can be used as a fungicide to control fungi growth in plants, as it is capable of promoting plant growth and eliminating the fungi without harming the plant (Kaur et al., 2016).

Streptomyces albidoflavus PMIC_2C12 was able to inhibit the growth of *E. coli* ATCC 25922 by 64.84%. It has been reported that this species can produce dibutyl phthalate, an antimicrobial agent (Roy et al., 2006). In the past, dibutyl phthalate was not bioactive against Gram negative bacteria like *E. coli* unless its concentration was above 300 µg/mL (El-Naggar, 1997). This species is also known for producing the cytotoxic agent antimycin A18 (Yan et al., 2010).

Streptomyces griseoflavus PMIC_1D9B inhibited *E. coli* ATCC 25922 in 64.60%. This species is known for the production of aborycin, which is a ribosomal synthesized peptide type I. (Helynck et al., 1993; Potterat et al., 1994). This data is consistent with the results of this study, as this strain amplified for PKS-I in the molecular analyses performed (Supplementary Table 1). Unlike the results obtained by Shao et al., (2019), the strain used in this study was not bioactive against *S. aureus*. Shao et al., (2019) also reported bioactivity from this species against other Gram positive bacteria such as *E. faecalis* and the Gram

negative bacteria *K. pneumoniae*. This species is also capable of producing colabomycin A (Grote & Zeeck, 1988), which is not efficient against fungi or Gram negative bacteria. However, growth inhibition against *E. coli* ATCC 25922 was obtained in this study. Colabomycin A belongs to the manumycin group, however, it shows less growth inhibition of Gram positive bacteria than manumycin, which is relatable to the results presented. It was also discovered that colabomycin A can have anticancer properties (Grote & Zeeck, 1988).

Streptomyces albogriseolus PMIC_2A12B.1 was bioactive against *S. aureus* inhibiting 87.36% of its growth, and also against *E. coli* ATCC 25922 (56.06% growth inhibition). This species is responsible for the production of several bioactive compounds including the anticancer drugs echinosporin and microeunicellols A and B (Cui et al., 2007; Ma et al., 2020). Furthermore, *S. albogriseolus* also produces the antibacterial methyl-4,8-dimethylundecanate and the antimicrobial albogrisin A (Gao et al., 2019; Thirumurugan et al., 2018).

Streptomyces setonii PMIC_1F12B was only bioactive against *E. coli* ATCC 25922 (62.07% growth inhibition) and there is no information about the bioactive potential of this species.

Streptomyces flavoviridis PMIC_1A8B was the most consistent strain in terms of bioactivity, presenting a strong growth inhibition of *S. aureus* ATCC 29213 (100%), comparable to the effect of ampicillin, and also being bioactive against *E. coli* ATCC 25922 (75.85% average bioactivity). The growth inhibition of *S. aureus* ATCC 29213 was 100% in the 3 replicates, as it is possible to see in Fig. 16 where the transparency in the well with the extract was similar to that of the ampicillin control and of the blank. *S. flavoviridis* is responsible for the production of zorbamycin, an antibiotic with anticancer activity (Wang et al., 2007). It was found that this antibiotic is active against several Gram negative and Gram positive bacteria as well as fungi, having a strong bioactivity against *S. aureus* (Argoudelis et al., 1971), just like what happened in the current study.

Strains PMIC_1A11B.2, PMIC_2A11B.1, PMIC_2H2C.2 and PMIC_1F6A.3 are all affiliated with *Nocardiopsis alba*. Most of them were only bioactive against *E. coli* ATCC 25922, however, PMIC_2H2C.2 was only bioactive against *S. aureus* 29213 (Supplementary Table 2). This species is known for the production of several bioactive compounds including isomethoxyneihumicin, a compound with cytotoxic activity against Jurkat cells, inhibiting the cell cycle (Fukuda et al., 2017). *N. alba* is also responsible for the production of

albonoursin, a cyclic dipeptide with antibacterial activity (Li et al., 2014) and nocazine D and E and nocarazepine A, three diketopiperazines (Zhang et al., 2013; Zhou et al., 2017).

Rhodococcus coprophilus strain PMIC_1E10C also displayed bioactivity against *E. coli* ATCC 25922, but not against *S. aureus* ATCC 29213. This species is able to biotransform hydrocortisone (Costa et al., 2020). In spite of this, it has not been reported bioactivity for this species.

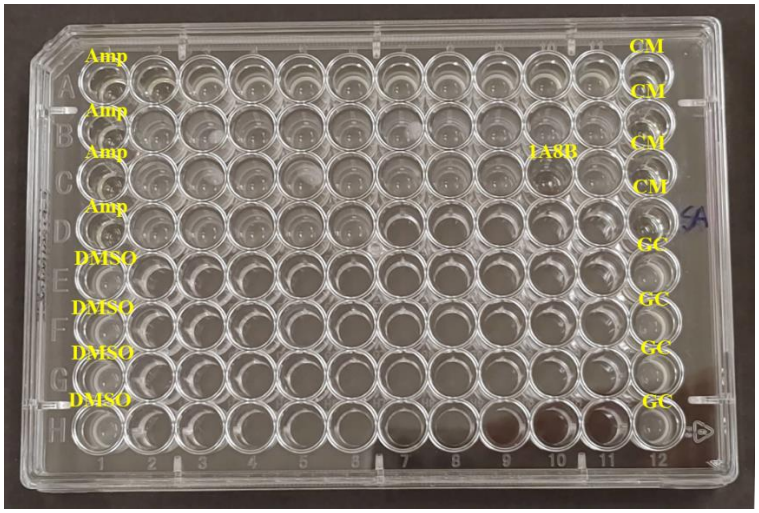

Figure 16 – 96 well plate of the second assay with *S. aureus* after incubating for 24 h. Amp – ampicillin; DMSO – DMSO control; CM – culture medium blank; GC - growth control; 1A8B – *S. flavoviridis* extract effect on the *S. aureus* growth were is visible that the culture medium is without any apparent growth.

Arenibacter aquaticus PMIC_1E11B.2 was bioactive against *E. coli* ATCC 25922 (64.96% bioactivity). For this species there is no data regarding its bioactivity. However, Chen et al., (2013) confirmed that *Arenibacter nanhaiticus* produced phenethylamine derivatives, despite having weak activity against *S. aureus* and *B. subtilis*. More recently, Romanenko et al., (2020) performed a biodiversity screening with strains of *Arenibacter* isolated from sediments in Russia. These *Arenibacter* strains possessed PKS-I just like the *Arenibacter aquaticus* that was used in this study (Supplementary Table 1). These authors also reported that the information regarding the *Arenibacter* genus is very limited.

Nocardia nova PMIC_1A10B presented activity against *E. coli* ATCC 25922 (50.64%). In the literature it is described the production of nocardimicins from this species, which inhibit the muscarinic receptors (Ikeda et al., 2005). Muscarinic receptors can be found in neurons and are important for the action of the parasympathetic nervous system (Abrams et al., 2006).

Strain PMO138_17 corresponds to a new species which is more closely associated with *Methylotenera mobilis* JLW8. This new species along with *Aquimarina algiphila* PMO90_19.1 and *Arthrobacter gandavensis* PMIC_1E12B were all bioactive against *E. coli* ATCC 25922 (68.76%; 70.75% and 64.82% bioactivity respectively) but were not bioactive against *S. aureus* ATCC 29213. For all these species there is no information about their bioactive potential.

In all the antimicrobial assays performed the antibiotic control worked as intended, as well as the growth and the DMSO control. Despite that, the culture medium extract possessed an average bioactivity of 25.76% against *E. coli* ATCC 25922 and 8.65% against *S. aureus* ATCC 29213. The bioactivity that was expressed in the culture medium might be due to the use of natural seawater in the medium. Natural seawater corresponds to a very complex mixture of components that limit the reproducibility of assays, since each batch is unique and, therefore, differs from the next one. There is also the limitation due to seawater collected in different locations to prepare the culture media, which has distinctive characteristics that constrain the replication of the results (Henson et al., 2016).

3.4.2. OSMAC approach

For the OSMAC approach the strains that previously showed bioactivity were selected. As so, all the strains that previously have inhibited the growth of *E. coli* ATCC 25922 or *S. aureus* ATCC 29213 above 50% were tested in 5 different culture media, with different nutrient richness (M600, M607, 1:10 M607, MA and CGY), except for strains PMO138_17 and PMO90_19.1 which did not present enough biomass in order to make the compound's extraction.

The bioactive strains and the respective controls are presented in Figs. 17 to 25. The average bioactivity and the bioactivity values of the 3 replicates as well as the information regarding the non-bioactive strains are shown on Supplementary Table 3.

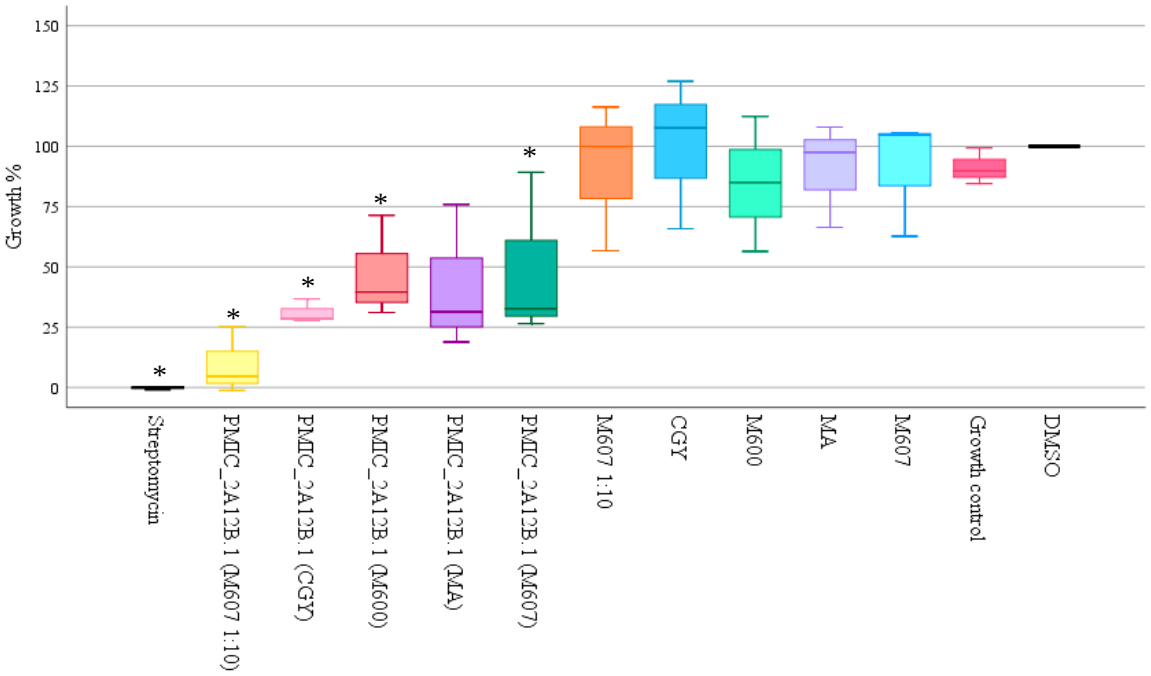

Figure 17 – Growth of *E. coli* ATCC 25922 when in contact with strain PMIC_2A12B.1 extracts made from 5 different culture media. It is possible to observe the streptomycin control, the culture media extract controls, the growth control and the DMSO control. The asterisks correspond to statistically significant conditions ($p < 0.05$).

This time, *S. albogriseolus* strain PMIC_2A12B.1 was bioactive against *E. coli* ATCC 25922 (Fig. 17). Despite testing 5 different culture media, including the M607 medium, which was initially used to assess the bioactivities of all the strains in a preliminary assay, none of them showed bioactivity against *S. aureus* ATCC 29213, when initially this strain in specific showed to be bioactive against both *E. coli* ATCC 25922 and *S. aureus* ATCC 29213. Regarding the variation between the different culture media for the *E. coli* ATCC 25922 assay, in 1:10 M607, which is a medium with less nutrient load, it was possible to observe the highest average bioactivity, with 90.4% growth inhibition. In CGY there was also a higher bioactivity than in the other media with 68.9% bioactivity. The other three media presented similar bioactivities of around 50%.

The OSMAC approach has already been applied to *S. albogriseolus*, but instead of experimenting with the culture media composition, this species was cultivated in co-culture

with *Bacillus cereus*. This interaction resulted in the production of a new peptide: dentigerumycin E which possesses anticancer activity (Shin et al., 2018).

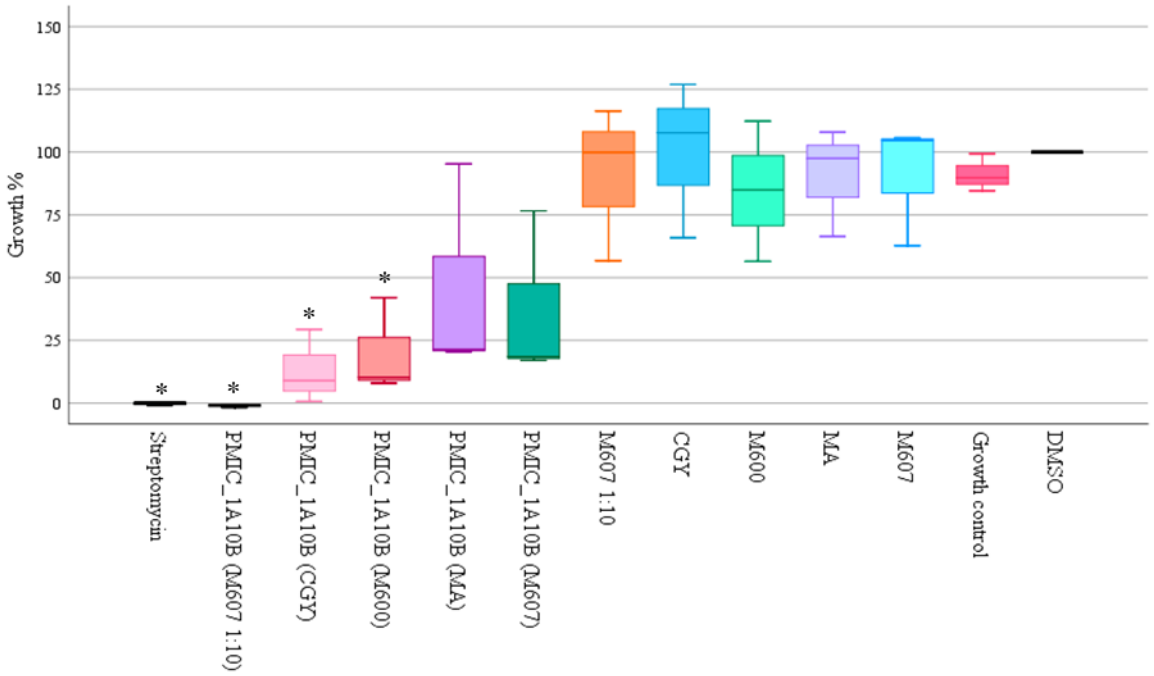

Figure 18 – Growth of *E. coli* ATCC 25922 when in contact with strain PMIC_1A10B extracts made from 5 different culture media. It is possible to observe the streptomycin control, the culture media extract controls, the growth control and the DMSO control. The asterisks correspond to statistically significant conditions ($p < 0.05$).

