

# **BIOPROSPECTING OF SOUTHERN OCEAN BACTERIA FOR BIOMEDICAL AND NON-MEDICAL APPLICATION**

Submitted in partial fulfilment of the requirements for the award of Master  
of Technology degree in Biotechnology

by

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### **BONAFIDE CERTIFICATE**

This is to certify that this Project Report is the bonafide work of HELEENA (41810007), who carried out the project entitled "**BIOPROSPECTING OF SOUTHERN OCEAN BACTERIA FOR BIOMEDICAL AND NON-MEDICAL APPLICATIONS**" under my supervision from January 2023 to May 2023.

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## DECLARATION

I HELEENA hereby declare that the Project Report entitled “**Bioprospecting of Southern Ocean Bacteria for Biomedical and Non-medical Application**” done by me under the guidance of **Dr.M. Radhakrishnan** (External) and **Dr.S. Usha Nandhini** (Internal) in the centre of Drug Discovery and Development at Sathyabama Institute of Science and Technology, Chennai is submitted in partial fulfilment of the requirement for the award of Master of Technology degree in Biotechnology.

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## ABSTRACT

Actinobacteria is one of the largest phyla in the Bacterial domain, and it can be found in both aquatic and terrestrial habitats. They effectively produce novel secondary metabolites with a variety of biological effects, such as antibacterial and anticancer properties, and it is also necessary from an industrial aspect for heavy metal degradation and siderophore production. The present work aimed to study the bacteria isolated from the Indian Sector of the Southern Ocean. For this study, about 25 bacterial cultures previously isolated from the Southern Ocean sector were obtained from the Actinobacterial Research lab, Centre for Drug Discovery and Development Department, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu. All the cultures were screened for siderophore production which revealed that the strain PP10 showed about 80% and 69 % production of siderophore in both psychrophilic and mesophilic conditions. For the chromium degradation study, 25 bacterial strains were spotted on chromium-incorporated ISP 2 and AIA media at various concentrations revealing that 19 strains could tolerate up to 1000 PPM. Among that PP68 strain was able to degrade the potassium dichromate in mesophilic conditions for about 63% at 1000 PPM. Based on their phenotypic and molecular characteristics, the potential strain PP10 and PP68 were identified as *Pseudonocardia*.

Keywords: Siderophore, Chromium, Southern Ocean, Potassium dichromate, *Pseudonocardia*.

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## CHAPTER-1

### INTRODUCTION

The marine ecosystem is a less well-known ecosystem in terms of microbial diversity and ecology. Between 106 and 108 bacterial species are expected to exist in the marine environment, which has salinity of 3.5% and a wide range of pressure and temperature (Murray and Grzyski, 2007). Pressure and temperature are just two of the extreme climatic conditions that marine life must endure. According to (Rasmussen and Morrissey 2007), (Lordan *et al.*, 2011), and (Gupta *et al.*, 2014), a variety of secondary metabolites that are bioactive are produced under such extreme conditions, which include Pigments, sterols, antioxidants, proteins, polysaccharides, polyunsaturated fats (PUFAS). The productivity and metabolism of bacteria are influenced by a number of variables, including hydrostatic pressure, salinity, temperature, dissolved oxygen, etc (Yoshida *et al.*, 2007). Endim-city theory states that some bacteria need particular environments (hot springs, cryospheres, and hyperhalophilic habitats) in order to produce secondary metabolites. Between the months of December and March, the marine environment undergoes a significant shift that ultimately has an impact on organism productivity (Campbell & Claridge, 1987).

The Southern Ocean or the Antarctic Circumpolar Current divide the Antarctic from other continents (Lewis Smith, 1991). Seawater is consistently at -1.8°C across the area of the Antarctic. Polar marine ecosystems see extreme seasonal fluctuations in sea-ice coverage, light levels, and day duration, in contrast to the deep ocean. It is a rocky landmass with areas of arid desert soils and subfreezing temperatures that is almost entirely covered with an ice sheet. Only 2% of Antarctica's continental interior is ice-free (Bratchkova, *et al.*, 2011). The enormous ice sheet reflects between 40 and 90 percent of incident solar energy, which results in an accumulation of cold, dense air on the northern plateau that maintains the freezing arctic temperatures. The ice mostly on soils is prone to thawing over the summer (Wynn-William, *et al.*, 1990) As a result, Antarctica has the reputation of being the world's freezing, driest, windiest, and also most inhospitable continent (Bratchkova *et al.*, 2011).

At the South Pole, wintertime lows of 80 to 90 degrees Celsius and summertime highs of 5 to 15 degrees Celsius are common. East Antarctica is colder than the west due to its higher elevation (Lewis smith,1991). Penguin, seal, worms, tardigrades, mites, tundra flora, several forms of algae, and other microbes are just a few of the cold-adapted creatures that can thrive in Antarctica. Despite the fact that the Antarctic region has traditionally been thought of as a natural, undeveloped environment, persistent pollutants, such as heavy metals, antibiotics, and pesticides, have been reported. These pollutants may have transported through natural phenomena by water and air currents, as well as through improper waste disposal practises and/or associated with the operation from research facilities (Corsolini, *et al.*,2009).

Actinobacteria, which are significant suppliers towards the pharmaceutical industry and the prolific manufacturers of antibiotics, can produce a wide range of secondary metabolites (Baltz 2005). The ability of Actinomycetaceae actinobacteria to create secondary metabolites, many of which are active towards pathogenic microbes, is well documented. earliest discovery of mycelium-forming actinobacterium from maritime sediments came some decades ago (Weyland 1969), these bacteria have typically being isolated from terrestrial sources. Marine actinobacteria are a source of new antibiotics and anticancer drugs with peculiar structures and characteristics, although this source has only lately come to light (Jensen *et al.*, 2005). The best sources for secondary metabolites are marine actinobacteria, and the vast majority of these substances come from species of the single genus *Streptomyces*, which is widely distributed in both aquatic and terrestrial habitats and is of commercial interest because of its special ability to produce novel metabolites (Pathom-Aree *et al.*, 2006a).

Microbial secondary metabolites have drawn a lot of attention due to their beneficial biological functions, particularly in relation to the positive effects on human health. Industrial biotechnology and metabolic engineering can be used to biosynthesize these secondary metabolites, which has many advantages over traditional techniques of extraction from biomass. Marine bacteria are among the microorganisms that produce distinctive and innovative secondary metabolites and exhibit intriguing biological behaviours. Actinomycetes, a group of marine bacteria, also play a significant role mostly in pharmaceutical and medical sectors due to their

ability to create secondary metabolites with a variety of chemical and biological properties. Many of the thousands of bioactive chemicals that have been identified and described have been turned into medicines to treat a variety of ailments in the human, animal, and agricultural sectors (Castillo, *et al.*, 2002., Singh, *et al.*, 2003., Shatoury *et al.*, 2009). Heavy metal contamination is one of the most important environmental issues facing the world today (Ali *et al.*, 2019). Heavy metals have spread globally in silt, soil, garbage, and wastewater as a result of the extensive use of these substances in different operations. Although agricultural, municipal landfills, and sewage disposal operations also contributed to the pollution, industrial activity was the primary source of toxic metal pollution in the environment (Ali *et al.*, 2019., Fernandez., *et al.*, 2014). Actinobacteria are among the environmental microbes that have been widely recognised to be potential bioremediation agents (Alvarez, *et al.*, 2017 & Aparicio, *et al.*, 2017).

The industries of electroplating, textile dyeing, and metal processing all utilise chromium. Chromium is present in industrial effluents and wastewater, which can be toxic to microbial consortia used in wastewater treatment systems (Stasinakis *et al.*, 2003). The buildup of potentially harmful heavy metals as Hg, Cd, Cr, Cu, and Zn in the body results in mental anomalies, neuromuscular disorders, cancer, and aberrant growth (Wuana and Okieimen, 2011). Industrial effluents need to be treated before being released into the environment to lessen the toxicity of chromium. Numerous conventional techniques, including chemical (precipitation, oxidation as well as reduction, ion exchange), physical (filtration and membrane technologies), and biological (membrane technologies), have been used to remove metals through industrial effluents (Ahluwalia and Goyal, 2007). In the soil and water that receive industrial effluents, there are a lot of naturally occurring microorganisms. To defend themselves against the effects of heavy metals, these bacteria have developed a variety of defence mechanisms by reducing the number of different soil microorganisms, Cr (VI) build up in agricultural lands lowers soil productivity (Karthik *et al.*, 2017; Wani *et al.*, 2018).

Microorganisms make siderophores, which are organic molecules with low molecular weights, in order to survive in such an iron-depleted environment (Ahmed and Holmstrom 2014). The primary purpose of siderophores, which are metal-chelating agents, is to draw insoluble ferric iron from various habitats (sideros, which means iron, and phores, which means carrier) (Nagoba and Vedpathak 2011). Although siderophores' primary function is to chelate Fe III, it may also have a significant impact on samples that have been contaminated with heavy metals by binding to a variety of hazardous metals, including Cr 3+, Cu2+, Eu3+, Al 3 + and Pb2+. (Rajkumar *et al.*, 2010, Brien *et al.*, 2014; Nair *et al.*, 2007). siderophores may prove to be a valuable environmentally friendly heavy metal remediation agent (Rajkumar *et al.*, 2010).In this paper the important roles and Biotechnology application of Marine Actiniobacteria in different sectores including Industries , medicine and agriculture and solving environmental issues such as Heavy metals degradations.