Regarding *N. nova* strain PMIC_1A10B, just like before, it was only bioactive against *E. coli* ATCC 25922 (Fig. 18). However, its bioactivity has increased when compared to its previous average activity (50.65%). In M607, its average *E. coli* ATCC 25922 growth inhibition was 61.95%. A lower value was obtained for MA and for all the other culture media, the activity was higher. Again, the 1:10 M607 culture medium which was considered a poorer medium showed better results, with a 100% growth inhibition of *E. coli* ATCC 25922.

N. alba strain PMIC_2H2C.2 was bioactive against *E. coli* ATCC 25922 (Fig. 19) but not against *S. aureus* ATCC 29213, unlike what was shown in the preliminary assay where this strain was only bioactive against *S. aureus* ATCC 29213. This strain showed the highest bioactivity in 1:10 M607 with 91.63% bioactivity against *E. coli* ATCC 25922, followed by CGY (68.1%) and M600 (54.68%), just like the majority of strains that were tested in the OSMAC approach. However, for MA and M607 this strain was not considered

bioactive against *E. coli* ATCC 25922.

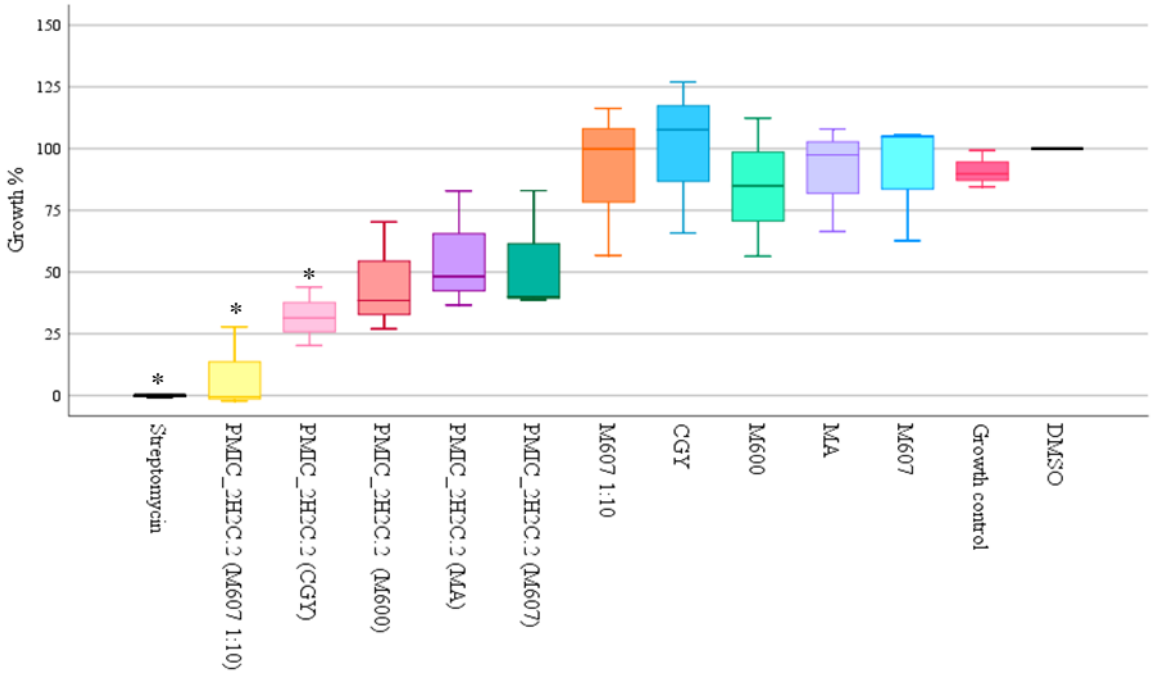

Figure 19 – Growth of *E. coli* ATCC 25922 when in contact with strain PMIC_2H2C.2 extracts made from 5 different culture media. It is possible to observe the streptomycin control, the culture media extract controls, the growth control and the DMSO control. The asterisks correspond to statistically significant conditions ($p < 0.05$).

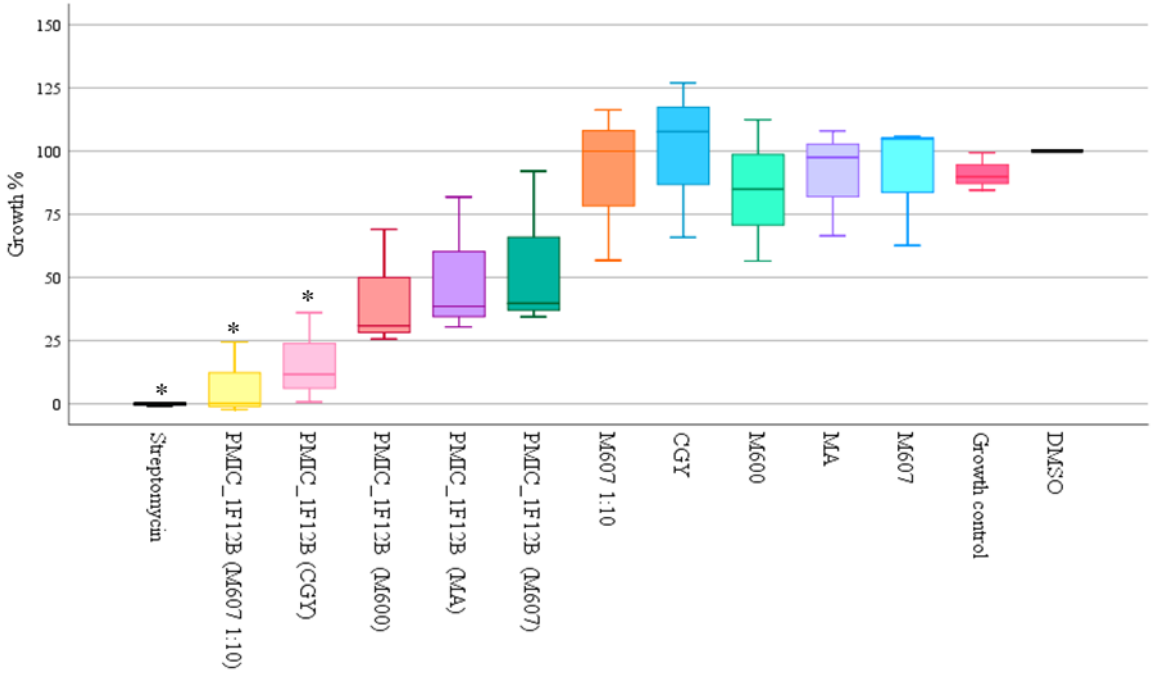

Figure 20 - Growth of *E. coli* ATCC 25922 when in contact with strain PMIC_1F12B extracts made from 5 different culture media. It is possible to observe the streptomycin control, the culture media extract controls,

the growth control and the DMSO control. The asterisks correspond to statistically significant conditions ($p < 0.05$).

Similarly, *S. setonii* strain PMIC_1F12B was most bioactive against *E. coli* ATCC 25922 (Fig. 20) when cultivated in 1:10 M607 (93.07%) and the culture medium that had the second highest activity was CGY (70.36%) followed by M600 (52.88%). Both MA and M607 did not display bioactivity, despite in the preliminary assay with M607 it has showed a 62.07% growth inhibition of *E. coli* ATCC 25922. In the literature it is referred that *Streptomyces* are unable to produce bioactive compounds when cultivated in culture medium that contain casein (Williams & Davies, 1965). However, this does not apply to this strain, as well as other *Streptomyces*, since in CGY some of the highest growth inhibitions for *E. coli* ATCC 25922 were obtained.

S. hydrogenans strain PMIC_1I1A was bioactive against both *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 (Figs. 21 and 22). For *E. coli* ATCC 25922, this strain was bioactive in the culture media 1:10 M607 and CGY, while for *S. aureus* ATCC 29213, the bioactivity occurred in 1:10 M607.

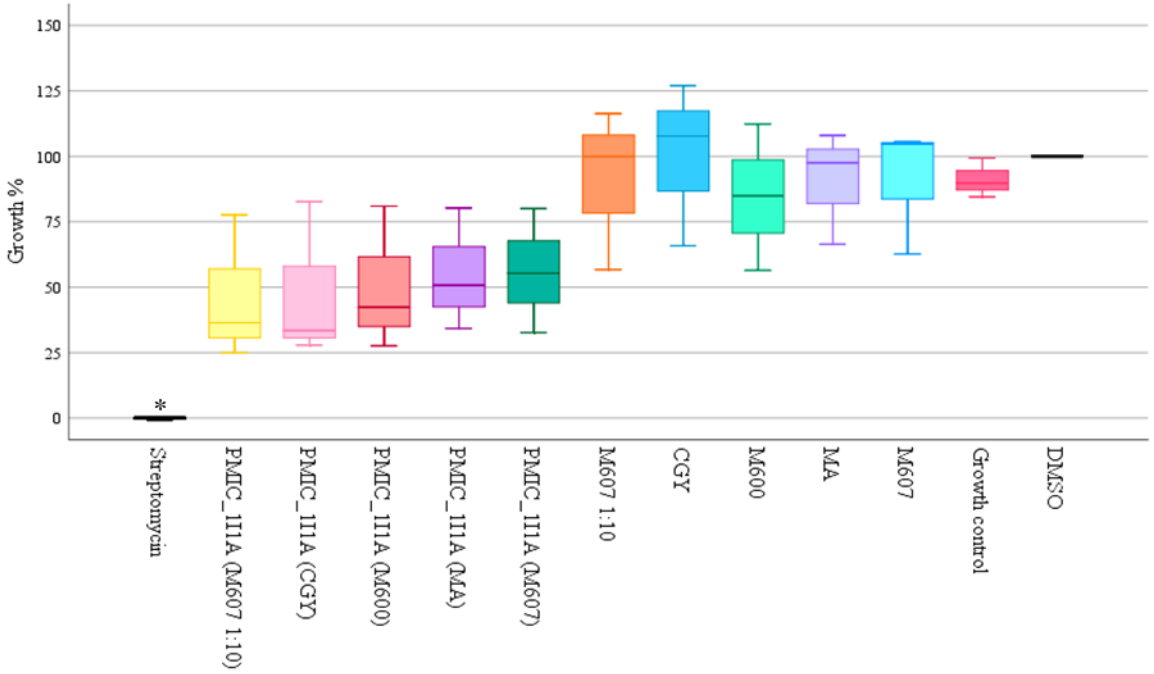

Figure 21 - Growth of *E. coli* ATCC 25922 when in contact with strain PMIC_1I1A extracts made from 5 different culture media. It is possible to observe the streptomycin control, the culture media extract controls, the growth control and the DMSO control. The asterisks correspond to statistically significant conditions ($p < 0.05$).

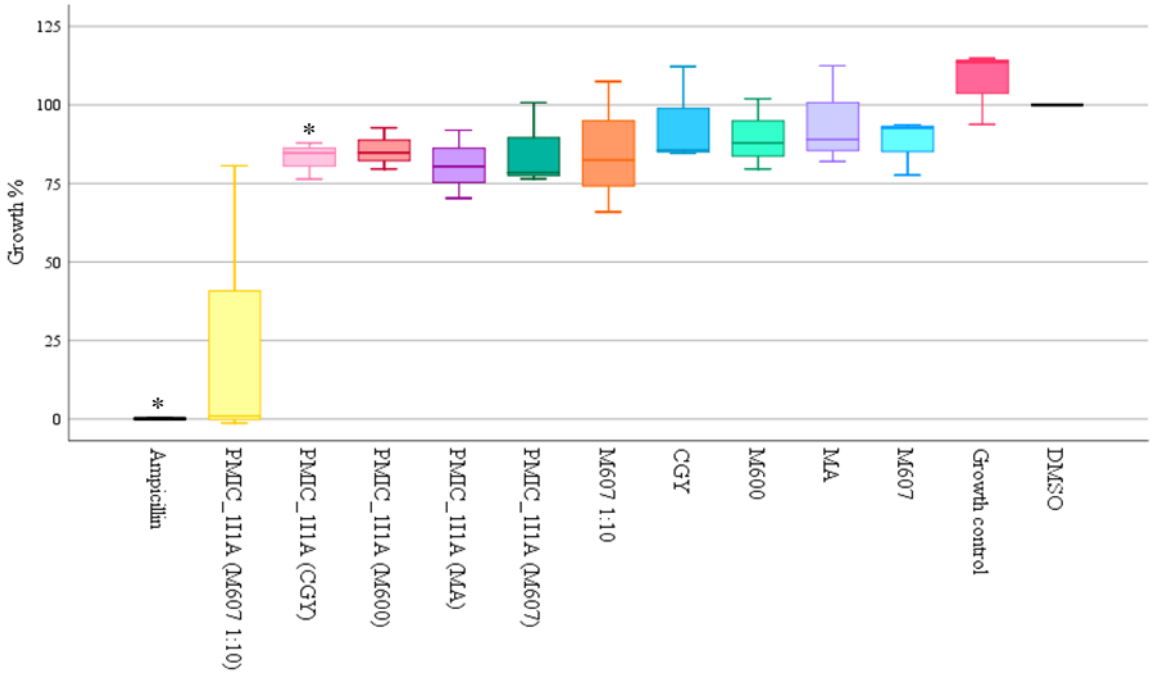

Figure 22 – Growth of *S. aureus* ATCC 29213 when in contact with strain PMIC_1I1A extracts made from 5 different culture media. It is possible to observe the streptomycin control, the culture media extract controls, the growth control and the DMSO control. The asterisks correspond to statistically significant conditions ($p < 0.05$).

S. griseoflavus strain PMIC_1D9B possessed bioactivity against *E. coli* ATCC 25922 in all culture media except in MA (Fig. 23), with the highest growth inhibition being observed in CGY (98.28%). For *S. aureus* ATCC 29213, growth inhibition was only observed in the CGY culture medium (91.06%) (Fig. 24).

S. flavoviridis strain PMIC_1A8B was again highly bioactive against *S. aureus* ATCC 29213 with 100% growth inhibition when cultivated in M600 and M607 culture media (Fig. 25). These results are contrary to the ones obtained for the other strains, where higher bioactivities were obtained in CGY and 1:10 M607.

The other strains that were initially considered bioactive that are not presented here were not bioactive in the OSMAC antimicrobial assay (Supplementary Table 3).

All the culture media extract controls tested were not considered bioactive, however in some cases the activity against the target strains was above 10%, namely in 1:10 M607 (14.72%), M600 (10.19%) and M607 (12.01%) for *S. aureus* and M600 (15.43%) and M607 (11.7%) for *E. coli* ATCC 2592.

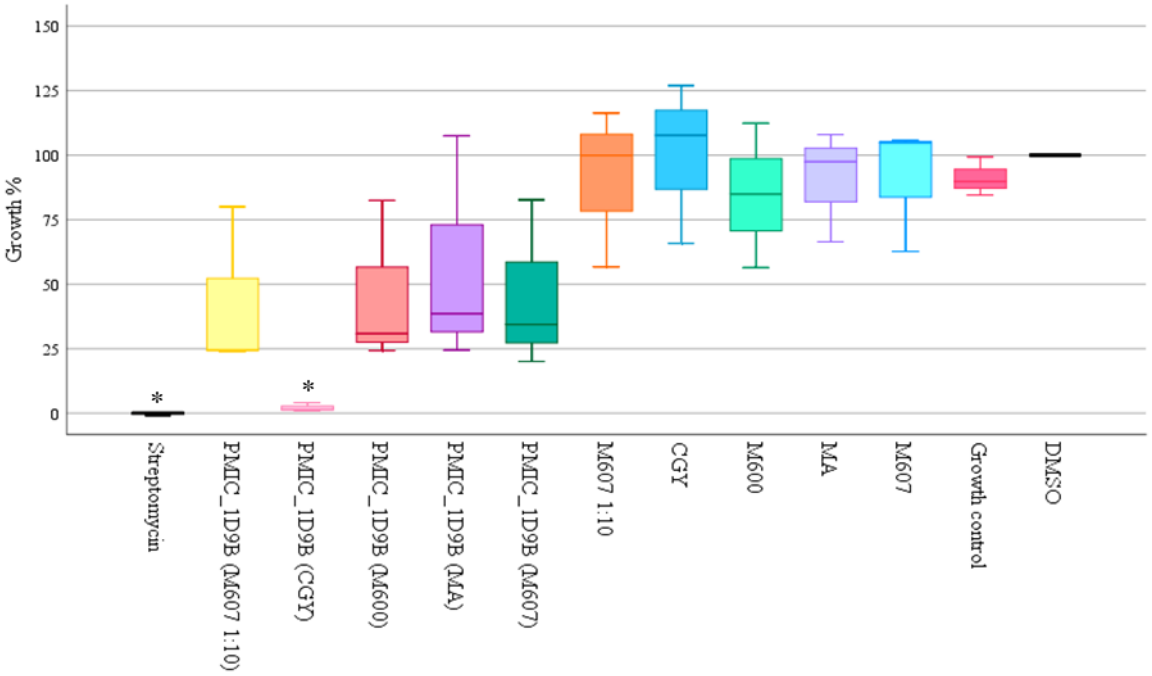

Figure 23 – Growth of *E. coli* ATCC 25922 when in contact with strain PMIC_1D9B extracts made from 5 different culture media. It is possible to observe the streptomycin control, the culture media extract controls, the growth control and the DMSO control. The asterisks correspond to statistically significant conditions ($p < 0.05$).

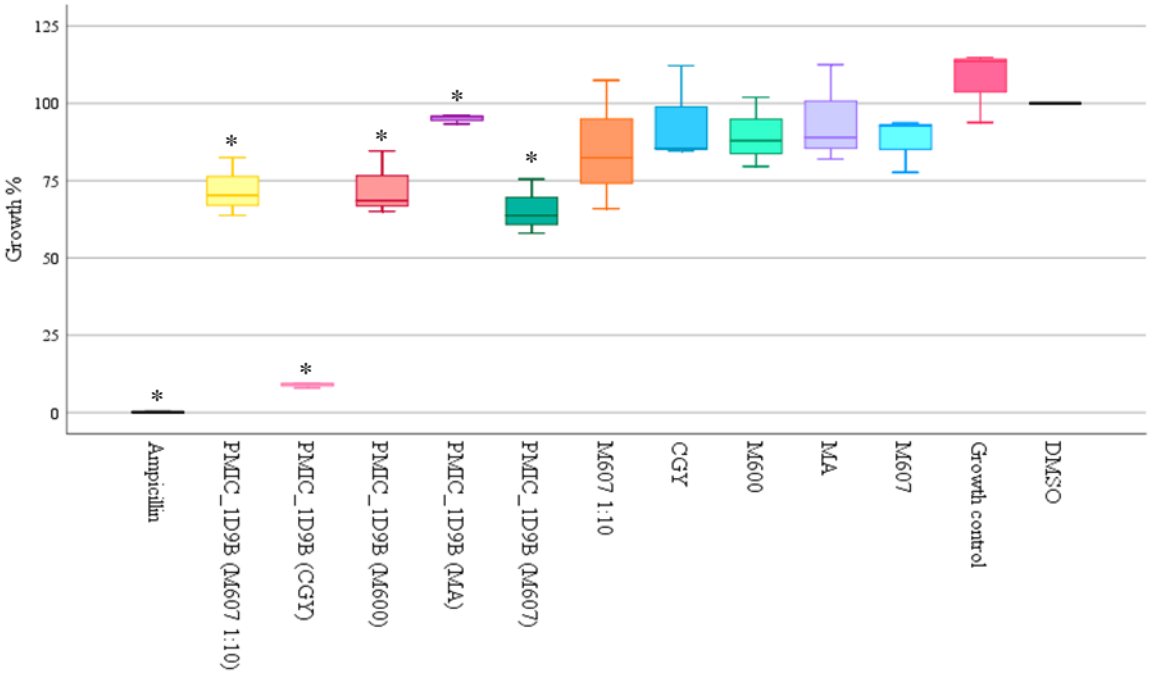

Figure 24 – Growth of *S. aureus* ATCC 29213 when in contact with strain PMIC_1D9B extracts made from 5 different culture media. It is possible to observe the streptomycin control, the culture media extract controls, the growth control and the DMSO control. The asterisks correspond to statistically significant conditions ($p < 0.05$).

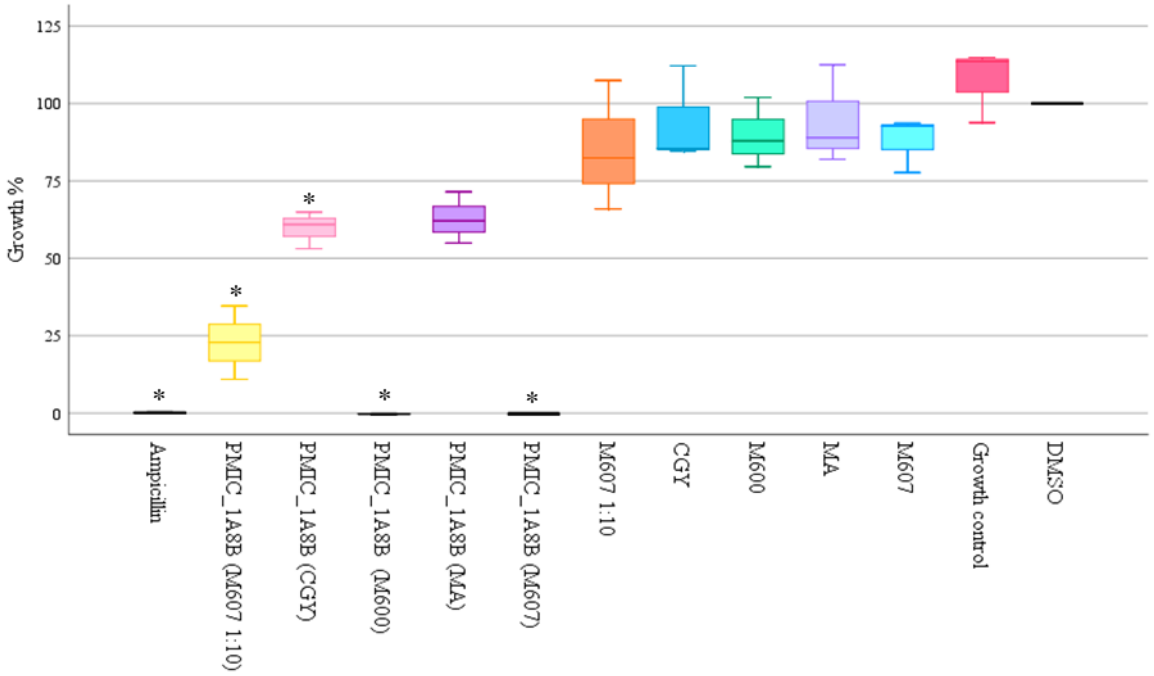

Figure 25 – Growth of *S. aureus* ATCC 29213 when in contact with strain PMIC_1A8B extracts made from 5 different culture media. It is possible to observe the streptomycin control, the culture media extract controls, the growth control and the DMSO control. The asterisks correspond to statistically significant conditions ($p < 0.05$).

As stated in the literature, different bioactive compounds require different conditions in order to activate the dormant genes that express them (Barrios-González, 2018). The 5 different culture media used in this study, differ in the proportion of nitrogen and carbon sources. Carbon sources might influence the production of secondary metabolites. For example, glucose has an influence in the production of antibiotics such as streptomycin and kanamycin by *Streptomyces* (Sánchez et al., 2010). Furthermore, antimycin, which has been proven to be produced by *S. albidoflavus* is also affected by the amount of glucose and glycerol present in the culture medium (Sanchez & Demain, 2002).

If the nitrogen source is constituted by amino acids, this might also affect the production of secondary metabolites, since NRPS and NRPS-PKS hybrids contain amino acids in their structure (Romano et al., 2018). In fungi, when the only nitrogen source is either peptone or yeast extract, there is a decrease in the quantity of antimicrobial compounds produced with the increment of biomass above certain levels. Lower concentrations of these nitrogen sources contributed to the production of secondary metabolites (Miao et al., 2006). This way, nutrient poor culture media also favour the production of anticancer and antimicrobial metabolites produced by diatoms and microalgae as well (Lauritano et al.,

2016; Wang et al., 2018). In this study, the highest bioactivity obtained for most of the strains was in fact obtained in the 1:10 M607 culture medium, which is nutritionally poorer when compared to the others. However, it was also possible to obtain very high activities in the CGY culture medium which is considered to be more nutrient rich. For example, Machushynets et al., (2019) discovered that high concentrations of glycerol, which is used as a carbon source in the CGY culture medium, could trigger the production of quinazolinone A and B by *Streptomyces* sp. MBT27. This result was not possible with other carbon sources such as glucose.

OSMAC approaches are normally based in certain cultivation conditions, with the most commonly used condition being the application of nutrient rich culture media (Mearns-Spragg et al., 1998; Rigali et al., 2008). In this study, 5 culture media with different composition and different nutrient concentrations were used.

Salinity is another aspect that might influence the production of secondary metabolites. Growth under high salinity conditions is prone to trigger the production of bioactive compounds (Wang et al., 2011), while low salt concentration might inhibit the production of those same compounds (Bose et al., 2015; Ng et al., 2014).

Regarding the differences between using solid culture medium *versus* liquid culture medium, it is said that in solid media the microorganisms are forced to develop another type of physiology and since the media is more concentrated than when in liquid culture the production of bioactive compounds is stimulated (Barrios-González, 2012). Besides, strains that are able to obtain higher growth rates are also better suited for cultivation in solid media (Barrios-González & Mejýa, 2008). This might indicate that bacterial groups such as *Planctomycetes* that were not bioactive in this study (Supplementary Table 2), are not suited for this kind of cultural strategy.

4. Conclusion

In principle, *R. ulvae* UC8^T transformation was achieved, but due to non-validation of controls, this process could not be confirmed. Furthermore, *R. ulvae* UC8^T extracts showed to be bioactive against *E. coli* ATCC 25922, which could interfere with the growth of the donor and helper *E. coli* DH5α involved in the transformation process in the triparental mating.

Regarding the antimicrobial assays using strains that previously presented the presence of PKS-I, NRPS or both genes, the majority of the bacteria that showed bioactivity belong to the phylum *Actinobacteria*, being the most bioactive strain *S. flavoviridis* strain PMIC_1A8B which was able to 100% inhibit *S. aureus*' growth. The only strains that did not belong to the *Actinobacteria* phylum, and that were bioactive, are the *Flavobacteriaceae A. aquaticus* PMIC_1E11B.2 and *A. algiphila* PMO90_19.1 and a novel taxon in the *Proteobacteria* phylum, strain PMO137_18, which is most closely affiliated with *Methylotenera mobilis* JLW8.

The OSMAC approach, performed using the bioactive strains from the preliminary assay, showed, in general, higher levels of bioactivity mainly when the strains were grown in 1:10 M607 and CGY culture media. The results also indicate that there are, in fact, differences between the cultivation in different culture media for the production of bioactive compounds.

Future perspectives include scale up studies in order to confirm the strains' bioactivity. It is also important to verify the bioactivity of extracts of culture medium, therefore, the use of sea salts or artificial seawater instead of natural seawater would be crucial, since the first batch of culture medium extracts showed some growth inhibition of the target strains. Natural seawater composition varies from batch to batch and these variations might influence the antimicrobial assays results. Furthermore, it would be interesting to explore the extracts' composition that showed bioactivity. In fact, dereplication of the extracts is already in progress.

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6. Annexes

Supplementary Table 1 – NRPS and PKS-I amplification results for the strains used in this study.