## CHAPTER-2

### LITERATURE REVIEW

#### 2.1. ACTINOBACTERIA

One of the largest phyla in the Bacteria domain, actinobacteria are present in both terrestrial and aquatic habitats. Gram-positive bacteria known as actinobacteria have DNA that contains more than 50% of cytosine and guanine (G + C). Actinobacteria are typically thought of as bacteria with filaments due to their capacity to produce aerial and substrate mycelium (Azman, *et al.*, 2015). Actinobacteria have been divided into 374 genera as of 2016 (Subramani, *et al.*, 2019). *Streptomyces*, the leading genus in the group, and other actinomycetes, including *Kitasatospora*, *Micromonospora*, *Nocardia*, *Micrococcus*, *Arthrobacter*, and *Rhodococcus*, are examples of actinobacteria (Amin *et al.*, 2020). *Streptomyces* includes a wide range of species with a wide variety of growth conditions (Shepherd *et al.*, 2010). Actinobacteria are currently recognised as part of the phylum Bacteria, one of the 18 major lineages, and are widely spread in both terrestrial and aquatic habitats (Ventura *et al.*, 2007). They live in areas with dirt or water. The class most well-known class of microorganisms with capacity to create bioactive substances is the actinobacteria. Actinobacteria have so attracted a lot of attention for a variety of uses Pharmaceutical, Agriculture, Biotechnology, food and enzyme sector (Mohanmadipanah *et al.*, 2016).

Additionally, there is interest in using actinobacteria to help with pollutant clean up. As they can metabolise the toxins for their own growth, actinobacteria offer a reasonable and secure biological technique for removing contaminants. Actinobacteria may manufacture a wide range of secondary metabolites, making them essential suppliers towards the pharmaceutical sector and the most prolific producers of antibiotics (Chaturvedi, *et al.*, 2019). In the recycling of carbon (Abbas, *et al.*, 1989). *Streptomyces* manufacture a wide variety of metal ions chelators, like siderophores, to offer defence against the harmful effects of heavy metals or particular metal absorption for specialised metabolic activities in order to thrive in a metal-contaminated environment (Locatelli *et al.*, 2016). They are of interest for taking heavy metals out of the environment along with siderophores.

### **2.1.2 Terrestrial Environment**

They are found both on the soil surface and at a depth of 2 m below ground and have been reported to be isolated from various locations, such as sandy soil, sandy loam soil, black alkaline soil, subtropical dessert soil, and alkaline dessert soil, where *Streptomyces* spp. was the most dominant actinobacteria followed by the other organisms, such as *Nocardia*, *Actinomycetes*. The ideal temperature range for actinobacteria, which are primarily mesophiles, is between 25 and 30°C. There have been a number of thermophilic as well as psychrophilic actinobacteria described, though. In cold region, icy ocean and ice-covered locations and lakes in the Antarctic, psychrophiles can thrive at temperatures between 0 and 20°C, with a temperature less than 10°C exhibiting optimal growth.

### **2.1.3 Aquatic Environment**

some actinobacteria are native to marine environments, others are wash-in elements that merely coexist there where they can survive for extended periods of time in spores or dormant propagules. Marine actinobacteria are capable of adapting to a wide range of environmental circumstances, from temperature just below 0°C at extremely high pressure as well as anaerobic conditions in the deep sea floor to temperatures of over 100°C at highly acidic conditions close to hydrothermal vents on the midocean ridges. The deep sea surface, marine animals, seashore soil, deep sea sediments, or marine snow are all examples of marine ecosystems. *Rhodococcus marinonascens*, the first marine actinobacteria species to be identified, provides evidence for their existence (*Helmke and Weyland, 1984*).

## **2.2. MARINE ACTINOBACTERIA**

Marine microorganisms are becoming more and more utilized as a source when looking for compounds with economic significance. Due to the discovery of novel and physiologically active compounds in aquatic life (*Jensen and William 1994; Imada 2004; Zhang et al., 2005*), the number of marine microorganisms is increasing. According to *Goodfellow and Williams (1983)*, actinomycetes can be found in a variety of ecological settings, including soil, fresh water, salt water, lakes, compost, sewage, and the marine environment. They are regarded as being

extremely helpful since they produce a variety of antibiotics and other therapeutically beneficial chemicals with a variety of biological functions. There are many different microorganisms in the marine environment that are not found in the terrestrial environment. Although there are some studies on the synthesis of antibiotics and enzymes by marine actinomycetes, the marine ecosystem is still an opportunity for new actinomycetes, which can produce novel bioactive chemicals and enzymes that are crucial for industry (Sharma and Pant 2001). Actinobacteria-related research that spanned nearly 80 years has ultimately led to the development of various essential medicines that continue to save many lives worldwide. However, the development of (antibiotic) resistance frequently renders the existing medications ineffective. It is clear that we need new medications (Jackson *et al.*, 2018). Since the late 1980s, there have been less and fewer new chemicals identified from terrestrial microbes. Whereas in marine actinobacteria they are unique tolerance for the bioremediation of heavy metals or organic pollutants due to their special resistance to these contaminants and their capacities for degradation, biostimulation, and bioaugmentation.

### 2.3. ACTINOBACTERIA SECONDARY METABOLITES

Over 500 species of *Streptomyces* are responsible for 70–80% of the secondary metabolites that are relevant and have a variety of biological activities, including antibacterial, antifungal, anticancer, antitumor, cytotoxic, anti-inflammatory, anti-parasitic, anti-malaria, antiviral, antioxidant, and anti-angiogenesis. To avoid the issue of resistant infections, which are no longer treatable with the medications currently in use, the discovery of novel secondary metabolites is crucial (Lam 2006; Ekwenye and Kazi 2007). These shrewd pathogenic microbes are causing an increasing number of fatalities. The manufacture of novel therapeutic medications that are effective against a variety of resistant germs may be based on secondary metabolites produced by marine actinobacteria (Fenical and Jensen 2006).

## 2.4 SIDEROPHORE

Greek words sideros, which translate to "iron," and phores, which means "carrier," are used to name siderophores (Nagoba and Vedpathak 2011). These 200–2000 kDa low-molecular-weight compounds. The primary goal is to chelate Fe (III) from various ecosystems so that it is available for plant and microbial cells (Schwyn *et al*; 1987). Although having a preference for iron, they can also combine with elements that are harm for the environment, including lead, cadmium, nickel, cobalt, and zinc. Siderophore-metal complexes are unable to enter bacterial cells, decreasing the number of free metals found in the environment. Microorganisms have the ability to produce a wide variety of organisms that can be categorised into the families of carboxylate, catecholate and hydroxamate based on the different functional groups (Schalk *et al*; 2011).

In recent years, siderophores have become more popular due to their prospective applications in a variety of environmental research sectors, bioremediation, as well as the chelate of heavy metals (Schalk *et al*; 2011). With siderophores, a wide range of natural metals can be mobilised or dissolved very effectively. Due to their strong affinity and selectivity for solid metal, they have become a useful tool in the cost-effective and environmentally beneficial process of bioremediation (other than iron). They play a critical role in removing metal from metal-polluted soils or industrial wastes (Schalk *et al*; 2011). It has been claimed that the production of certain siderophores is stimulated by the presence of harmful metals in the environment, indicating that these chelators may play a part in bacterial heavy metal resistance. Free heavy metal concentrations or their toxicity are decreased by binding heavy metal to the siderophore extracellularly (Lutkenhaus *et al.*,1977) Environmental, Agriculture, Food industry, medical, bioremediation (Boukhalfa *et al.*, 2003). The role of siderophores has been investigated by scientists for a number of biotechnology uses due to the pertinent impacts of iron at various levels within the tree of life.

## 2.5 TYPES OF SIDEROPHORE

### **2.5.1. Hydroxamate siderophore**

The most typical class of siderophores to be found in nature is the hydroxamate group. Microorganisms like bacteria and fungus are responsible for producing these siderophores (Hofte 1993; Winkelmann and Drechsel 1997). The iron forms a bidentate ligand with two oxygen molecules from each hydroxamate group. In order to create a hexadentate octahedral complex with  $\text{Fe}^{3+}$ , each siderophore is therefore able to do so. The binding constants between the hydroxamates and ferric iron range from  $10^{22}$  to  $10^{32} \text{ M}^{-1}$  (Winkelmann 2007). The complexes are shielded from environmental hydrolysis and enzymatic breakdown by the tight interaction between ferric iron and siderophore (Winkelmann 2007).

### **2.5.2. Catecholate (Phenolates) Siderophore**

According to (Dave *et al.*, 2006), certain bacteria primarily create siderophores of the catecholate. To create a hexadentate octahedral complex, iron is chelated with two oxygen atoms from each catecholate group. The Neilands spectrophotometric assay (Neilands 1981), in which the catecholate type of siderophore combines with  $\text{FeCl}_3$  and forms a wine-colored complex with the maximum absorbance at 495 nm, is one of the most widely used assays for catecholate identification (Neilands 1981).

### **2.5.3. Carboxylate (Complexones) Siderophore**

The majority of bacteria including *Rhizobium* and *Staphylococcus* as well as fungus like *Mucorales* create carboxylate-type siderophores. According to (Dave and Dube 2000), the hydroxyl and carboxyl groups on this kind of siderophore bond to iron. An analysis using spectrophotometry can identify carboxylate siderophores in This results in the formation of the siderophore copper complex, which is between 190 to 280 nm, scanned for maximum absorption.

## 2.6. SIDEROPHORE PRODUCED BY ACTINOBACTERIA

The ability of members of the streptomyces to create several siderophores that are individually regulated and function in concert to compete more effectively in the

environment is well known (Challis and Hopwood 2003). Actinobacteria are among the most abundant producers of siderophores (Bhatti *et al.*, 2017). According to (Nakouti *et al.*, 2012), a CAS plate assay on actinobacteria shows that about 44% of them are positive in fig 3. All siderophores are chelating substances that have stability constants of roughly  $10^{30} \text{ M}^{-1}$  and a very strong affinity for  $\text{Fe}^{3+}$ . Solubilizing extracellular iron from minerals so that it is accessible to bacteria is one of the key functions of siderophores. A family of at least 500 distinct molecules called siderophores has a variety of chemical configurations (Schalk *et al.*, 2011). Additionally, siderophores are being investigated for the development of antibiotics against bacterial strains that are multi-drug resistant. Plant growth-promoting (PGP) substances such as siderophores as well as indole acetic acid are also extraordinarily produced by actinobacteria (Anwar *et al.*; 2016 & Anilkumar *et al.*, 2017).

Many strains of actinobacteria (either as a single strain or as consortia) or their related chemicals have been used as biopesticides, biofertilizer and biological control agents in agriculture, making them a significant contributor to the sector (Law *et al.*; 2017 & Zamoum *et al.*; 2015). Actinobacterial management of plant diseases and pests that impair agricultural crops is efficient, economical, and environmentally benign; as a result, they can replace and reduce the usage of hazardous chemical fertilisers and pesticides. Around 80% of antibiotics that are used in medicine come from actinobacteria, many of which are isolated from genus *Streptomyces* (Lee *et al.*; 2014 & Hopwood *et al.*; 2007). Moreover, this genus produces 17% of all known active secondary metabolites, several of which have significant medical use (Tan *et al.*; 2017 & Lee *et al.*; 2020). As a result, it is now less likely to isolate a brand-new strain of *Streptomyces* from terrestrial settings.

## 2.7. ROLE OF SIDEROPHORE IN MARINE ENVIRONMENT

According to (Rue and Bruland 1995) & (Wu and Luther 1995), 99 percent of the dissolved iron in open ocean surface waters is bonded to organic ligands. It has long been hypothesised that either one or all of such ligands may constitute siderophores. The conditional binding strengths of many isolated ocean siderophores, which are often created in response to iron stress, are comparable to those of natural iron ligands. Throughout the ocean, the quantities of dissolved iron

& ligand are positively associated, with ligand concentrations in iron-limited areas being either lower or more similar to those in iron-rich regions (Buck and Bruland 2007). where iron-rich shelf waters and oceanic waters with low iron content mingled. Although these findings unmistakably point to a biological origin for the ligands produced, the distribution seen does not seem to be in line with what would be predicted for siderophores. The natural iron-binding ligands probably come from many places. According to (Laglera & vanden Berg 2009), dissolved organic material is a complex mixture of new and chemically altered molecules, some of which may accidentally bind iron. This notion is supported by the discovery that numerous trace metals are complexed to biological materials across the ocean. By releasing organic exudates with iron-binding properties, phytoplankton and other organisms may potentially add to the pool of ligands.

As photosynthetic rates increase after iron limitation is lifted, such ligands could explain observed increases in ligand concentrations. Since marine heterotrophic bacteria synthesise siderophores, it is likely that the more hydrophilic those contribute to the natural iron ligand pool. The first study to convincingly document the discovery and concentration of particular siderophores in the seawater is a recent one by (Mawji *et al.*, 2008). The North and South Atlantic both included ferrioxamines and amphibactins, two kinds of siderophores known to be generated by marine bacteria. were discovered binding up to 5% of the total amount of dissolved iron in surface waters at concentrations of up to 10 pM apiece. These siderophores were shown to be associated with the quantity of heterotrophic bacteria, suggesting that these organisms are producing locally. Siderophores, organic exudates, or humic substances likely make up the complex mixture of iron binding ligands that work together to stabilise dissolved iron in saltwater.

## 2.8. MICROBIAL SIDEROPHORE

The siderophores produced by microorganisms are diverse. Catecholates make up the majority of bacterial siderophores, while hydroxamates and carboxylates are also present. Different siderophore-mediated Fe transport mechanisms are used by microorganisms. The transport methods used by gram-positive and gram-negative bacteria are different. The complex crosses the membrane through an energy-

dependent system made up of the outer membrane receptors proteins, periplasmic binding proteins, and inner membrane transport proteins once the Fe (III)-siderophore attaches to the outer membrane receptor (Matzanke, 1991). After that, a high-affinity periplasmic bind protein that was attached to the Fe (III)-siderophore complex when it was transported to the cytoplasmic membrane releases it into the space around the cytoplasm (Noinaj et al., 2010). A subsequent ATP-binding cassette (ABC) transfer pathway allows the complex to penetrate the cytoplasmic membrane and enter the cytoplasm. At some point, Fe (III) is reduced, releasing Fe (II) from the siderophore complex. Contrarily, the membrane's outer receptors are entirely lacking in gram-positive bacteria (such as *Bacillus* sp), which have the outer membrane. Because there is no periplasmic gap, periplasmic siderophore protein binders that are linked to the cell membrane are responsible for binding the Fe(III)-siderophore complexes (Fukushima *et al.*, 2013). Similar to gram-negative bacteria, ATP-dependent transporters then carry the Fe(III)-siderophore complexes to the cytoplasm in accordance with the ABC transport systems.

## 2.9 CHROMIUM

The increase in concentration of various heavy metals over the past few decades has had a disastrous impact on public health and environmental degradation. Because there are more heavy metals being used in industries which mainly fall under Textile dyeing, Tanning, painting, leather (Stasinakis et al., 2003). people are being exposed to more metals. These industries' discharged effluents have a significant impact on both the environment and human health (Raskin and Ensley, 2000). Hg, Cd, Cr, Cu, and Zn (Wuana and Okieimen, 2011). The electroplating, tanning, textile applying dye, and metal processing industries all require chromium. Chromium is present in industrial wastewater and effluents, and it has harmful effects on the microbial consortia used in wastewater treatment system. (Stasinakis *et al.*, 2003). Industrial wastewater must be cleaned before release in order to decrease the harmful effects of chromium on the environment. Chemical (precipitation, oxidation as well as reduction, ion exchange), physical (filter and membrane technologies), and other conventional methods have all been used to remove metals from industrial waste water (Ahluwalia and Goyal, 2007).

## 2.10. HEAVY METALS IN WASTEWATER

Toxic metal ions are present in significant quantities in the effluents from small- and large-scale metal extraction and refining operations as well as the textile, leather, tannery, plating, galvanizing, pigment and dye, metallurgical, and paint industries. Mercury, lead, cadmium, and chromium are the four most hazardous metals. (VI). Heavy metals' effects on people are frequently poorly understood. Environmental metal ions bioaccumulate and become biomagnified as they go up the food chain. Their harmful effects are therefore deeper in animals at greater trophic levels. The primary environmental sources of heavy metals are mine tailings and non-ferrous metals industry effluents (Moore and Ramamoorthy, 1984).

## 2.11. HARMFUL EFFECT OF CHROMIUM ON HUMAN

Human exposure to Cr (VI) can result in lung cancer, eczema, rashes, nasal or skin irritations, eardrum perforations, allergies, irritations, rashes, eczema, ulcerations, respiratory tract problems, and skin irritations (Focardi *et al.*, 2013). Exposure to Cr (VI) may result in oxidative protein alterations, DNA mutations, chromosomal damage, genotoxicity, and carcinogenicity of Cr (VI)-containing compounds, as well as all of the above. In addition to other respiratory issues like wheezing, coughing, nasal blockage, and facial erythema, can also result from inhaling a high level of hexavalent chromium (Engwa *et al.*, 2019). Eventually, exposure to chromium and its associated compounds, particularly those that include hexavalent ions, on a regular basis seriously increase the risk of dangers in human body which are Cancer growth, Neuromuscular disorder, growth abnormal, mental abnormalities. (Wuana and Okieimen, 2011).

## 2.12. CHROMIUM TREATMENT USING MICROORGANISMS

When treating metal concentrations in wastewater within the range of 100 mg/L, these methods are useless and extremely expensive. They work best when treating large concentrations. (Nourbakhsh *et al.*, 1994). In order to efficiently remove very

low concentrations of harmful heavy metals, existing technologies must be replaced by highly effective, less expensive, and environmentally acceptable alternatives. Waters and soil that receive industrial effluents inherently contain a huge number of microorganisms. To defend themselves against the toxic effects of heavy metals, these bacteria have adapted and developed a variety of defence mechanisms. (Adsorption, uptake, methylation, oxidation, and reduction). Cr (VI) buildup in agricultural regions lowers soil productivity by reducing the population of several soil microorganisms. (Karthik *et al.*, 2017; Wani *et al.*, 2018).

#### **2.12.1 Removal of chromium**

Hexavalent Cr(VI) & trivalent Cr(III) are the two most prevalent oxidation states of chromium, an element that occurs naturally. Cr(III) is a crucial ingredient for healthy sugar and fat metabolism, whereas Cr(VI) is 1,000 times more poisonous, mobile, and soluble than Cr(III). Numerous bacteria, including streptomycetes, have been shown to be capable of reducing Cr(VI) (Laxman *et al.*, 2002)

### **2.13. NON-MEDICAL APPLICATIONS**

#### **2.13.1. Heavy metals**

Actinobacteria have been discovered to be possibly the second or third significant group in contaminated soils (Hemmat *et al.*, 2018) making them a prospective group that can be exploited for removing heavy metal pollution alongside the native microorganisms in soil have also been shown to be effective at eliminating heavy metals, particularly from samples of water (Halttunen *et al.*, 2007) have been found to be resistant to heavy metals, and some of them are also capable of removing heavy metals.

#### **2.13.2. Bioaugmentation**

The actinobacteria with the ability to resist or accept high concentrations of contaminants will have better adaptability as well as survival in these stressful environments, making them acceptable to be used in heavy metal biological remediation. Despite the fact that actinobacteria that are resistant to heavy metals

have been found in numerous investigations, some of them even have the ability to remove heavy metals. There hasn't been much research done on the possibilities of using actinobacteria that are resistant to heavy metals in bioaugmentation methods.

#### **2.13.3. Chromium**

The harmful effects and health impacts of chromium (Cr), the main heavy metal from tanneries, among other heavy metals produced by various industrial processes, caught the curiosity of researchers. The leather tanning industry's one of its main Polluted environments, including wastewater, have heavy metals like chromium removed. Microbial species can remove chromium in a safe and environmentally friendly way, contributing to sustainable development.