Strain designation	Taxon	Phylum	NRPS	PKS-I
ABPL45_1	99.92% *Streptomyces antimycoticus* NBRC 12839	*Actinobacteria*	Non amplified	Non amplified
ABPP45_1	98.79% *Sporosarcina aquimarina* SW28(T)	*Firmicutes*	Non amplified	Non amplified
ICM_A1	100% *Hirschia litorea* strain M-M23	*Proteobacteria*	Non amplified	Non amplified
ICM_A11	99.77% *Streptomyces xiamenensis* MCCC1A01550	*Actinobacteria*	Non amplified	Non amplified
ICM_A11.2	100% *Staphylococcus epidermidis* strain 3039	*Firmicutes*	Amplified	Non amplified
ICM_A5	99.9% *Tritonibacter mobilis*	*Proteobacteria*	Amplified	Amplified
ICM_D7A.1	99.92% *Streptomyces lienomycini* LMG 20091	*Actinobacteria*	Non amplified	Non amplified
ICM_D7A.2	99.92% *Streptomyces lienomycini* LMG 20092	*Actinobacteria*	Non amplified	Non amplified
ICM_D7A.3	100% *Streptomyces hydrogenans* NBRC 13475	*Actinobacteria*	Non amplified	Amplified
ICM_D7A.4	100% *Streptomyces rubrogriseus* LMG 20318	*Actinobacteria*	Non amplified	Non amplified
ICM_G4	99.9% *Novipirellula caenicola*	*Planctomycetes*	Non amplified	Amplified
ICM_H10	96.7% *Rubinisphaera italica*	*Planctomycetes*	Non amplified	Amplified
ICM_H12	99.04 % *Erythrobacter lutimaris* strain S-5	*Proteobacteria*	Amplified	Amplified
ICM_H5	99.9% *Novipirellula caenicola*	*Planctomycetes*	Non amplified	Amplified
M600PL15_2	99.92% *Dermacoccus nishinomiyaensis* DSM 20448	*Actinobacteria*	Non amplified	Non amplified
M600PL45_2	98.52% *Streptomyces nanshensis* SCSIO 01066	*Actinobacteria*	Non amplified	Non amplified
M600PL45_3	98.52% *Streptomyces nanshensis* SCSIO 01066	*Actinobacteria*	Non amplified	Non amplified
M600PP15_1	99.53% *Qipengyuania aquimaris* SW-110	*Proteobacteria*	Non amplified	Amplified
MAPL30_1	100% *Micromonospora matsumotoense* DSM 44100	*Actinobacteria*	Non amplified	Non amplified
MEMO13_5	98.62 % *Tenacibaculum gallaicum* strain A37.1	*Bacteroidetes*	Amplified	Non amplified
MEMO17_8	99.9% *Novipirellula caenicola*	*Planctomycetes*	Non amplified	Amplified
MEMO26_1	99.92% *Rhodopirellula baltica* SH 1	*Planctomycetes*	Non amplified	Non amplified
MEMO3_10.2	99.92% *Rhodopirellula baltica* SH 1	*Planctomycetes*	Non amplified	Non amplified
MEMO3_5	100% *Rhodopirellula baltica* SH 1	*Planctomycetes*	Non amplified	Non amplified

MEMO3_5.2	99.92% *Rhodopirellula baltica* SH 1	*Planctomycetes*	Non amplified	Non amplified
MEMO3_6	99.82% *Rhodopirellula baltica* SH1	*Planctomycetes*	Non amplified	Amplified
MEMO4_5	99.92% *Hellea balneolensis* DSM 19091	*Proteobacteria*	Amplified	Amplified
PMI12_1B	99.91% *Sphingorhabdus* sp. Alg231_15	*Proteobacteria*	Amplified	Non amplified
PMI12_2	100% *Altererythrobacter ishigakiensis* strain H93616	*Proteobacteria*	Non amplified	Amplified
PMI18_1	100% *Algihabitans albus* HHTR 118	*Proteobacteria*	Non amplified	Non amplified
PMI25_3	99.68% *Vibrio coralliirubri* strain DS1904-S1125	*Proteobacteria*	Non amplified	Non amplified
PMI29_1	99.4% *Erythrobacter* sp. B809	*Proteobacteria*	Non amplified	Amplified
PMI30_1	98.64% *Roseobacter cerasinus*	*Proteobacteria*	Amplified	Non amplified
PMI30_4	99.83% *Vibrio* sp. strain 6c	*Proteobacteria*	Non amplified	Amplified
PMI30_9	98.62% *Roseobacter cerasinus*	*Proteobacteria*	Amplified	Non amplified
PMI36_2	99.84% *Denitrobaculum tricleocarpae*	*Proteobacteria*	Non amplified	Non amplified
PMI37_3A	99.90% *Sphingopyxis ummariensis* strain 258-LNR4	*Proteobacteria*	Amplified	Non amplified
PMI37_4	100% *Paracoccus lutimaris* strain HDM-25	*Proteobacteria*	Amplified	Amplified
PMI38_1	100% *Algihabitans albus* strain HHTR118	*Proteobacteria*	Non amplified	Amplified
PMI41_5	100% *Ochrobactrum* sp. strain FA75	*Proteobacteria*	Amplified	Amplified
PMI41_6	100% *Staphylococcus hominis* strain FDAARGOS_748	*Firmicutes*	Amplified	Non amplified
PMI45_2	99.91% *Sphingopyxis ummariensis* strain 258-LNR4	*Proteobacteria*	Amplified	Non amplified
PMIC_1A1	99.06% *Aquimarina muelleri* KMM 6020	*Bacteroidetes*	Amplified	Amplified
PMIC_1A10A	100% *Microbacterium flavum* YM18-098	*Actinobacteria*	Non amplified	Amplified
PMIC_1A10B	99.82% *Nocardia nova* NBRC 15556	*Actinobacteria*	Non amplified	Amplified
PMIC_1A11B.1	99.92% *Nocardiopsis alba* DSM 43377	*Actinobacteria*	Non amplified	Non amplified
PMIC_1A11B.2	100% *Nocardiopsis alba* DSM 43377	*Actinobacteria*	Non amplified	Amplified
PMIC_1A11C	100% *Bacillus mycoides* DSM 2048	*Firmicutes*	Non amplified	Non amplified
PMIC_1A3A.1	99.85% *Streptomyces xiamenensis* MCCC 1A01550	*Actinobacteria*	Non amplified	Non amplified
PMIC_1A3A.2	99.82% *Streptomyces xiamenensis* MCCC 1A01550	*Actinobacteria*	Non amplified	Non amplified
PMIC_1A3A.3	99.82% *Streptomyces xiamenensis* MCCC 1A01550	*Actinobacteria*	Non amplified	Non amplified
PMIC_1A3B.1	100% *Streptomyces albogriseolus* NRRL B-1305	*Actinobacteria*	Non amplified	Non amplified
PMIC_1A3B.2	100% *Streptomyces albogriseolus* NRRL B-1305	*Actinobacteria*	Non amplified	Non amplified

PMIC_1A3C	100.00% *Streptomyces albogriseolus* NRRL B-1305	*Actinobacteria*	Non amplified	Non amplified
PMIC_1A8A	100% *Phaeobacter porticola* P97	*Proteobacteria*	Non amplified	Non amplified
PMIC_1A8B	99.43% *Streptomyces flavoviridis* NBRC 12772	*Actinobacteria*	Non amplified	Amplified
PMIC_1A8C	99.66% *Streptomyces albogriseolus* NRRL B-1305	*Actinobacteria*	Non amplified	Amplified
PMIC_1B2	99.92% *Catalinimonas alkaloidigena* CNU-914	*Bacteroidetes*	Amplified	Amplified
PMIC_1B3A.1	99.52% *Streptomyces xiamenensis* MCCC 1A01550	*Actinobacteria*	Non amplified	Amplified
PMIC_1B3A.2	99.54% *Streptomyces xiamenensis* MCCC 1A01550	*Actinobacteria*	Non amplified	Non amplified
PMIC_1B3A.3	99.55% *Streptomyces xiamenensis* MCCC 1A01550	*Actinobacteria*	Non amplified	Amplified
PMIC_1B3B	100% *Pseudoalteromonas carrageenovora* IAM 12662	*Proteobacteria*	Amplified	Non amplified
PMIC_1B5B.1	100% *Pseudoalteromonas tetraodonis* GFC	*Proteobacteria*	Amplified	Amplified
PMIC_1B5B.2	100% *Pseudoalteromonas tetraodonis* GFC	*Proteobacteria*	Amplified	Amplified
PMIC_1B9A	99.46% *Bacillus horikoshii* DSM 8719	*Firmicutes*	Non amplified	Non amplified
PMIC_1B9B.1	100% *Bacillus aryabhattai* B8W22	*Firmicutes*	Non amplified	Non amplified
PMIC_1B9B.2	100% *Bacillus aryabhattai* B8W22	*Firmicutes*	Non amplified	Non amplified
PMIC_1C10	100% *Pseudoalteromonas carrageenovora* IAM12662	*Proteobacteria*	Amplified	Amplified
PMIC_1C10	100% *Pseudoalteromonas carrageenovora* IAM12662	*Proteobacteria*	Non amplified	Non amplified
PMIC_1C11A	100% *Dietzia maris* DSM 43672	*Actinobacteria*	Non amplified	Non amplified
PMIC_1C11B	100% *Marinobacter litoralis* SW-45	*Proteobacteria*	Amplified	Amplified
PMIC_1C12A	99.67% *Streptomyces albidoflavus* DSM 40455	*Actinobacteria*	Amplified	Non amplified
PMIC_1C12B	99.68% *Streptomyces albidoflavus* DSM 40455	*Actinobacteria*	Non amplified	Non amplified
PMIC_1C1B	98.48% *Microbacterium diaminobutyricum* RZ63	*Actinobacteria*	Non amplified	Non amplified
PMIC_1C5B.1	100% *Dietzia maris* DSM 43672	*Actinobacteria*	Non amplified	Non amplified
PMIC_1C5B.2	99.35% *Psychrobacter cryohalolentis* K5	*Proteobacteria*	Non amplified	Non amplified
PMIC_1C7A	99.92% *Microbacterium phyllosphaerae* DSM 13468	*Actinobacteria*	Non amplified	Non amplified
PMIC_1C8A	99.69% *Streptomyces ardesiacus* NRRL B-1773	*Actinobacteria*	Non amplified	Amplified
PMIC_1D11A.1	99.85% *Streptomyces hydrogenans* NBRC 13475	*Actinobacteria*	Non amplified	Non amplified
PMIC_1D11A.2	99.84% *Streptomyces hydrogenans* NBRC 13475	*Actinobacteria*	Non amplified	Non amplified
PMIC_1D11B	99.84% *Nocardiopsis prasina* DSM 43845	*Actinobacteria*	Non amplified	Non amplified
PMIC_1D12	100% *Bacillus toyonensis* BCT-7112	*Firmicutes*	Amplified	Non amplified

PMIC_1D1B.1	100% *Sulfitobacter pontiacus* DSM 10014	*Proteobacteria*	Amplified	Non amplified
PMIC_1D2B.1	99.85% *Cobetia marina* JCM 21022	*Proteobacteria*	Amplified	Amplified
PMIC_1D2B.2	100% *Pseudoalteromonas atlantica* NBRC 103033	*Proteobacteria*	Amplified	Non amplified
PMIC_1D2B.3	100% *Cobetia marina* JCM 21022	*Proteobacteria*	Non amplified	Amplified
PMIC_1D8B	100% *Corynebacterium marinum* DSM 44953	*Actinobacteria*	Non amplified	Non amplified
PMIC_1D8D.1	100% *Psychrobacter nivimaris* 88/2-7	*Proteobacteria*	Non amplified	Amplified
PMIC_1D9A	99.80% *Streptomyces griseoflavus* LMG 19344	*Actinobacteria*	Non amplified	Amplified
PMIC_1D9B	99.75% *Streptomyces griseoflavus* LMG 19344	*Actinobacteria*	Non amplified	Amplified
PMIC_1E10A	99.71% *Bacillus licheniformis* ATCC 14580	*Firmicutes*	Non amplified	Non amplified
PMIC_1E10C	99.69% *Rhodococcus coprophilus* NBRC 100603	*Actinobacteria*	Non amplified	Amplified
PMIC_1E11A.1	100% *Bacillus aryabhattai* B8W22	*Firmicutes*	Non amplified	Non amplified
PMIC_1E11B.1	100% *Bacillus aryabhattai* B8W22	*Firmicutes*	Non amplified	Non amplified
PMIC_1E11B.2	99.59% *Arenibacter aquaticus* GUO666	*Bacteroidetes*	Non amplified	Amplified
PMIC_1E12A	99.44% *Micromonospora citrea* DSM 43903	*Actinobacteria*	Non amplified	Non amplified
PMIC_1E12B	100% *Arthrobacter gandavensis* R 5812	*Actinobacteria*	Non amplified	Amplified
PMIC_1E1A.1	99.52% *Aquimarina algiphila* 9Alg 151	*Bacteroidetes*	Non amplified	Non amplified
PMIC_1E1A.2	99.91% *Aquimarina amphilecti* 92V	*Bacteroidetes*	Non amplified	Non amplified
PMIC_1E1A.3	100% *Marinobacter litoralis* SW-45	*Proteobacteria*	Amplified	Non amplified
PMIC_1E1B.1	100% *Bacillus aryabhattai* B8W22	*Firmicutes*	Non amplified	Amplified
PMIC_1E1B.2	100% *Bacillus aryabhattai* B8W22	*Firmicutes*	Non amplified	Non amplified
PMIC_1E8A	99.33% *Limimaricola cinnabarinus* LL-001	*Proteobacteria*	Non amplified	Non amplified
PMIC_1E8C	99.35% *Limimaricola cinnabarinus* LL-001	*Proteobacteria*	Non amplified	Non amplified
PMIC_1E9A	99.82% *Streptomyces xiamenensis* MCCC1A01550	*Actinobacteria*	Non amplified	Non amplified
PMIC_1E9A	99.82% *Streptomyces xiamenensis* MCCC1A01550	*Actinobacteria*	Non amplified	Non amplified
PMIC_1E9B	100% *Rhodococcus erythropolis* NBRC 15567	*Actinobacteria*	Amplified	Amplified
PMIC_1E9C	99.84% *Streptomyces xiamenensis* MCCC 1A01550	*Actinobacteria*	Non amplified	Non amplified
PMIC_1E9D	99.81% *Streptomyces* xiamenensis MCCC 1A01550	*Actinobacteria*	Non amplified	Non amplified
PMIC_1F10B.1	100% *Arthrobacter gandavensis* R 5812	*Actinobacteria*	Non amplified	Non amplified
PMIC_1F10B.2	99.93% Bacillus megaterium NBRC 15308	*Firmicutes*	Non amplified	Non amplified