#### **2.13.4. Siderophore**

Siderophores have attracted more interest in recent years due to their prospective uses in a variety of environmental research fields, bioremediation, and the chelation of heavy metals. They have also developed into an important instrument in the economical and environmentally benign process of bioremediation. Some siderophores are said to be produced more frequently when hazardous heavy metals are present in the environment, indicating that these chelators may contribute to bacterial resistance to heavy metals. Free heavy metal concentrations and their harmful effects are decreased through the extracellular binding of heavy metal to the siderophore.

## 2.14. MEDICAL APPLICATIONS

### **2.14.1. Antifungal activity**

Marine actinobacteria are advantageous biological agents for the synthesis of compounds that fight fungus (Okami and Hotta 1988) *Aspergillus niger* & *Candida albicans* were resistant to the antifungal effects of marine *Streptomyces* sp. DA11, which was discovered to be connected with the sponge *Craniella australiensis*.

### **2.14.2. Anticancer**

Marine actinobacteria are the source of many anticancer chemicals found in marine medications, and their metabolites are crucial for identifying medicinal molecules (Ravikumar *et al.*, 2012a). There are currently only a small number of studies that appear to be focused on discovering bioactive substances produced from marine actinobacteria that can be employed as anticancer drugs as well as agents against pathogenic organisms.

### **2.14.3. Iron overload Therapy**

Some siderophores may be used to treat disorders caused by an excess of iron. Periodic whole transfusion is necessary in the management of thalassemia and several other anemias, such as sickle cell anaemia (Hershko *et al.*, 2002). Humans lack particular physiological systems for iron elimination, hence repeated transfusion therapy causes an iron accumulation over time. By removing iron from the system, particularly the liver, the body should be able to reduce both this extra iron and the original iron overload. A medication based on siderophores can be used to treat illnesses linked to iron overload. Desferal is a medication used to treat thalassemia major and sickle cell anaemia (Propper *et al.*, 1977).

### **2.14.4. Antimalarial Activity**

several siderophores have antimalarial efficacy against *Plasmodium falciparum*. For instance, desferrioxamine B, a siderophore generated by *Streptomyces pilosus*, and the siderophore made by *Klebsiella pneumoniae* both exhibit

antimalarial action towards *Plasmodium falciparum* (Nagoba and Vedpathak 2011). Desferrioxamine B enters the parasite and causes iron to be depleted intracellularly. This substance exhibits a ten-fold increase in anti-*Plasmodium falciparum* in vitro activity when conjugated with methyl anthranilic acid, and this activity can be increased even more when nalidixic acid is used as a conjugate.

#### **2.14.5. Agriculture**

Many siderophore-producing bacteria may promote plant growth through a variety of processes, such as by giving plants iron, creating phytohormones, and producing organic acids which solubilize phosphate. It is difficult to define the precise function of siderophores in the development of plants because of the system's complexity. However, a number of researchers have looked into the interactions between siderophores and plants and/or microbes in the presence of a Fe deficiency. According to the results of these investigations, siderophore-producing bacteria or siderophores derived from bacteria can be used as biofertilizers to give plants iron directly (Fernandez *et al.*, 2005; Radzki *et al.*, 2013).

## **CHAPTER-3**

### **AIM AND SCOPE**

#### **Aim:**

To explore Southern Ocean Bacteria for Biomedical and Nonbiomedical Applications.

#### **Objectives:**

1. To retrieve and characterize bacterial cultures [n=25] previously isolated from the Indian Sector of Southern Ocean
2. In vitro evaluation of bacterial cultures for antibacterial activity against drug resistant bacterial pathogens
3. Production, partial purification and characterization of active molecules from potential bacterial culture
4. Evaluation of heavy metal remediation and siderophore production properties of Southern Ocean Bacteria

## CHAPTER-4

### MATERIAL AND METHODS

#### 4.1. CULTURE COLLECTION

Around 200 bacterial cultures were isolated from the Indian sector of Southern Ocean CDDD – SIST during the 11th ISESO in 2020. Around 25 cultures from this collection will be used for the proposed study.

##### **4.1.1. Media Preparation**

50% Actinomycetes Isolation Agar (AIA) was prepared and autoclaved. 25 bacterial cultures were streaked and incubated in both mesophilic (30°C) and psychrophilic conditions (4-10°C).

##### **4.1.2. Antibacterial activity**

Antimicrobial activity was tested against a set of 8 pathogens of *Escherichia coli*, *Candida albicans*, *Candida non-albicans*, *Enterococcus faecalis*, *Bacillus cereus*, *Aeromonas salmonella* and *Aeromonas caviae* and incubated in 37°C for 24 hours to observe the zone of clearance around the colony.

##### **4.1.3. Co-Culture Method**

*Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Candida non-albicans* were inoculated in MHB broth and incubated at 37°C for 2 days. After incubation cultures were centrifuged at 8000rpm for 10 minutes supernatant was discarded and pellets were collected. Four bacterial cultures were grown on MHB broth after collected cells were inoculated in freshly grown MHB broth and incubated at 37°C for 7 days. Then these strains tested for an antimicrobial activity for a set of pathogens

#### **4.1.4. Minimum Inhibitory Concentration Test**

The inoculum was prepared in accordance with the CSLI recommendation where the OD 600 value was adjusted to the equivalent of 108 CFU ml<sup>-1</sup>, which was determined from a calibration curve for each microorganism. Preparation of 96 well-plates for testing standard antibiotics Biosurfactants were dissolved in MHB at twice the concentration of the final test, with PH adjusted to 7.

##### **Preparation of Standard Inoculum**

200µl of the Test sample was added in Column 1. Columns 2-10 contain 100µl of MHB broth only. Column 11 contains 200µl of diluted standardised inoculum. Column 12 contains 200µl of the medium broth (as a monitor control for sterility). A multichannel pipette was used to mix the wells 1-10 resting in 100µl of test samples per well. 100µl of bacterial pathogens was added to all the wells and stored in an incubator for 24 hrs. at 37 c. Resazurin (0.0151) was added to all the wells and 30 µl per and further incubated for 2-4 hrs for the observation of colour change.

#### **4.1.5 Media Optimization**

Modified Isp2 was using different carbon sources for media optimization in this method peptone, d-glucose, and mannitol, were used as carbon sources. The freshly grown culture was inoculated in different carbon sources and incubated at 37°C for 24 hours after this will be tested for AMA for test pathogens.

## **4.2. SIDEROPHORE PRODUCTION**

Isp2 broth was Prepared 5 different cultures were inoculated and incubated at 37 °c for 24hrs. CAS dye was prepared and 10ml of CAS dye was added to 40ml of ISP2 broth then freshly prepared grown culture was inoculated in the dye. The dyeshould be stored in both Mesophilic at 37 ° c and Psychrophilic at 4 ° c condition. Every day we need to check the colour in the medium. That will indicate the presence of siderophore production.

#### **4.2.1. Detection of Siderophore Production in Micro Broth Dilution Assay**

In this assay 4 bacterial cultures were inoculated in 45ml Isp2 broth and 5ml of CAS dye was added inoculated in a Rotatory shaker for over 7 days and also control flask was set in without inoculum. After 24 hrs 100µl culture and control sample was added in 96 well plates the samples were read at 630nm by uv-Spectroscopy. This assay was continued for 10 days to detect the siderophore production.

#### **Formula**

Siderophore units (%SU) =  $Ar - AS \div Ar \times 100$

Ar = absorbance of reference (uninoculated sample SM+CAS reagent)

AS= absorbance of a sample (Supernatant of production medium +CAS reagent)

### **4.3. CHROMIUM RESISTANCE ASSAY**

Isp2 Agar with 50% seawater was prepared with different concentrations (1000ppm, 500ppm, 250ppm, 100ppm, and 50ppm). 25 bacterial cultures were streaked in all the concentrations. Incubated at 37 °c for 48 hrs and observed the results.

#### **4.3.1. Chromium Degradation in Micro Broth Dilution Assay**

To detect the potency of chromium degradation culture 50ml of Isp2 broth was prepared with different concentrations of (1000ppm, 500ppm, 250ppm, 100ppm, and 50ppm) of Potassium Dichromate ( $K_2Cr_2O_7$ ) and incubated in 37 °c for 2 hrs Diphenyl Sulfoxide, Sulphuric acid, and water mix was also added in Eppendorf and this was made up to 1ml Initial day reading was taken by using 96 well plates 100µl of the test sample and control samples were added in each well. The colour degradation also indicates chromium degradation.

#### **4.3.2. Chromium Degradation in Different Temperature**

Isp2 broth was prepared and transferred to a conical flask. Different concentration of PDC was added (0.025g, 0.0125g, 0.006g and 0.003g) and autoclaved at 121 ° c for 15 minutes. After autoclaving 5ml freshly prepared bacterial inoculum was added in 50 ml of broth. The culture is kept in a Rotatory shaker for culture growth at different temperatures Mesophilic at 37 ° c and Psychrophilic at 4 ° c Initial reading was taken using a DPC Mixture containing (1600µl, of DH<sub>2</sub>O, and 600µL of Sulphuric acid) then this mix has added the value of 150µl of DPC mix 830µl of DH<sub>2</sub>O and 20µl of superannuated of Different cultures. 100µl of mixed samples were added in 96 well plates and observed the reading at 540nm using uv -photo spectrometer.

#### **Formula**

$$(E_2 - F_2) \div (E_2) \times 100$$

E<sub>2</sub> = Positive Control

F<sub>2</sub> = Average Control

#### **4.4. MOLECULAR CHARACTERIZATION**

The Potential Actinobacteria, SOE-11-GC1-PP10 and SOE-11-PP68 were subjected to 16s rRNA sequencing. The 16s rRNA gene was amplified by PCR using Universal primers.