PMIC_1F10B.3	99.92% *Bacillus megaterium* NBRC 15308	*Firmicutes*	Non amplified	Non amplified
PMIC_1F10C.1	100% *Arthrobacter gandavensis* R 5812	*Actinobacteria*	Non amplified	Amplified
PMIC_1F10C.2	99.92% *Bacillus megaterium* NBRC 15308	*Firmicutes*	Non amplified	Non amplified
PMIC_1F12A	100% *Streptomyces setonii* NRRL ISP-5322	*Actinobacteria*	Non amplified	Non amplified
PMIC_1F12B	100% *Streptomyces setonii* NRRL ISP-5322	*Actinobacteria*	Non amplified	Amplified
PMIC_1F6A.1	99.77 *Nocardiopsis alba* DSM 43377	*Actinobacteria*	Non amplified	Non amplified
PMIC_1F6A.2	99.76 *Nocardiopsis alba* DSM 43377	*Actinobacteria*	Non amplified	Non amplified
PMIC_1F6A3	99.76% *Nocardiopsis alba* DSM 43377	*Actinobacteria*	Non amplified	Amplified
PMIC_1F6B	100% *Pseudoalteromonas neustonica* PAMC28425	*Proteobacteria*	Amplified	Amplified
PMIC_1H7A	100% *Kocuria polaris* CMS 76or	*Actinobacteria*	Amplified	Non amplified
PMIC_1H7B	99.09% *Pseudoalteromonas prydzensis* MB8-11	*Proteobacteria*	Non amplified	Non amplified
PMIC_1H8A	99.30% *Limimaricola soesokkakensis* CECT 8367	*Proteobacteria*	Non amplified	Non amplified
PMIC_1I1A	100% *Streptomyces hydrogenans* NBRC 13475	*Actinobacteria*	Non amplified	Amplified
PMIC_1I1B	100% *Streptomyces hydrogenans* NBRC 13475	*Actinobacteria*	Non amplified	Amplified
PMIC_2A10A	99.85% *Fictibacillus phosphorivorans* Ca7	*Firmicutes*	Amplified	Non amplified
PMIC_2A10B.1	100% *Bacillus megaterium* NBRC 15308	*Firmicutes*	Non amplified	Non amplified
PMIC_2A10B.3	100% *Bacillus aryabhattai* B8W22	*Firmicutes*	Non amplified	Non amplified
PMIC_2A11A.1	99.92% *Nocardiopsis alba* DSM 43377	*Actinobacteria*	Non amplified	Amplified
PMIC_2A11A.2	100% *Nocardiopsis alba* DSM 43377	*Actinobacteria*	Non amplified	Non amplified
PMIC_2A11B.1	100% *Nocardiopsis alba* DSM 43377	*Actinobacteria*	Non amplified	Amplified
PMIC_2A11B.2	100% *Nocardiopsis alba* DSM 43377	*Actinobacteria*	Non amplified	Amplified
PMIC_2A11B.3	100% *Nocardiopsis alba* DSM 43377	*Actinobacteria*	Non amplified	Amplified
PMIC_2A11C	100% *Bacillus mycoides* DSM 2048	*Firmicutes*	Non amplified	Non amplified
PMIC_2A12A.1	99.68% *Streptomyces albidoflavus* DSM 40455	*Actinobacteria*	Non amplified	Non amplified
PMIC_2A12A.2	99.69% *Streptomyces albidoflavus* DSM 40455	*Actinobacteria*	Non amplified	Non amplified
PMIC_2A12B.1	99.77% *Streptomyces albogriseolus* NRRL B-1305	*Actinobacteria*	Non amplified	Non amplified
PMIC_2A12B.1	99.77% *Streptomyces albogriseolus* NRRL B-1305	*Actinobacteria*	Non amplified	Non amplified
PMIC_2A12B.2	99.76% *Streptomyces albogriseolus* NRRL B-1305	*Actinobacteria*	Non amplified	Non amplified
PMIC_2A12B.2	99.76% *Streptomyces albogriseolus* NRRL B-1305	*Actinobacteria*	Non amplified	Non amplified

PMIC_2B1A	99.85% *Nocardiopsis prasina* DSM 43845	*Actinobacteria*	Non amplified	Amplified
PMIC_2B1C	99.82% *Nocardiopsis prasina* DSM 43845	*Actinobacteria*	Non amplified	Amplified
PMIC_2B1D	99.85% *Nocardiopsis prasina* DSM 43845	*Actinobacteria*	Non amplified	Amplified
PMIC_2B9A	100% *Bacillus horikoshii* DSM 8719	*Firmicutes*	Amplified	Non amplified
PMIC_2B9A.2	100% *Bacillus horikoshii* DSM 8719	*Firmicutes*	Non amplified	Non amplified
PMIC_2B9A.3	100% *Bacillus horikoshii* DSM 8719	*Firmicutes*	Non amplified	Non amplified
PMIC_2C11	100% *Alkalihalobacillus algicola* KMM 3737	*Firmicutes*	Amplified	Non amplified
PMIC_2C12	99.69% *Streptomyces albidoflavus* DSM 40455	*Actinobacteria*	Non amplified	Amplified
PMIC_2C2A	99.84% *Streptomyces xiamenensis* MCCC1A01550	*Actinobacteria*	Non amplified	Non amplified
PMIC_2C2B	99.81% *Streptomyces xiamenensis* MCCC1A01550	*Actinobacteria*	Non amplified	Non amplified
PMIC_2C3A	100% *Pseudoalteromonas carrageenovora* IAM 12662	*Proteobacteria*	Amplified	Non amplified
PMIC_2C3B.1	99.11% *Nocardiopsis umidischolae* 66/93	*Actinobacteria*	Non amplified	Non amplified
PMIC_2C3B.2	99.11% *Nocardiopsis umidischolae* 66/93	*Actinobacteria*	Non amplified	Amplified
PMIC_2C3B.3	99.19% *Nocardiopsis umidischolae* 66/93	*Actinobacteria*	Non amplified	Amplified
PMIC_2C3B.4	99.2% *Nocardiopsis umidischolae* 66/93	*Actinobacteria*	Non amplified	Amplified
PMIC_2C5A	99.93% *Vibrio toranzoniae* Vb 10.8	*Proteobacteria*	Amplified	Non amplified
PMIC_2C8A	99.69% *Streptomyces ardesiacus* NRRL B-1773	*Actinobacteria*	Non amplified	Amplified
PMIC_2C8B	99.7% *Streptomyces ardesiacus* NRRL B-1773	*Actinobacteria*	Amplified	Amplified
PMIC_2C8C	99.57% *Streptomyces hydrogenans* NBRC 13475	*Actinobacteria*	Non amplified	Amplified
PMIC_2C8D	99.13% *Pseudoalteromonas prydzensis* MB8-11	*Proteobacteria*	Non amplified	Non amplified
PMIC_2D10A	99.84% *Nocardiopsis prasina* DSM 43845	*Actinobacteria*	Non amplified	Amplified
PMIC_2D10B.1	99.75% *Nocardiopsis prasina* DSM 43845	*Actinobacteria*	Non amplified	Amplified
PMIC_2D10B.2	99.84% *Nocardiopsis prasina* DSM 43845	*Actinobacteria*	Non amplified	Amplified
PMIC_2D10C	99.84% *Nocardiopsis prasina* DSM 43845	*Actinobacteria*	Non amplified	Amplified
PMIC_2D11A.1	99.77% *Streptomyces hydrogenans* NBRC 13475	*Actinobacteria*	Non amplified	Non amplified
PMIC_2D11A.2	99.69% *Streptomyces hydrogenans* NBRC 13475	*Actinobacteria*	Non amplified	Amplified
PMIC_2D11B	99.84% *Streptomyces hydrogenans* NBRC 13475	*Actinobacteria*	Non amplified	Non amplified
PMIC_2D11C	99.70% *Streptomyces hydrogenans* NBRC 13475	*Actinobacteria*	Amplified	Amplified
PMIC_2D1A	100% *Sulfitobacter pontiacus* DSM 10014	*Proteobacteria*	Non amplified	Non amplified

PMIC_2D8A	100% *Streptomyces ardesiacus* NRRL B-1773	*Actinobacteria*	Non amplified	Amplified
PMIC_2D8B	100% *Streptomyces ardesiacus* NRRL B-1774	*Actinobacteria*	Non amplified	Amplified
PMIC_2D8E	100% *Psychrobacter nivimaris* 88/2-7	*Proteobacteria*	Non amplified	Non amplified
PMIC_2E10	99.69% *Rhodococcus coprophilus* NBRC 100603	*Actinobacteria*	Amplified	Amplified
PMIC_2E5A	99.67% *Tenacibaculum gallaicum* A37.1	*Bacteroidetes*	Non amplified	Non amplified
PMIC_2E5B	100% *Sulfitobacter pontiacus* DSM 10014	*Proteobacteria*	Non amplified	Amplified
PMIC_2E9A.1	99.84% *Streptomyces xiamenensis* MCCC 1A01550	*Actinobacteria*	Non amplified	Non amplified
PMIC_2E9A.2	99.85% *Streptomyces xiamenensis* MCCC 1A01550	*Actinobacteria*	Non amplified	Non amplified
PMIC_2E9A.3	99.85% *Streptomyces xiamenensis* MCCC 1A01550	*Actinobacteria*	Non amplified	Non amplified
PMIC_2E9B.1	100% *Rhodococcus erythropolis* NBRC 15567	*Actinobacteria*	Non amplified	Non amplified
PMIC_2E9B.2	100% *Psychrobacter nivimaris* 88/2-7	*Proteobacteria*	Amplified	Non amplified
PMIC_2E9C	99.76% *Rhodococcus qingshengii* JCM 15477	*Actinobacteria*	Amplified	Non amplified
PMIC_2F12A	99.82% *Nocardiopsis prasina* DSM 43845	*Actinobacteria*	Non amplified	Amplified
PMIC_2F12B	100% *Streptomyces setonii* NRRL ISP-5322	*Actinobacteria*	Non amplified	Amplified
PMIC_2F12C	100% *Streptomyces setonii* NRRL ISP-5322	*Actinobacteria*	Non amplified	Non amplified
PMIC_2F12E	99.23% *Henriciella algicola* CCUG 67844	*Proteobacteria*	Non amplified	Non amplified
PMIC_2F6A	99.76% *Nocardiopsis alba* DSM 43377	*Actinobacteria*	Non amplified	Amplified
PMIC_2F6B	99.76% *Nocardiopsis alba* DSM 43377	*Actinobacteria*	Non amplified	Amplified
PMIC_2F6C	99.77% *Nocardiopsis alba* DSM 43377	*Actinobacteria*	Non amplified	Amplified
PMIC_2F9	100% *Arthrobacter gandavensis* R 5812	*Actinobacteria*	Non amplified	Non amplified
PMIC_2G1A.1	100% *Tritonibacter mobilis* subsp. *Pelagius* NBRC 102038	*Proteobacteria*	Non amplified	Non amplified
PMIC_2G1A.2	100% *Tritonibacter mobilis* subsp. *Pelagius* NBRC 102038	*Proteobacteria*	Non amplified	Non amplified
PMIC_2G1B	100% *Tritonibacter mobilis* subsp. *Pelagius* NBRC 102038	*Proteobacteria*	Non amplified	Non amplified
PMIC_2G2A	99.68% *Streptomyces ambofaciens* ATCC 23877	*Actinobacteria*	Non amplified	Non amplified
PMIC_2G2B	99.74% *Arenibacter aquaticus* GUO666	*Bacteroidetes*	Amplified	Non amplified
PMIC_2G8A.1	100% *Alkalihalobacillus hwajinpoensis* SW-72	*Firmicutes*	Non amplified	Non amplified
PMIC_2G8A.2	99.52% *Psychrobacter cryohalolentis* K5	*Proteobacteria*	Non amplified	Non amplified
PMIC_2G8B	99.53% *Psychrobacter cryohalolentis* K5	*Proteobacteria*	Non amplified	Non amplified
PMIC_2G8C	99.68% *Streptomyces ambofaciens* ATCC 23877	*Actinobacteria*	Non amplified	Amplified

PMIC_2H10A	99.92% *Bacillus pumilus* ATCC 7061	*Firmicutes*	Amplified	Non amplified
PMIC_2H2A	100% *Nocardiopsis alba* DSM 43377	*Actinobacteria*	Non amplified	Amplified
PMIC_2H2C.1	100% *Nocardiopsis alba* DSM 43377	*Actinobacteria*	Non amplified	Amplified
PMIC_2H2C.2	100% *Nocardiopsis alba* DSM 43377	*Actinobacteria*	Non amplified	Amplified
PMIC_2H3	100% *Cobetia amphilecti* KMM 1561	*Proteobacteria*	Amplified	Amplified
PMIC_2H5B	99.84% *Pseudoalteromonas carrageenovora* IAM12662	*Proteobacteria*	Non amplified	Non amplified
PMIC_2H5C	99.92% *Providencia vermicola* OP1	*Proteobacteria*	Amplified	Non amplified
PMIC_2H6	99.81% *Plantibacter flavus* VKM Ac-2504	*Actinobacteria*	Non amplified	Amplified
PMO?verde	98.01% *Aquimarina algiphila* strain 9Alg 151	*Bacteroidetes*	Non amplified	Amplified
PMO_112_11.3 Laranja	100% *Rubinisphaera brasiliensis* DSM 5305	*Planctomycetes*	Non amplified	Amplified
PMO100_1.1	100% *Algihabitans albus* strainHHTR118	*Proteobacteria*	Non amplified	Non amplified
PMO102_1.1	99.2% *Sphingopyxis litoris* strain FR1093	*Proteobacteria*	Amplified	Non amplified
PMO102_6.2	98.4% *Sphingopyxis litoris* strain FR1093	*Proteobacteria*	Amplified	Non amplified
PMO107_3	99.3% *Labrenzia alba* strain 5OM6	*Proteobacteria*	Amplified	Non amplified
PMO107_8.1	100% *Vibrio cyclitrophicus* strain LMG 21359	*Proteobacteria*	Non amplified	Non amplified
PMO108_1.1.laranja	100% *Shewanella colwelliana* strain ATCC 39565	*Proteobacteria*	Non amplified	Non amplified
PMO110_1.1	100% *Algihabitans albus* strainHHTR118	*Proteobacteria*	Non amplified	Amplified
PMO110_11	98.46% *Litoreibacter meonggei* strain MA1-1	*Proteobacteria*	Non amplified	Non amplified
PMO110_18.1	100% *Pelagicola litoralis* strain CL-ES2	*Proteobacteria*	Non amplified	Amplified
PMO110_18.2	100% *Pelagicola litoralis* strain CL-ES2	*Proteobacteria*	Amplified	Non amplified
PMO110_3.2	100% *Amphritea ceti* strain RA1	*Proteobacteria*	Non amplified	Non amplified
PMO111_13.1	99.41% *Roseovarius aestuarii* strain SMK-122	*Proteobacteria*	Non amplified	Amplified
PMO111_2.1	98.96% *Ruegeria faecimaris* strain HD-28	*Proteobacteria*	Non amplified	Non amplified
PMO111_27.1	99.43% *Roseovarius aestuarii* strain SMK-122	*Proteobacteria*	Non amplified	Non amplified
PMO111_4.3.1.rosa	98.65% *Roseobacter cerasinus* strain AI77	*Proteobacteria*	Amplified	Amplified
PMO111_5.1.1	98.76% *Roseovarius aestuarii* strain SMK-122	*Proteobacteria*	Amplified	Non amplified
PMO111_6.1	99.39% *Roseovarius aestuarii* strain SMK-122	*Proteobacteria*	Non amplified	Amplified
PMO111_7.1	99.44% *Roseovarius aestuarii* strain SMK-122	*Proteobacteria*	Amplified	Non amplified
PMO111_8.5.1	98.86% *Roseovarius aestuarii* strain SMK-122	*Proteobacteria*	Non amplified	Non amplified