#### **4.5. PHYLOGENETIC ANALYSIS**

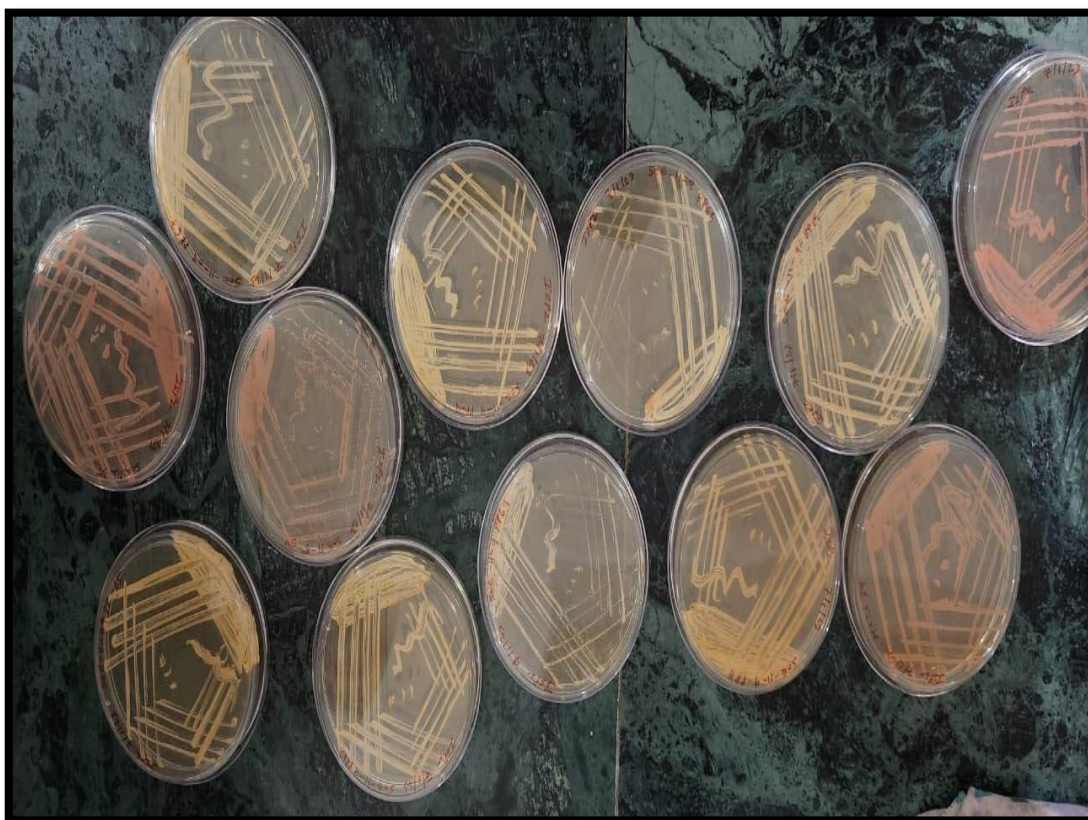
16s rRNA gene sequence of the isolates were compared with 16srRNA sequences available in the BLAST search in NCBI, Gene bank Database. The program Mega 11 was used for tree rendering (Dereeper et al.,2008) The 16s rRNA sequence of potential strains SOE-11-GC1-PP10 and SOE-11-PP68 were deposited in the GenBank database.

## CHAPTER-5

### RESULTS

#### 5.1. CULTURE PREPARED

50% seawater Actinomycetes Isolation Agar (AIA) and 50% Sea water International Streptomyces Project – 2 medium (ISP-2) was prepared and autoclaved. 25 bacterial cultures were streaked on AIA and Isp2 agar plate observed after the result in 2 days Fig 5.1.

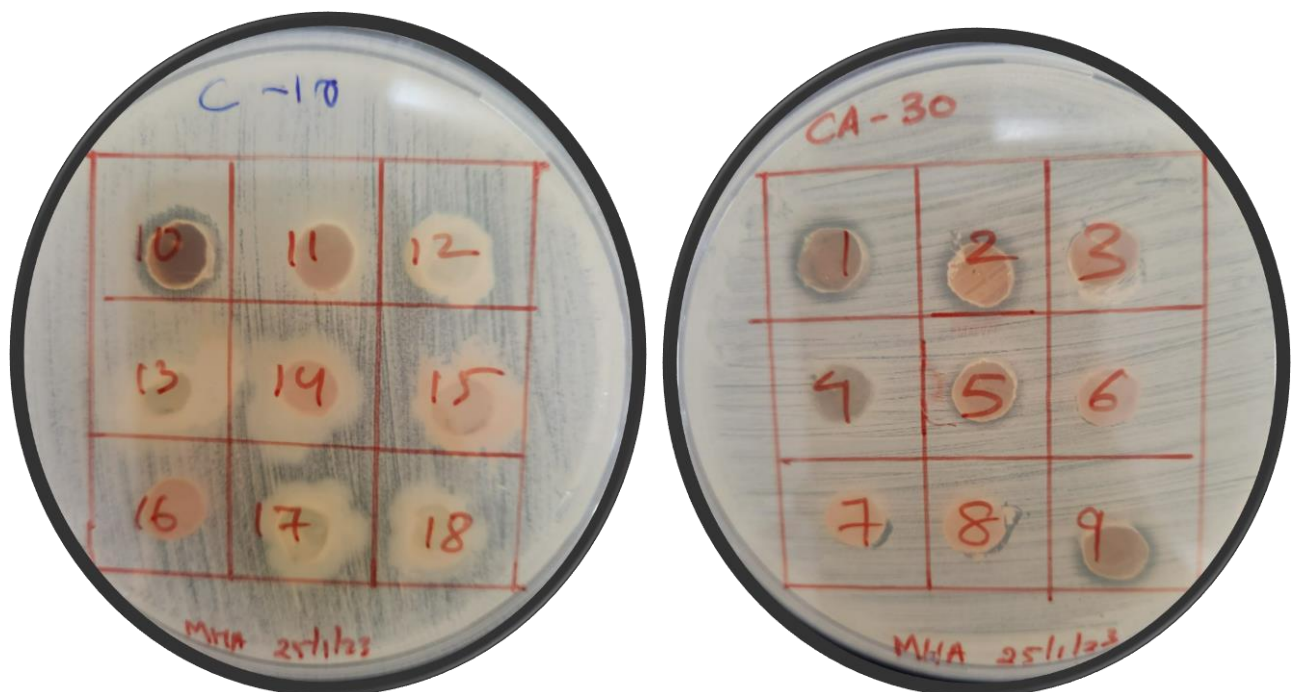


***Figure 5.1. AIA and Isp2 50% sea water Southern Ocean culture.***

#### 5.2. ANTIMICROBIAL ACTIVITY

The Bacterial culture for antibacterial activity against a drug-resistant bacterial pathogen. Eight different pathogens, including *Escherichia coli*, *Candida albicans*<sup>10</sup>, *Candida albicans* 30, *Enterococcus faecalis*, *Bacillus cereus*,

*Aeromonas salmonella*, and *Aeromonas caviae* were swabbed into MHA agar plates. applying the plug technique. After 1-day a Mild zone formed 4 well in C10 and C30. C10 and C30 both are fungal pathogens Fig 5.2



**Figure 5.2: Antibacterial activity of *Candida albicans*10 and *Candida albicans* 30 against the bacterial pathogen.**

### **5.2.1 Co-Culture Method**

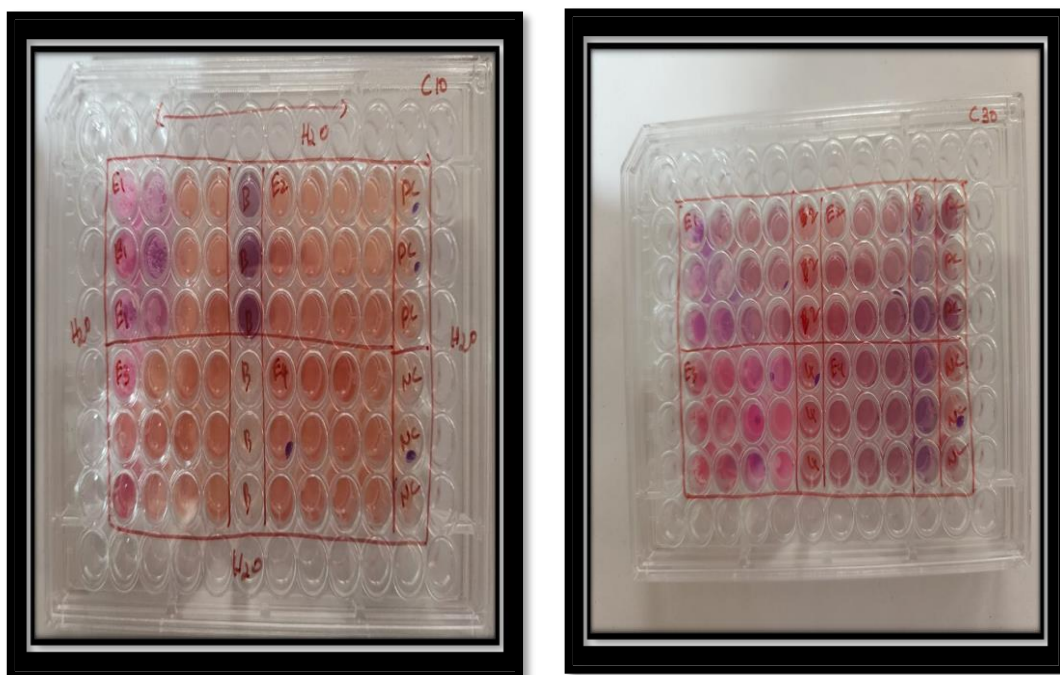
In which multiple bacterial strains are grown together to induce antibiotic production. Four cultures were used (PP68, PP69, PP65, and PP9). And pathogens (*S. aureus*, *E. coli*, C10, and C30) were swabbed in the MHA agar plate. The culture broth is added to each agar well. The mild zone formed for *E. coli* Pathogen in Fig 5.3.