PMO112_8.2	98.38% *Erythrobacter longus* strain DSM 6997	*Proteobacteria*	Non amplified	Non amplified
PMO113_2	99.06% *Pseudoalteromonas prydzensis* strain MB8-11	*Proteobacteria*	Non amplified	Non amplified
PMO113_7	93.54% *Fodinicurvata halophila* strain BA45AL	*Proteobacteria*	Non amplified	Non amplified
PMO113_9	100% *Pseudoalteromonas arctica* A 37-1-2	*Proteobacteria*	Amplified	Non amplified
PMO114_1.a	98.77% *Sphingopyxis litoris* strain FR1093	*Proteobacteria*	Non amplified	Non amplified
PMO114_12	99.83% *Kiloniella spongiaestrain* JCM 19930	*Proteobacteria*	Non amplified	Non amplified
PMO114_13	99.14% *Sphingopyxis litoris* strain FR1093	*Proteobacteria*	Non amplified	Non amplified
PMO114_16.1	99.27% *Sphingopyxis litoris* strain FR1093	*Proteobacteria*	Amplified	Non amplified
PMO114_18	98.64% *Roseobacter cerasinus* strain AI77	*Proteobacteria*	Amplified	Non amplified
PMO114_2.a	98.61% *Flagellimonas aquimarina* sp. strain ECD12	*Bacteroidetes*	Non amplified	Non amplified
PMO114_2T	94.61% *Alteromonas alba* strain 190	*Proteobacteria*	Amplified	Amplified
PMO114_7.2	99.38% *Aquimarina algiphila* strain 9Alg 151	*Bacteroidetes*	Non amplified	Non amplified
PMO114_8.v	98.75% *Erythrobacter aquimaris* strain SW-110	*Proteobacteria*	Non amplified	Amplified
PMO115_16.1	98.48% *Sphingopyxis litoris* strain FR1093	*Proteobacteria*	Amplified	Non amplified
PMO115_17.2.1	100% *Maribacter dokdonensis* strain DSW-8	*Bacteroidetes*	Non amplified	Amplified
PMO115_3.2	100% *Pseudoalteromonas carrageenovora* IAM 12662 strain ATCC43555T	*Proteobacteria*	Amplified	Non amplified
PMO115_5.1	99.92% *Cellulophaga lytica* strain DSM 7489	*Bacteroidetes*	Non amplified	Amplified
PMO117_1.2	100% *Pseudoalteromonas carrageenovora* IAM 12662 strain ATCC43555T	*Proteobacteria*	Non amplified	Amplified
PMO117_3.1	99.46% *Labrenzia alba* strain 5OM6	*Proteobacteria*	Non amplified	Amplified
PMO117_4.3	99.44% *Labrenzia alba* strain 5OM6	*Proteobacteria*	Non amplified	Non amplified
PMO117_7	100% *Cellulophaga fucicola* strain cfHf10-1	*Bacteroidetes*	Non amplified	Non amplified
PMO117_8.1	99.44% *Labrenzia alba* strain 5OM6	*Proteobacteria*	Amplified	Non amplified
PMO118_11.2	99.84% *Kiloniella spongiae* strain JCM 19930	*Proteobacteria*	Non amplified	Amplified
PMO118_11.3	99.55% *Granulosicoccus undariae* strain W-BA3	*Proteobacteria*	Non amplified	Non amplified
PMO118_13.2	99.68% *Denitrobaculum tricleocarpae*	*Proteobacteria*	Non amplified	Non amplified
PMO118_2.2	100% *Kiloniella spongiae* strain JCM 19930	*Proteobacteria*	Non amplified	Non amplified
PMO118_9.1	100% *Kiloniella spongiaestrain* JCM 19930	*Proteobacteria*	Amplified	Non amplified
PMO119_11	92.33% *Inquilinus ginsengisoli* strain Gsoil 080	*Proteobacteria*	Non amplified	Amplified
PMO119_6.1.2	93.56% *Fodinicurvata halophila* strain BA45AL	*Proteobacteria*	Non amplified	Non amplified

PMO119_7.1	99.92% *Pelagibius litoralis* strain CL-UU02	*Proteobacteria*	Amplified	Non amplified
PMO119_7.2	99.92% *Pelagibius litoralis* strain CL-UU02	*Proteobacteria*	Non amplified	Non amplified
PMO120_1	99.84% *Citrobacter murliniae* strain CIP 104556	*Proteobacteria*	Amplified	Non amplified
PMO121_14	96.2% *Aquimarina algiphila* strain 9Alg 151	*Bacteroidetes*	Non amplified	Non amplified
PMO121_15.2	98.21% *Jannaschia seosinensis* strain CL-SP26	*Proteobacteria*	Non amplified	Non amplified
PMO121_15.5	98.16% *Jannaschia seosinensis* strain CL-SP26	*Proteobacteria*	Non amplified	Non amplified
PMO122_2.a	98.78% *Yoonia maritima* strain KMM 9530	*Proteobacteria*	Non amplified	Non amplified
PMO122_3.1	99.66% *Brevundimonas vesicularis* strain NBRC 12165	*Proteobacteria*	Non amplified	Amplified
PMO122_4.1	99.67% *Brevundimonas vesicularis* strain NBRC 12165	*Proteobacteria*	Non amplified	Amplified
PMO122_5.1	99.92% *Paracoccus mutanolyticus* strain RSP-02	*Proteobacteria*	Non amplified	Non amplified
PMO122_6.rosa	99.67% *Pseudomonas caeni* strain HY-14	*Proteobacteria*	Non amplified	Non amplified
PMO123_1	98.46% *Sphingopyxis litoris* strain FR1093	*Proteobacteria*	Amplified	Non amplified
PMO123_2	98.44% *Sphingopyxis litoris* strain FR1093	*Proteobacteria*	Non amplified	Amplified
PMO123_3.2	98% *Pyruvatibacter mobilis* strain CGMCC 1.15125	*Proteobacteria*	Non amplified	Non amplified
PMO124_1	100% *Acinetobacter johnsonii* strain HAMBI 1969	*Proteobacteria*	Non amplified	Amplified
PMO124_2.1	99.52% *Vibrio splendidus* strain LMG 4042	*Proteobacteria*	Non amplified	Non amplified
PMO126_2	99.84% *Kiloniella spongiae* strain JCM 19930	*Proteobacteria*	Amplified	Non amplified
PMO127_3	95.45% *Tepidicaulis marinus* strain MA2	*Proteobacteria*	Non amplified	Amplified
PMO128_2.1	93.38% *Fodinicurvata halophila* strain BA45AL	*Proteobacteria*	Non amplified	Amplified
PMO128_3	100% *Algihabitans albus* strain HHTR118	*Proteobacteria*	Non amplified	Amplified
PMO131_14.1	98.6% *Roseobacter cerasinus* strain AI77	*Proteobacteria*	Non amplified	Amplified
PMO131_17.1	99.27% *Roseovarius aestuarii* strain SMK-122	*Proteobacteria*	Non amplified	Amplified
PMO132_19.1	99.83% *Kiloniella spongiae* strain JCM 19930	*Proteobacteria*	Amplified	Amplified
PMO132_2	100% *Ruegeria meonggei* strain MA-E2-3	*Proteobacteria*	Non amplified	Non amplified
PMO132_20.1	99.84% *Kiloniella spongiae* strain JCM 19930	*Proteobacteria*	Non amplified	Non amplified
PMO132_20.2.3	100% *Aquimarina macrocephali* JAMB N27	*Bacteroidetes*	Amplified	Non amplified
PMO132_3	99.79% *Kiloniella spongiae* strain JCM 19930	*Proteobacteria*	Non amplified	Non amplified
PMO132_8.1	98.34% *Roseobacter cerasinus* strain AI77	*Proteobacteria*	Non amplified	Non amplified
PMO133_10.1	95.95% *Aliiglaciecola litoralis* strain Sd 2-38	*Proteobacteria*	Amplified	Amplified

PMO133_6.2	99.39% *Aquimarina algiphila* strain 9Alg 151	*Bacteroidetes*	Non amplified	Amplified
PMO135_10	99.5% *Thalassospira lohafexi* strain 139Z-12	*Proteobacteria*	Amplified	Amplified
PMO135_5.1	100% *Ruegeria meonggei* strain MA-E2-3	*Proteobacteria*	Non amplified	Amplified
PMO135_6	99.84% *Kiloniella spongiae* strain JCM 19930	*Proteobacteria*	Non amplified	Non amplified
PMO135_8	99.67% *Kiloniella spongiae* strain JCM 19930	*Proteobacteria*	Non amplified	Non amplified
PMO135_9	99.43% *Labrenzia alba* strain 5OM6	*Proteobacteria*	Amplified	Amplified
PMO136_11	100% *Vibrio atlanticus* strain DS1904-S1116	*Proteobacteria*	Amplified	Non amplified
PMO136_9.1	100% *Kiloniella spongiae* strain JCM 19930	*Proteobacteria*	Non amplified	Non amplified
PMO138_12	94.67% *Methylotenera mobilis* JLW8	*Proteobacteria*	Amplified	Non amplified
PMO138_15.2	94.82% *Methylotenera versatilis* strain 301	*Proteobacteria*	Amplified	Non amplified
PMO138_17	94.3% *Methylotenera mobilis* JLW8	*Proteobacteria*	Amplified	Non amplified
PMO138_18	93.88% *Methylotenera mobilis* JLW8	*Proteobacteria*	Amplified	Non amplified
PMO138_2	94.9% *Methylotenera versatilis* strain 301	*Proteobacteria*	Non amplified	Non amplified
PMO138_4.1	98.79% *Sulfitobacter marinus* strain SW-265	*Proteobacteria*	Amplified	Amplified
PMO138_9.1	99.75% *Pseudomonas nitrititolerans* strain GL14	*Proteobacteria*	Non amplified	Non amplified
PMO139_11.2	97.61% *Thalassobius gelatinovorus* strain NBRC15761	*Proteobacteria*	Non amplified	Amplified
PMO139_12.5.a	98.76% *Roseobacter cerasinus* strain AI77	*Proteobacteria*	Non amplified	Non amplified
PMO139_21.1	99.44% *Labrenzia alba* strain 5OM6	*Proteobacteria*	Non amplified	Non amplified
PMO139_7.1	98.46% *Erythrobacter longus* strain DSM 6997	*Proteobacteria*	Amplified	Non amplified
PMO140_12	98.14% *Sulfitobacter marinus* strain SW-265	*Proteobacteria*	Amplified	Amplified
PMO140_13	97.3% *Defluviimonas aestuarii* strain BS14	*Proteobacteria*	Non amplified	Non amplified
PMO140_15.2	98.45% *Erythrobacter longus* strain DSM 6997	*Proteobacteria*	Non amplified	Non amplified
PMO140_2.rosa	97.48% *Jannaschia seosinensis* strain CL-SP26	*Proteobacteria*	Non amplified	Non amplified
PMO140_4	100% *Microbulbifer echini* strain AM134	*Proteobacteria*	Non amplified	Non amplified
PMO140_9.1.2	99.2% *Sphingopyxis litoris* strain FR1093	*Proteobacteria*	Non amplified	Non amplified
PMO141_1.v	100% *Pseudoalteromonas translucida* KMM 520	*Proteobacteria*	Non amplified	Amplified
PMO85_2	97.94% *Microbulbifer echini* strain AM134	*Proteobacteria*	Non amplified	Non amplified
PMO85_3.1	100% *Pseudoalteromonas translucida* KMM 520	*Proteobacteria*	Non amplified	Non amplified
PMO86_4	100% *Colwellia meonggei* strain MA1-3	*Proteobacteria*	Non amplified	Amplified

PMO87_15.4.1	99.37% *Mycolicibacterium frederiksbergense* strain DSM 44346	*Actinobacteria*	Non amplified	Non amplified
PMO87_18.1.3	99.68% *Denitrobaculum tricleocarpaestrain* R148	*Proteobacteria*	Non amplified	Non amplified
PMO87_21	96.19% *Aliiglaciecola litoralis* strain Sd 2-38	*Proteobacteria*	Non amplified	Non amplified
PMO87_22	99.92% *Zobellia russellii* strain KMM 3677	*Bacteroidetes*	Amplified	Amplified
PMO87_4.a	95.6% *Aliiglaciecola litoralis* strain Sd 2-38	*Proteobacteria*	Non amplified	Non amplified
PMO87_5.2	100% *Pseudoalteromonas carrageenovora* IAM 12662 strain ATCC43555T	*Proteobacteria*	Amplified	Amplified
PMO88_1	99.7% *Pseudoalteromonas arctica* A 37-1-2	*Proteobacteria*	Non amplified	Non amplified
PMO90_13	98.1% *Sneathiella aquamaris* 216LB-ZA1-12	*Proteobacteria*	Amplified	Amplified
PMO90_19.1	99.15% *Aquimarina algiphila* strain 9Alg 151	*Bacteroidetes*	Amplified	Non amplified
PMO94_4	99.86% *Brevundimonas bullata* strain NBRC 13290	*Proteobacteria*	Non amplified	Non amplified
PMO94_5	99.35% *Pseudomonas caeni* strain HY-14	*Proteobacteria*	Non amplified	Non amplified
PMO95_10	99.27% *Sphingopyxis litoris* strain FR1093	*Proteobacteria*	Non amplified	Non amplified
PMO95_11	99.06% *Sphingopyxis litoris* strain FR1093	*Proteobacteria*	Non amplified	Amplified
PMO95_13.2	99.28% *Sphingopyxis litoris* strain FR1093	*Proteobacteria*	Amplified	Amplified
PMO95_8.1	99.2% *Sphingopyxis litoris* strain FR1093	*Proteobacteria*	Non amplified	Non amplified
PMO96_5.1	99.33% *Sphingopyxis litoris* strain FR1093	*Proteobacteria*	Non amplified	Non amplified
R2APL15_2	100% *Actinomadura maheshkhaliensis* 13-12-50	*Actinobacteria*	Non amplified	Non amplified
R2APL30_1.2	100% *Streptomyces diastaticus* NBRC 3714	*Actinobacteria*	Non amplified	Non amplified
R2APL45_1	98.34% *Streptomyces marinus* DSM 41970	*Actinobacteria*	Non amplified	Non amplified

Supplementary Table 2 – Initial antimicrobial assay results from each replicate for the selected strains extracts against *E. coli* ATCC 25922 and *S. aureus* ATCC 29213. The growth inhibition values that were considered bioactive are highlighted in emerald green.