**Figure 5.3. Antibacterial activity of *E. coli* against the bacterial pathogen**

### **5.2.2. Minimum Inhibitory Concentration Test (MIC)**

Four cultures broth pp65, pp68, pp69, and pp9 and two pathogen C10 and C30. The lowest concentration of an antimicrobial will inhibit the visible growth of a microorganism. Positive control is broth and negative control is broth, culture and antibiotic were added. The sample were read at 600nm for C10 pathogens the growth of microorganisms is high which means the activity is low in Table 5.1 whereas for the C30 pathogen, the growth rate is high in Table 5.2. The pink colour represents the growth of bacteria and the purple represents no growth of bacteria Fig 5.4



**Figure 5.4: Minimal inhibitory Concentration Assay for C10 and C30**

**Table 5.1. Pathogen C10 MIC Assay Reading by UV Spectroscopy**

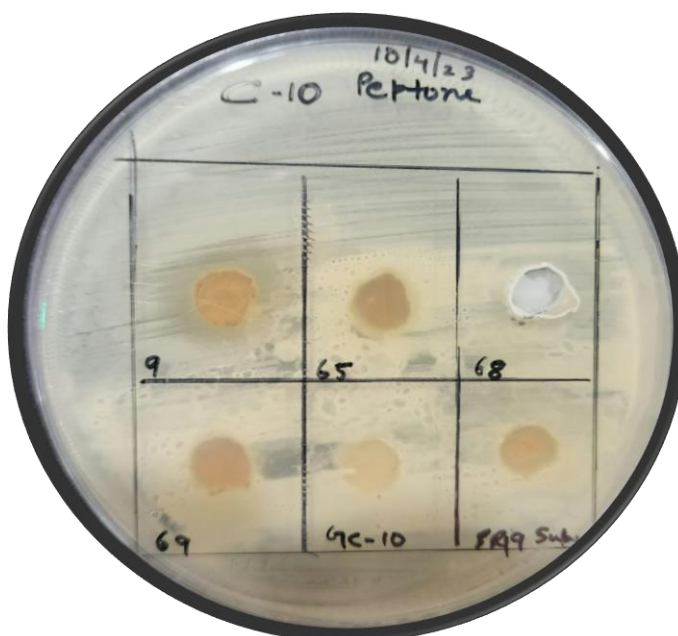
| Culture name     | Percentage |
|------------------|------------|
| PP69             | -34.6043   |
| PP9              | -13.7366   |
| PP68             | -15.8388   |
| PP65             | -12.1599   |
| Positive control | 0.808667   |
| Negative control | 0.556      |

**Table 5.2. Pathogen C30 MIC Assay Reading by UV Spectroscopy**

| Culture name | Percentage |
|--------------|------------|
| PP69         | -278.014   |
| PP9          | -178.055   |
| PP68         | -396.543   |
| PP65         | -211.937   |
| Positive     | 0.20733    |
| Negative     | 0.403333   |

### **5.2.3. Media Optimization**

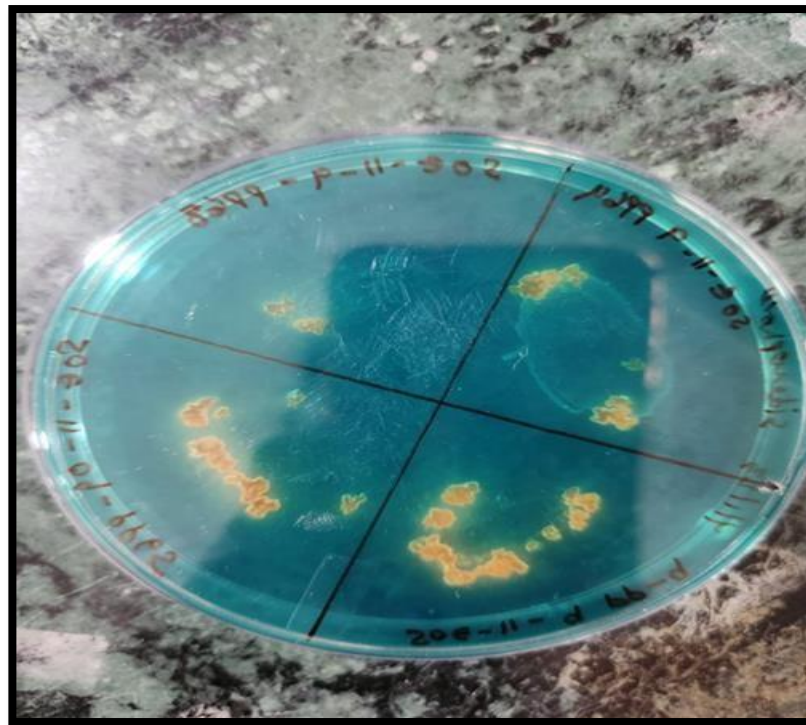
media optimization for carbon sources in a 24-well plate, four cultures of pp68, pp69, pp10, and pp9 are streaked on the media. Three pathogens are swabbed E. coli, Aeromonas salmonella, and C10 using the agar plug technique. 1 day later mild zone against the C10 pathogen in culture 9 formed in Fig 5.5



**Fig 5.5 Media optimization carbon source pathogen C10 against pp9 bacterial culture**

### 5.3. SIDEROPHORE

23 Southern Ocean bacterial cultures were streaked on CAS agar plates, after 7 days it was observed that siderophore colonies, which are orange and yellow in colour, were formed. It is confirmed that siderophore production on CAS agar plates in fig5.6



***Figure 5.6: Screening of siderophore-producing actinobacteria using chrome azurol s agar***

#### **5.3.1. Siderophore Mesophile Condition**

5 culture broth PP68, pp69, pp65, pp9 and pp10 are kept at mesophile temperature ranges between 68°F (20°C) and 113°F (45°C) the bacteria are growing at a moderate temperature. Three days later it was observed that the 5 cultures are colour change in Fig 5.7. Using micro broth assay the culture broth sample is read at 630nm by uv spectroscopy for 10 days in Table 5.3



Siderophore dye and Broth



Culture Broth



**Figure 5.7: Siderophore mesophile temperature colour changes after 3 days**

**Table 5.3. Siderophore Mesophile Condition reading**

| Culture       | Initial | Percentage |
|---------------|---------|------------|
| PP69          | 0.26    | 64         |
| PP9           | 0.299   | 77         |
| PP10          | 0.294   | 69         |
| PP68          | 0.28    | 60         |
| Pp65          | 0.302   | 81         |
| Broth control | 0.105   | 63         |

**Formula**

Siderophore units(%SU) =  $Ar - As \div Ar \times 100$

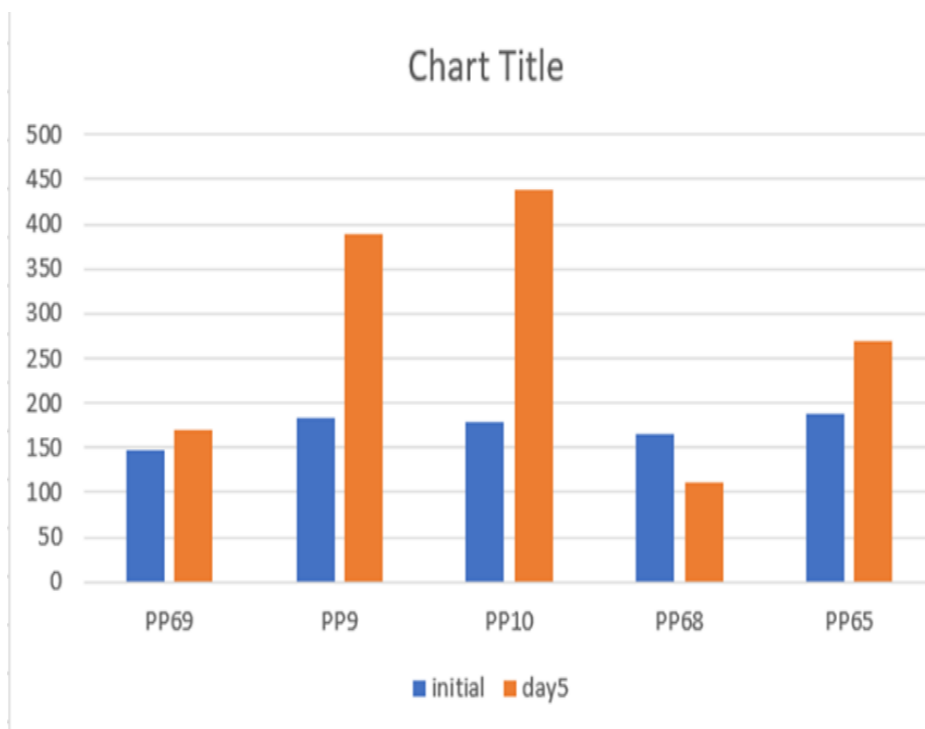
Ar= absorbance of reference (uninoculated sample SM+CAS reagent)

As=absorbance of Sample (supernatant of production medium + CAS reagent)

Table 5.4. Siderophore production is estimated by using the formula  $[Ar - As \div Ar \times 100]$   
The concentration of siderophore determine in the sample. The graph is plotted in Fig5.8. siderophore production PP10 culture gradually increased 1ml of sample in 100µl in micro broth assay by uv spectroscopy measurement at 630nm.

**Table 5.4. Siderophore Mesophile condition Absorbance unit**

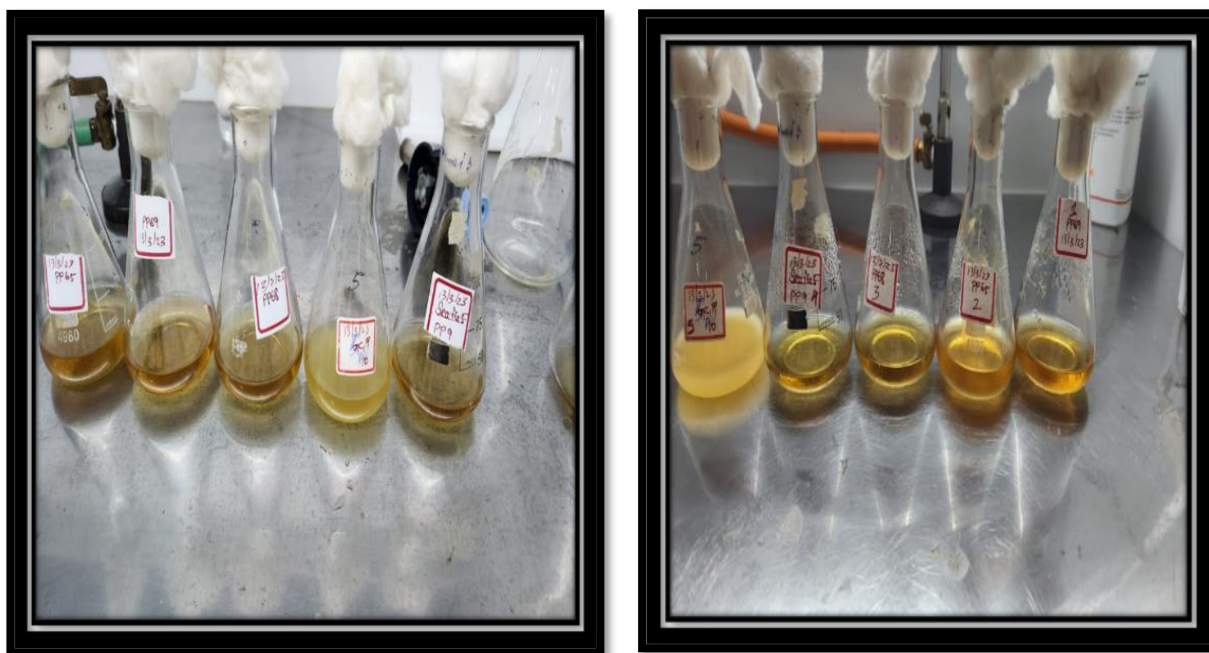
| Culture | Initial | Siderophore units |
|---------|---------|-------------------|
| PP69    | 147     | 169               |
| PP9     | 184     | 390               |
| PP10    | 180     | 438               |
| PP68    | 166     | 111               |
| PP65    | 187     | 269               |



**Figure 5.8.: Detection of siderophore in mesophile condition PP10 culture is gradually increased.**

### **5.3.2. Siderophore Psychrophile Condition**

5 culture broth PP68, pp69, pp65, pp9 are kept at Psychrophile the bacteria grow optimally at less than 15 c and 20 c. Three days it was observed that the pp10 culture colour changed Fig 5.9. Using micro broth assay the culture broth sample is read at 630nm by uv spectroscopy for 10 days in Table 5.5



**Figure 5.9: siderophore Psychrophile condition pp10 colour change**

**Table 5.5. Siderophore Psychrophile Condition reading**

| Culture       | Initial | Day |
|---------------|---------|-----|
| PP69          | 0.056   | 79  |
| PP65          | 0.055   | 75  |
| PP68          | 0.06    | 75  |
| PP9           | 0.058   | 70  |
| PP10          | 0.26    | 40  |
| Broth control | 0.143   | 43  |

### Formula

$$\text{Siderophore units}(\% \text{SU}) = \frac{Ar - As}{Ar} \times 100$$

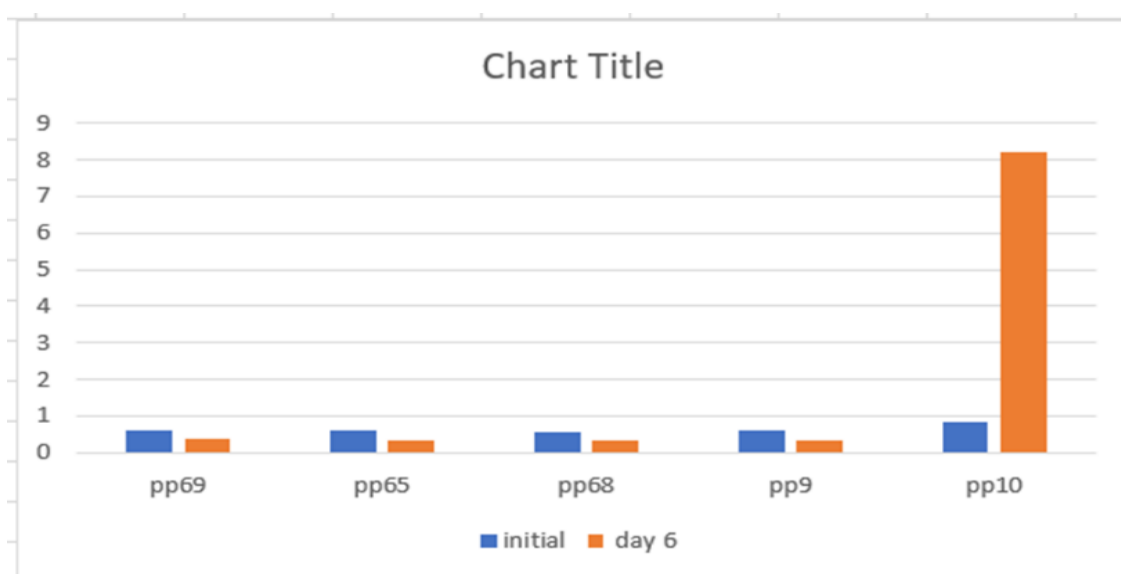
Ar= absorbance of reference (uninoculated sample SM+CAS reagent)

As=absorbance of Sample (supernatant of production medium + CAS reagent)

Table 5.6. Siderophore production is estimated by using the formula  $[Ar-As \div Ar \times 100]$  The concentration of siderophore determine in the sample. The graph is plotted siderophore production PP10 culture gradually increased 1ml of sample in 100 $\mu$ l Fig: 5.10. Micro broth assay by uv spectroscopy measurement at 630nm.

**Table 5.6. Siderophore Psychrophile Condition Absorbance unit**

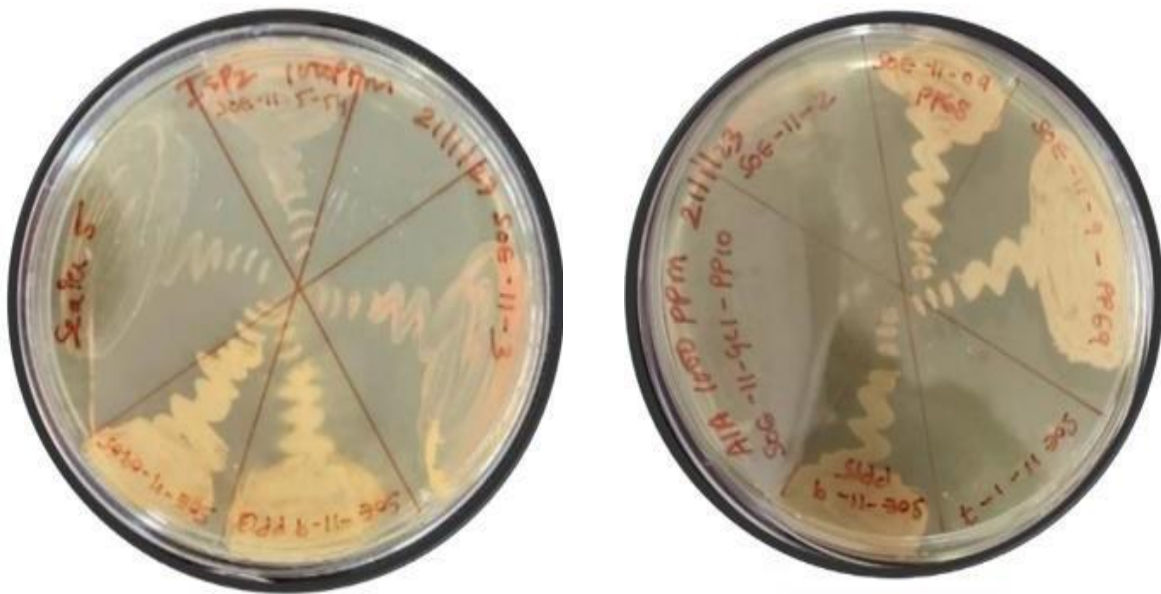
| Culture | Initial | Siderophore unit |
|---------|---------|------------------|
| PP69    | 0.608   | 0.397            |
| PP65    | 0.615   | 0.326            |
| PP68    | 0.58    | 0.336            |
| PP9     | 0.59    | 0.336            |
| PP10    | 0.818   | 8.193            |



**Figure 5.10. Detection of siderophore in Psychrophile temperature PP10 culture is gradually increased.**

## 5.4. CHROMIUM

Isp2 and AIA media were combined with 50% seawater and various concentrations of potassium dichromate before being autoclaved. After two days, it was discovered that the chromium is resistant to bacteria on the agar plate of Isp2 and AIA, which had been streaked with 25 Southern Ocean bacterial strains 19 cultures are tolerated to 1000ppm whereas in AIA 9 culture tolerate to 1000ppm Table 5.11. Numerous studies have noted the presence of metal-resistant bacteria in wastewater and soil that has been contaminated with various heavy metals (Alam et al., 2008). Fig 5.11.