Strain designation	**Taxon**	***E. coli* activity (%)**				***S. aureus* activity (%)**			
		Replicate 1	**Replicate 2**	**Replicate 3**	**Mean**	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Mean**
PMIC_1I1A	100% *Streptomyces hydrogenans* NBRC 13475	**99.676**	**79.031**	**62.022**	**80.243**	-12.187	42.209	5.664	11.895
PMIC_2C12	99.69% *Streptomyces albidoflavus* DSM 40455	**76.640**	**55.800**	**62.093**	**64.844**	-1.522	32.218	8.587	13.095
PMIC_2H2A	100% *Nocardiopsis alba* DSM 43377	24.332	31.674	23.973	26.660	12.322	42.288	25.709	26.773
PMIC_2C3B.2	99.11% *Nocardiopsis umidischolae* 66/93	28.947	43.376	**51.930**	41.418	-2.330	18.994	7.160	7.942
PMIC_2A11A.1	99.92% *Nocardiopsis alba* DSM 43377	31.498	38.464	48.935	39.632	14.530	36.829	6.290	19.216
PMIC_2C8C	99.57% *Streptomyces hydrogenans* NBRC 13475	31.457	43.550	34.350	36.452	-0.875	38.366	9.318	15.603
PMIC_1B3A.1	99.52% *Streptomyces xiamenensis* MCCC 1A01550	25.506	32.946	29.821	29.424	-0.229	38.684	7.995	15.484
PMIC_1C8A	99.69% *Streptomyces ardesiacus* NRRL B-1773	31.255	37.337	33.316	33.969	5.373	23.791	7.752	12.305
PMIC_1F10B	100% *Arthrobacter gandavensis* R 5812	26.721	36.528	29.215	30.821	5.535	29.356	5.629	13.507
PMIC_1D9A	99.80% *Streptomyces griseoflavus* LMG 19344	29.757	32.454	28.002	30.071	-9.386	20.770	3.089	4.824
PMIC_1A11B.2	100% *Nocardiopsis alba* DSM 43377	**71.093**	**99.863**	**57.921**	**76.292**	-0.768	43.772	9.875	17.627
PMIC_1D9B	99.75% *Streptomyces griseoflavus* LMG 19344	**63.036**	**75.650**	**55.140**	**64.609**	-15.904	43.428	1.975	9.833
Culture medium	N/A	22.470	**61.579**	38.665	40.904	-24.791	12.104	16.591	1.301
PMO107_3	99.3% *Labrenzia alba* strain 5OM6	9.352	29.450	26.255	21.686	-18.274	7.599	22.577	3.967
PMO123_2	98.44% *Sphingopyxis litoris* strain FR1093	5.182	30.779	21.655	19.205	-10.302	35.478	3.889	9.688
PMIC_2D8A	100% *Streptomyces ardesiacus* NRRL B-1773	14.008	29.883	18.481	20.791	-12.510	34.497	9.353	10.447
PMIC_2D10A	99.84% *Nocardiopsis prasina* DSM 43845	12.510	23.266	24.080	19.952	-10.840	43.110	12.450	14.907
PMIC_1C11B	100% *Marinobacter litoralis* SW-45	5.182	20.724	22.760	16.222	-6.639	48.198	6.638	16.066
PMIC_2B1A	99.85% *Nocardiopsis prasina* DSM 43845	11.619	18.903	11.884	14.136	-21.290	13.191	18.192	3.364
PMIC_2C8B	99.7% *Streptomyces ardesiacus* NRRL B-1773	7.814	15.552	8.746	10.704	-19.459	9.295	4.272	-1.964
PMIC_1E10C	99.69% *Rhodococcus coprophilus* NBRC 100603	**101.134**	**98.794**	**60.096**	**86.675**	-53.717	**71.810**	31.660	16.584
PMIC_1E11B.2	99.59% *Arenibacter aquaticus* GUO666	**65.547**	**73.252**	**56.102**	**64.967**	13.614	35.716	9.109	19.480
PMIC_1D12	100% *Bacillus toyonensis* BCT-7112	36.640	**56.320**	47.330	46.763	11.945	38.101	40.047	30.031

PMIC_1B5B.1	100% *Pseudoalteromonas tetraodonis* GFC	11.700	29.421	30.213	23.778	1.064	28.217	12.833	14.038
PMIC_1B3B	100% *Pseudoalteromonas carrageenovora* IAM 12662	-1.336	20.406	8.175	9.082	18.139	30.522	2.149	16.937
PMIC_1B2	99.92% *Catalinimonas alkaloidigena* CNU-914	4.737	15.118	14.273	11.376	28.858	32.404	11.998	24.420
PMI30_9	98.62% *Roseobacter cerasinus*	-4.534	4.428	17.054	5.649	-44.129	18.835	24.700	-0.198
PMO136_11	100% *Vibrio atlanticus* strain DS1904-S1116	-1.296	16.939	4.288	6.644	-25.653	6.566	0.409	-6.226
PMO90_13	98.1% *Sneathiella aquamaris* 216LB-ZA1-12	-7.571	10.958	11.171	4.853	-38.527	22.704	30.407	4.862
PMIC_1A8C	99.66% *Streptomyces albogriseolus* NRRL B-1305	-7.247	1.307	11.884	1.981	-26.784	47.138	**99.487**	39.947
PMIC_2A11B.1	100.00% *Nocardiopsis alba* DSM 43377	**99.069**	**78.713**	**55.247**	**77.676**	-59.803	20.955	14.016	-8.277
PMIC_1A10B	99.82% *Nocardia nova* NBRC 15556	**53.036**	**67.155**	31.746	**50.646**	-53.178	20.028	-4.846	-12.665
PMIC_2G8C	99.68% *Streptomyces ambofaciens* ATCC 23877	29.393	44.936	18.338	30.889	1.764	4.048	30.198	12.003
PMIC_2C5A	99.93% *Vibrio toranzoniae* Vb 10.8	-20.121	16.303	2.683	-0.378	-29.370	-0.272	26.649	-0.998
PMIC_1E9B	100% *Rhodococcus erythropolis* NBRC 15567	-10.486	17.632	19.515	8.887	-35.672	-5.254	38.307	-0.873
PMIC_1H7A	100% *Kocuria polaris* CMS 76or	-12.632	13.240	5.037	1.882	-41.058	-12.170	4.411	-16.273
PMIC_1C12A	99.67% *Streptomyces albidoflavus* DSM 40455	-16.194	13.732	8.175	1.904	-59.157	-12.170	16.626	-18.234
MEMO17_8	99.9% *Novipirellula caenicola*	-23.360	11.622	3.147	-2.864	-55.386	8.924	13.285	-11.059
PMIC_2E9B.2	100% *Psychrobacter nivimaris* 88/2-7	-16.640	4.023	0.401	-4.072	-41.382	0.470	0.896	-13.338
PMIC_2G2B	99.74% *Arenibacter aquaticus* GUO666	-16.478	5.121	9.281	-0.692	-63.843	-0.537	12.346	-17.345
PMIC_2C11	100% *Alkalihalobacillus algicola* KMM 3737	-16.194	-1.033	21.013	1.262	-90.183	1.265	-21.794	-36.904
PMIC_1E1B.1	100% *Bacillus aryabhattai* B8W22	-3.279	6.393	15.343	6.152	-14.449	36.458	9.074	10.361
PMO114_2T	94.61% *Alteromonas alba* strain 190	-10.081	18.961	36.703	15.195	-14.449	10.249	15.269	3.690
PMI12_1B	99.91% *Sphingorhabdus* sp. Alg231_15	-17.935	12.836	14.630	3.177	-38.096	-19.511	11.406	-15.400
PMI29_1	99.4% *Erythrobacter* sp. B809	-17.126	18.210	7.640	2.908	-32.709	-16.039	13.459	-11.763
PMI41_5	100% *Ochrobactrum* sp. strain FA75	-14.980	17.054	7.676	3.250	-20.644	-10.342	5.803	-8.394
PMIC_2H10A	99.92% *Bacillus pumilus* ATCC 7061	-15.344	14.425	10.172	3.084	-39.119	-9.229	2.462	-15.295
PMIC_2E9C	99.76% *Rhodococcus qingshengii* JCM 15477	-14.494	13.992	10.172	3.223	-42.567	2.564	5.490	-11.504
PMI45_2	99.91% *Sphingopyxis ummariensis* strain 258-LNR4	-20.526	10.264	15.878	1.872	-36.911	4.869	14.329	-5.904
PMIC_1D2B.3	100% *Cobetia marina* JCM 21022	-12.591	4.688	9.780	0.626	-59.588	-1.994	-7.908	-23.164
PMIC_1C10	100% *Pseudoalteromonas carrageenovora* IAM12662	-14.696	4.630	2.398	-2.556	-87.759	7.944	-3.802	-27.873
PMIC_1A10A	100% *Microbacterium flavum* YM18-098	-24.980	6.624	8.853	-3.168	-41.543	-11.375	-7.352	-20.090

PMIC_1D8D.1	100% *Psychrobacter nivimaris* 88/2-7	-21.781	11.536	25.684	5.146	-28.023	-2.206	3.541	-8.896
PMIC_2H6	99.81% *Plantibacter flavus* VKM Ac-2504	-40.405	13.789	16.555	-3.353	-18.597	-11.720	0.792	-9.842
PMIC_2H5C	99.92% *Providencia vermicola* OP1	-12.996	14.801	7.212	3.006	-33.625	-5.969	1.418	-12.725
PMIC_1D1B.1	100% *Sulfitobacter pontiacus* DSM 10014	3.077	20.666	19.765	14.503	-19.513	21.538	10.571	4.199
PMO114_18	98.64% *Roseobacter cerasinus* strain AI77	-10.810	14.396	16.234	6.607	-32.332	6.566	5.942	-6.608
PMI30_4	99.83% *Vibrio* sp. strain 6c	-6.478	9.918	9.566	4.335	-45.960	30.920	7.439	-2.534
PMI37_3A	99.90% *Sphingopyxis ummariensis* strain 258-LNR4	-1.538	18.528	13.595	10.195	-34.864	16.901	6.151	-3.937
PMI37_4	100% *Paracoccus lutimaris* strain HDM-25	-8.664	-1.986	11.064	0.138	-43.105	17.431	3.889	-7.262
PMI41_6	100% *Staphylococcus hominis* strain FDAARGOS_748	-29.879	2.983	14.915	-3.993	-34.595	33.172	2.671	0.416
PMIC_1E1A.3	100% *Marinobacter litoralis* SW-45	9.879	7.028	12.240	9.716	-44.937	35.557	1.801	-2.526
PMIC_1F10C.1	100% *Arthrobacter gandavensis* R 5812	4.413	-6.754	7.462	1.707	-11.163	14.781	5.037	2.885
MEMO4_5	99.92% *Hellea balneolensis* DSM 19091	6.073	15.061	17.554	12.896	-14.126	12.581	3.263	0.573
PMI38_1	100% *Algihabitans albus* strain HHTR118	-11.741	16.505	10.921	5.228	-31.901	6.460	7.995	-5.815
ICM_H12	99.04 % *Erythrobacter lutimaris* strain S-5	-9.676	9.889	6.820	2.344	-34.756	-2.418	-0.322	-12.499
ICM_G4	99.9% *Novipirellula caenicola*	-13.036	16.881	13.274	5.706	-31.093	8.606	3.645	-6.281
ICM_H5	99.9% *Novipirellula caenicola*	-6.923	18.557	18.624	10.086	-41.058	10.037	2.427	-9.531
PMI12_2	100% *Altererythrobacter ishigakiensis* strain H93616	0.081	7.057	11.313	6.150	-30.178	19.259	5.420	-1.833
ICM_A5	99.9% *Tritonibacter mobilis*	0.567	3.532	13.453	5.851	-18.489	17.510	2.323	0.448
PMO135_10	99.5% *Thalassospira lohafexi* strain 139Z-12	-25.668	18.672	15.271	2.759	-15.203	25.593	-1.714	2.892
PMIC_2E10	99.69% *Rhodococcus coprophilus* NBRC 100603	45.466	20.117	6.356	23.980	-21.290	9.375	-16.469	-9.462
PMIC_2D8B	100% *Streptomyces ardesiacus* NRRL B-1774	44.332	17.690	13.809	25.277	-26.892	14.330	0.235	-4.109
PMIC_2A10A	99.85% *Fictibacillus phosphorivorans* Ca7	2.955	26.618	22.724	17.433	-18.597	10.461	7.334	-0.267
PMIC_2H3	100% *Cobetia amphilecti* KMM 1561	30.081	31.588	15.628	25.766	-8.363	24.904	15.025	10.522
PMIC_ 1H8A	99.30% *Limimaricola soesokkakensis* CECT 8367	34.251	27.803	19.693	27.249	-24.737	24.029	30.442	9.911
PMIC_2C3A	100% *Pseudoalteromonas carrageenovora* IAM 12662	30.891	19.713	16.377	22.327	-13.156	28.058	30.164	15.022
PMIC_2F12A	99.82% *Nocardiopsis prasina* DSM 43845	-34.089	13.240	4.609	-5.413	-3.515	24.718	30.616	17.273
PMIC_2B9A.2	100% *Bacillus horikoshii* DSM 8719	47.976	13.038	6.606	22.540	-4.592	20.902	**59.118**	25.143
PMIC_2D11C	99.70% *Streptomyces hydrogenans* NBRC 13475	22.632	27.572	13.916	21.373	-3.784	22.386	**59.605**	26.069
PMIC_2A12B.1	99.77% *Streptomyces albogriseolus* NRRL B-1305	**74.192**	**57.456**	36.532	**56.060**	**89.206**	**89.203**	**83.691**	**87.367**

PMIC_2F6A	99.76% *Nocardiopsis alba* DSM 43377	**75.025**	26.233	34.401	45.220	27.562	19.226	45.700	30.829
PMIC_2A11B.2	100% *Nocardiopsis alba* DSM 43377	15.983	11.647	10.935	12.855	29.817	-14.051	-3.131	4.211
PMO112_11.1 Laranja	100% *Rubinisphaera brasiliensis* DSM 5305	-3.504	34.665	11.234	14.132	2.394	7.045	18.582	9.341
PMIC_2C2B	99.81% *Streptomyces xiamenensis* MCCC1A01550	**58.145**	25.159	24.049	35.784	11.383	17.435	27.674	18.831
PMIC_2F6B	99.76% *Nocardiopsis alba* DSM 43377	14.099	14.480	18.424	15.668	-13.491	-14.887	31.235	0.952
PMIC_2B1C	99.82% *Nocardiopsis prasina* DSM 43845	0.226	24.605	13.697	12.843	35.112	2.985	12.955	17.017
R2APL15_2	100% *Actinomadura maheshkhaliensis* 13-12-50	7.036	43.749	19.389	23.391	8.735	31.963	34.223	24.974
PMIC_2D11C	99.70% *Streptomyces hydrogenans* NBRC 13475	**56.805**	**55.177**	30.174	47.385	6.480	-3.543	20.140	7.692
PMIC_2H2C.2	100% *Nocardiopsis alba* DSM 43377	40.143	32.419	27.744	33.435	**55.965**	44.701	**55.238**	**51.968**
PMIC_2F6C	99.77% *Nocardiopsis alba* DSM 43377	10.405	39.386	8.272	19.354	**51.389**	0.557	3.958	18.635
ABPL45_1	99.92% *Streptomyces antimycoticus* NBRC 12839	18.627	41.600	19.289	26.506	24.261	46.890	39.819	36.990
PMIC_1F6A.3	99.76% *Nocardiopsis alba* DSM 43377	33.007	**57.423**	33.769	41.400	7.591	27.625	31.966	22.394
PMIC_2C8A	99.69% *Streptomyces ardesiacus* NRRL B-1773	45.105	21.382	32.870	33.119	22.038	25.873	47.767	31.893
UC8	*Roseimaritima ulvae* UC8	**58.145**	**61.004**	45.519	**54.890**	**51.422**	**50.234**	43.125	48.260
MAPL30_1	100% *Micromonospora matsumotoense* DSM 44100	7.869	33.200	19.189	20.086	31.517	-8.956	35.654	19.405
PMIC_2D11A.2	99.69% *Streptomyces hydrogenans* NBRC 13475	40.614	**61.981**	35.633	46.076	11.677	43.387	40.168	31.744
PMIC_2D10B.1	99.75% *Nocardiopsis prasina* DSM 43845	43.765	37.303	25.747	35.605	17.364	8.598	40.677	22.213
UC8 1:10	*Roseimaritima ulvae* UC8	33.514	14.252	28.044	25.270	48.578	-1.552	26.053	24.360
ABPP45_1	98.79% *Sporosarcina aquimarina* SW28(T)	17.142	37.856	13.731	22.910	17.723	-0.199	26.403	14.642
PMIC_1I1B	100% *Streptomyces hydrogenans* NBRC 13475	35.978	34.014	20.454	30.149	25.633	20.340	33.810	26.595
PMIC_2B1D	99.85% *Nocardiopsis prasina* DSM 43845	33.188	**54.753**	27.345	38.429	**55.671**	48.363	44.238	49.424
PMO128_3	100% *Algihabitans albus* strain HHTR118	6.855	24.345	**60.930**	30.710	-14.831	-13.335	17.183	-3.661
PMIC_2C3B.4	99.2% *Nocardiopsis umidischolae* 66/93	16.816	19.038	14.962	16.939	23.803	0.279	16.547	13.543
PMIC_2D10C	99.84% *Nocardiopsis prasina* DSM 43845	48.836	**54.330**	29.109	44.092	33.739	37.695	36.894	36.109
PMO138_17	94.3% *Methylotenera mobilis* JLW8	**57.675**	**63.739**	**84.863**	**68.759**	18.443	35.148	45.700	33.097
PMIC_2C3B.3	99.19% *Nocardiopsis umidischolae* 66/93	41.846	45.865	29.708	39.140	17.004	25.197	27.039	23.080
PMIC_2F12B	100% *Streptomyces setonii* NRRL ISP-5322	10.006	49.935	25.481	28.474	43.283	42.512	36.576	40.790
PMO90_19.1	99.15% *Aquimarina algiphila* strain 9Alg 151	**61.224**	**60.711**	**90.322**	**70.753**	24.882	19.266	47.735	30.627
PMIC_2H2C.1	100% *Nocardiopsis alba* DSM 43377	44.272	41.665	30.207	38.715	**56.880**	37.536	**51.168**	48.528

PMIC_2D10B.2	99.84% *Nocardiopsis prasina* DSM 43845	-2.526	42.447	19.389	19.770	20.404	-22.450	24.400	7.451
PMIC_1A8B	99.43% *Streptomyces flavoviridis* NBRC 12772	**73.286**	**68.004**	**86.261**	**75.851**	**100.025**	**101.343**	**100.827**	**100.732**
PMIC_2A11B.3	100% *Nocardiopsis alba* DSM 43377	45.069	41.568	25.647	37.428	24.195	17.076	26.435	22.569
PMIC_2D8B	100% *Streptomyces ardesiacus* NRRL B-1774	1.784	47.461	33.636	27.627	32.987	36.302	15.912	28.400
Culture medium 2	N/A	**78.538**	**89.492**	**96.646**	**88.226**	14.259	-12.061	49.324	17.174
PMIC_ 1E12B	100% *Arthrobacter gandavensis* R 5812	**73.963**	**77.901**	42.607	**64.824**	38.520	39.586	40.711	39.606
PMIC_1F6A.3	99.76% *Nocardiopsis alba* DSM 43377	**70.939**	**99.657**	**67.085**	**79.227**	32.828	30.738	36.590	33.386
PMIC_1F12B	100% *Streptomyces setonii* NRRL ISP-5322	**69.211**	**58.630**	**58.381**	**62.074**	42.033	16.847	5.780	21.553
Culture medium 2	N/A	-15.892	-1.916	-29.418	15.742	20.509	35.254	18.435	24.732
Culture medium 3	N/A	-1.087	-6.992	-22.957	10.345	15.872	-11.415	-14.748	-3.430
M600PL45_2	98.52% *Streptomyces nanshensis* SCSIO 01066	-10.041	-19.023	-2.357	10.473	**59.763**	**62.210**	25.219	49.064

Supplementary Table 3 – OSMAC approach results for the antimicrobial assays from each replicate for the selected strains extracts against *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 The growth inhibition values highlighted in emerald green were considered to be bioactive.

Strain designation	Taxon	Culture medium	*E. coli* activity (%)				*S. aureus* activity (%)			
			Replicate 1	Replicate 2	Replicate 3	Mean	Replicate 1	Replicate 2	Replicate 3	Mean
PMIC_2A12B.1	99.77% *Streptomyces albogriseolus* NRRL B-1305	M607 1:10	**95.397**	**74.637**	**101.184**	**90.406**	37.092	**62.757**	48.284	49.378
		CGY	**72.033**	**71.384**	**63.284**	**68.900**	44.405	37.520	37.169	39.698
		M600	**60.464**	**68.887**	28.602	**52.651**	-7.514	10.717	-9.978	-2.258
		MA	**81.157**	**68.591**	24.147	**57.965**	27.112	30.664	-4.756	17.673
		M607	**73.499**	**68.591**	10.748	**50.946**	32.102	25.180	20.160	25.814
PMIC_1A10B	99.82% *Nocardia nova* NBRC 15556	M607 1:10	**100.546**	**100.854**	**101.750**	**101.050**	34.578	25.865	26.016	28.820
		CGY	**91.063**	**99.343**	**70.674**	**87.027**	-3.552	6.113	15.983	6.181
		M600	**92.041**	**89.717**	**58.052**	**79.936**	10.694	29.424	27.695	22.604
		MA	**78.615**	**79.499**	4.667	**54.260**	-56.842	-9.819	-9.493	-25.385
		M607	**82.949**	**79.499**	23.405	**61.951**	15.646	31.807	33.663	27.039
PMIC_1F12B	100% *Streptomyces setonii* NRRL ISP-5322	M607 1:10	**99.927**	**76.969**	**102.316**	**93.071**	26.807	37.651	29.933	31.464
		CGY	**88.358**	**58.768**	**63.956**	**70.361**	44.062	31.252	25.084	33.466
		M600	**74.379**	**53.248**	31.006	**52.878**	15.989	29.913	19.937	21.946
		MA	**69.621**	**58.242**	18.208	48.690	15.913	31.056	22.958	23.309
		M607	**60.269**	**58.242**	7.919	42.144	-2.638	10.129	-52.611	-15.040
PMIC_2H2C.2	100% *Nocardiopsis alba* DSM 43377	M607 1:10	**100.448**	**72.205**	**102.245**	**91.633**	21.893	36.900	20.608	26.467
		CGY	**79.723**	**56.041**	**68.552**	**68.105**	36.749	45.976	40.489	41.071
		M600	**72.945**	**61.462**	29.627	**54.678**	37.701	42.287	40.787	40.258
		MA	**63.332**	**51.737**	17.147	44.072	25.588	37.520	31.947	31.685
		M607	**60.008**	**51.737**	17.005	42.917	20.255	45.846	26.464	30.855
PMIC_1E10C	99.69% *Rhodococcus coprophilus* NBRC 100603	M607 1:10	**55.739**	23.975	13.753	31.156	17.741	35.007	18.818	23.855
		CGY	**54.110**	39.548	5.586	33.081	15.265	33.113	14.677	21.019
		M600	**50.916**	**74.111**	18.490	47.839	10.389	16.854	-4.345	7.633

		MA	**52.187**	**59.885**	15.980	42.684	13.932	27.236	16.729	19.299
		M607	**56.782**	**59.885**	18.101	44.923	17.779	33.374	23.219	24.791
PMIC_2C12	99.69% *Streptomyces albidoflavus* DSM 40455	M607 1:10	26.672	22.661	16.652	21.995	15.798	27.138	16.542	19.826
		CGY	-6.534	17.142	-10.748	-0.047	-27.664	5.787	-24.002	-15.293
		M600	20.481	**57.848**	14.672	31.000	-6.866	13.198	-33.999	-9.222
		MA	27.031	**60.575**	9.051	32.219	-5.000	-4.954	-1.250	-3.734
		M607	38.892	**60.575**	6.753	35.407	-45.834	14.047	-12.104	-14.630
PMIC_1F6A.3	99.76% *Nocardiopsis alba* DSM 43377	M607 1:10	10.802	4.493	28.779	14.691	-44.920	37.651	-29.373	-12.214
		CGY	10.933	8.731	28.142	15.935	-25.683	23.351	-7.255	-3.196
		M600	9.988	11.984	24.359	15.444	-29.911	37.259	-57.796	-16.816
		MA	11.193	32.320	34.824	26.113	-6.218	35.757	-1.175	9.455
		M607	11.585	32.320	20.364	21.423	-37.758	-2.506	-0.205	-13.490
PMIC_1I1A	100% *Streptomyces hydrogenans* NBRC 13475	M607 1:10	**75.063**	**63.598**	22.379	**53.680**	19.341	**98.996**	**101.250**	**73.196**
		CGY	**72.130**	**66.587**	17.324	**52.014**	15.341	23.645	12.104	17.030
		M600	**72.391**	**57.651**	19.056	49.699	15.189	20.380	7.292	14.287
		MA	**65.776**	49.273	19.728	44.926	29.702	19.564	8.038	19.101
		M607	**67.373**	44.641	19.975	43.996	23.531	21.523	-0.727	14.776
PMIC_1D9B	99.75% *Streptomyces griseoflavus* LMG 19344	M607 1:10	**76.106**	**75.589**	20.011	**57.235**	17.513	29.718	36.199	27.810
		CGY	**99.340**	**99.310**	**96.199**	**98.283**	**90.414**	**90.772**	**91.985**	**91.057**
		M600	**75.780**	**69.150**	17.571	**54.167**	15.379	31.448	34.931	27.253
		MA	**75.552**	**61.462**	-7.495	43.173	4.295	3.861	6.621	4.925
		M607	**79.984**	**65.602**	17.324	**54.303**	36.292	24.527	42.018	34.279
PMIC_2A11B.1	100% *Nocardiopsis alba* DSM 43377	M607 1:10	**61.475**	47.598	13.647	40.906	0.409	32.003	25.196	19.203
		CGY	**63.397**	**53.676**	17.854	44.976	28.369	47.902	33.215	36.495
		M600	**61.442**	**57.585**	18.172	45.733	11.037	29.456	17.251	19.248
		MA	**61.181**	48.715	15.238	41.711	19.074	36.900	-5.987	16.663
		M607	**63.560**	49.010	16.864	43.145	25.093	32.427	29.336	28.952
PMIC_1A8B		M607 1:10	**59.943**	**50.390**	14.955	41.763	**65.394**	**77.155**	**89.053**	**77.200**

	99.43% *Streptomyces flavoviridis* NBRC 12772	CGY	**62.713**	**60.838**	16.546	46.699	35.073	46.890	39.034	40.332
		M600	**55.739**	**50.686**	14.955	40.460	**100.092**	**100.237**	**100.466**	**100.265**
		MA	**56.130**	**54.037**	17.112	42.426	28.483	45.062	37.915	37.153
		M607	**56.815**	48.057	12.515	39.129	**100.248**	**100.269**	**100.205**	**100.241**
PMIC_1E11B.2	99.59% *Arenibacter aquaticus* GUO666	M607 1:10	39.544	**59.786**	17.147	38.826	11.228	14.341	14.081	13.216
		CGY	28.790	24.830	-22.980	10.213	-17.722	8.529	-19.899	-9.697
		M600	12.106	29.856	-25.066	5.632	-23.093	-6.946	-31.835	-20.625
		MA	31.756	13.823	-26.693	6.295	-12.504	11.533	-20.310	-7.093
		M607	33.906	38.957	-5.515	22.449	2.009	7.484	-14.640	-1.715
PMIC_1E12B	100% *Arthrobacter gandavensis* R 5812	M607 1:10	44.106	38.004	-8.344	24.589	-33.454	4.938	8.933	-6.528
		CGY	42.346	42.045	5.197	29.863	-17.227	-9.656	-4.308	-10.397
		M600	13.116	31.893	3.889	16.299	4.752	17.605	-11.358	3.667
		MA	43.226	46.480	-0.177	29.843	-0.467	3.534	5.427	2.832
		M607	33.222	30.119	-11.278	17.354	-14.713	10.292	-24.897	-9.773
PMIC_1A11B.2	100% *Nocardiopsis alba* DSM 43377	M607 1:10	9.629	29.758	31.536	23.641	-46.938	35.464	-18.706	-10.060
		CGY	42.346	42.045	5.197	29.863	-17.227	-9.656	-4.308	-10.397
		M600	13.116	31.893	3.889	16.299	4.752	17.605	-11.358	3.667
		MA	43.226	46.480	-0.177	29.843	-0.467	3.534	5.427	2.832
		M607	33.222	30.119	-11.278	17.354	-14.713	10.292	-24.897	-9.773
UC8	*Roseimaritima ulvae* UC8	M607 1:10	**59.715**	46.185	19.940	41.947	16.141	41.210	32.693	30.015
		M607	**53.230**	16.386	9.652	26.423	31.607	-11.876	31.388	17.039
		M600	28.601	17.953	7.542	18.032	15.212	-20.877	26.082	6.806
Culture medium	N/A	M607 1:10	43.291	0.090	-16.298	9.028	34.082	-7.468	17.549	14.721
		CGY	34.167	-7.696	-26.975	-0.168	14.465	-12.202	15.386	5.883
		M600	43.552	15.072	-12.339	15.428	12.104	-1.951	20.421	10.191
		MA	33.580	2.522	-7.955	9.382	18.008	-12.496	11.022	5.511
		M607	37.328	2.522	-4.737	11.704	22.312	6.374	7.367	12.018