**Isp2 1000 ppm**

**AIA 1000 ppm**

**Figure 5.11: Screening of chromium resistance on bacterial strain at 1000ppm Concentration**

**Table 5.7. Chromium Resistance Bacteria on Isp2 and AIA positive**

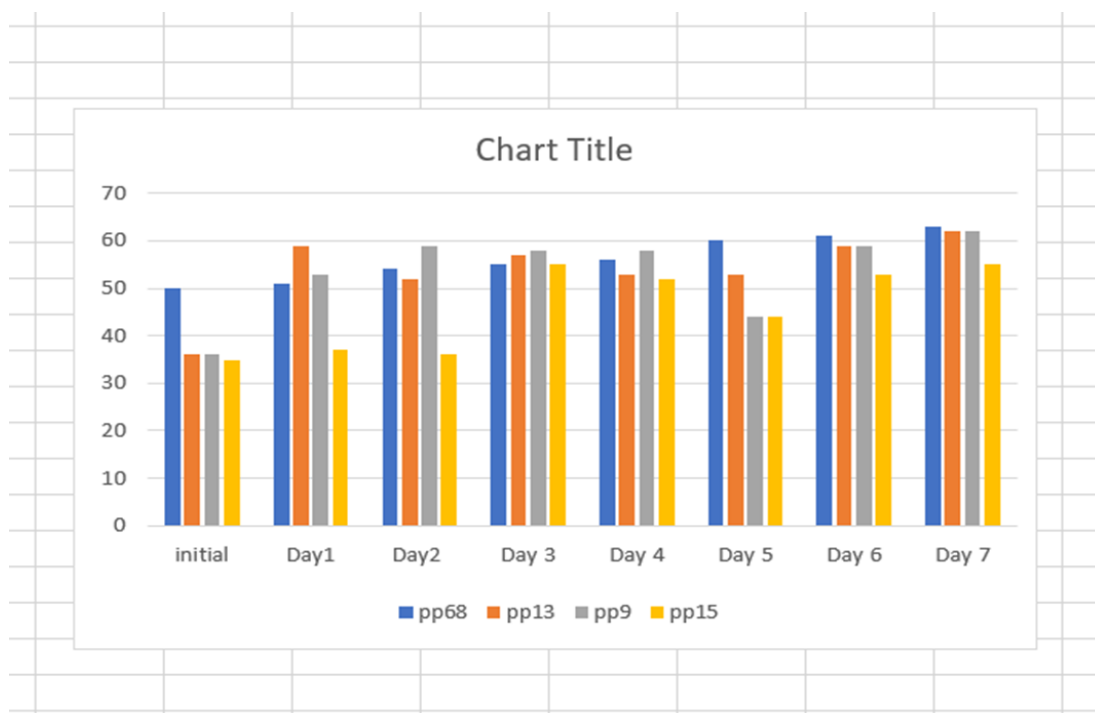
| S. NO | Concentration | Resistance of Bacteria Isp2 | Resistance of Bacteria AIA |
|-------|---------------|-----------------------------|----------------------------|
| 1     | 1000 ppm      | 19                          | 9                          |
| 2     | 500ppm        | 18                          | 8                          |
| 3     | 250ppm        | 18                          | 17                         |
| 4     | 100ppm        | 16                          | 19                         |
| 5     | 50ppm         | 16                          | 21                         |

**5.4.1. Chromium Degradation Determination test for 1000ppm at mesophile condition**

The four culture pp68, pp13, pp9, pp15 combined with 1000ppm potassium dichromate. The bacteria grow at mesophile temperature that ranges from 20 -45 c. Day wise read at 540nm in uv spectroscopy Table 5.8. PP68 in 1000ppm concentration mesophile temperature gradually increasing it as the capability to degrade the chromium graph plotted in fig 5.12. Effect on bacterial strains' ability to withstand metals (Shiva et al., 2014). According to Soni et al. (2013), the mean of a number of parameters, including pH, temperature, chromium content, incubation period, and inoculum size, has an impact on the reduction of Cr(VI) (direct or indirect).

**Table 5.8 Chromium mesophile temperature day-wise reading at 1000ppm in percentage**

| Culture | Initial | Percentage |
|---------|---------|------------|
| PP68    | 50      | 63         |
| PP13    | 36      | 62         |
| PP9     | 36      | 62         |
| PP15    | 35      | 55         |



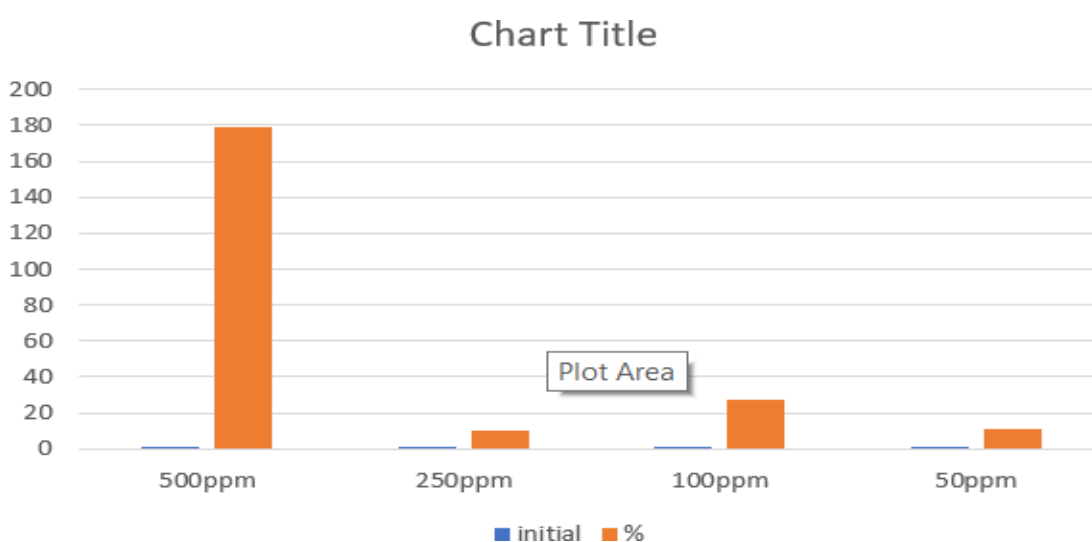
**Figure 5.12. Mesophile temperature PP68 in 1000ppm gradually increasing**

#### 5.4.2. Chromium degradation test in Mesophiles temperature

PP68 culture used it as the capability to degrade chromium in 1000ppm. PP68 culture is checked into four different concentrations 500ppm, 250ppm, 100pp, and 50ppm in Mesophile temperature which ranges from 20- 45 c. To degrade the chromium day-wise, the sample read at 540nm in uv spectroscopy Table 5.9. PP68 in 500ppm concentration it as the capability to degrade the chromium 1ml in 100µl. In mesophile temperature which ranges from 20-45 c in Fig 5.13.

**Table 5.9 Chromium mesophile reading in percentage**

| Culture | Initial | Percentage |
|---------|---------|------------|
| 500ppm  | 0.045   | 179.4715   |
| 250ppm  | 0.057   | 9.82801    |
| 100ppm  | 0.063   | 27.03777   |
| 50ppm   | 0.05    | 10.67708   |



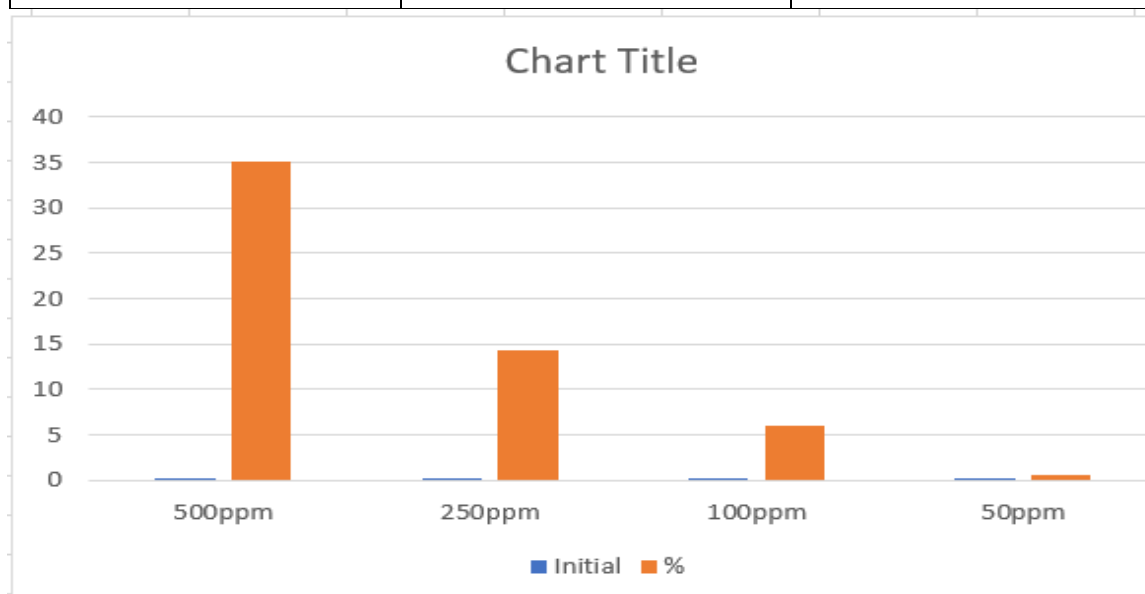
**Figure 5.13. pp68 in 500ppm concentration gradually increased.**

#### 5.4.3. chromium degradation test in Psychrophilic temperature

PP68 culture used it as the capability to degrade chromium in 1000ppm. PP68 culture is checked into four different concentrations 500ppm, 250ppm, 100pp, and 50ppm in Psychrophilic temperature which ranges from 15-20 c. To degrade the chromium day-wise, the sample read at 540nm in uv spectroscopy Table 5.10. PP68 in 500ppm concentration it as the capability to degrade the chromium 1ml in 100μl. In mesophile temperature which ranges from 20-45 c in fig 5.14.

**Table 5.10. Chromium psychrophilic temperature reading**

| Culture | Initial | Percentage |
|---------|---------|------------|
| 500ppm  | 0.045   | 35.08946   |
| 250ppm  | 0.057   | 14.31718   |
| 100ppm  | 0.063   | 5.970149   |
| 50ppm   | 0.05    | 0.664452   |



**Fig 5.14. Pp68 in 500ppm concentration psychrophile temperature gradually increased.**

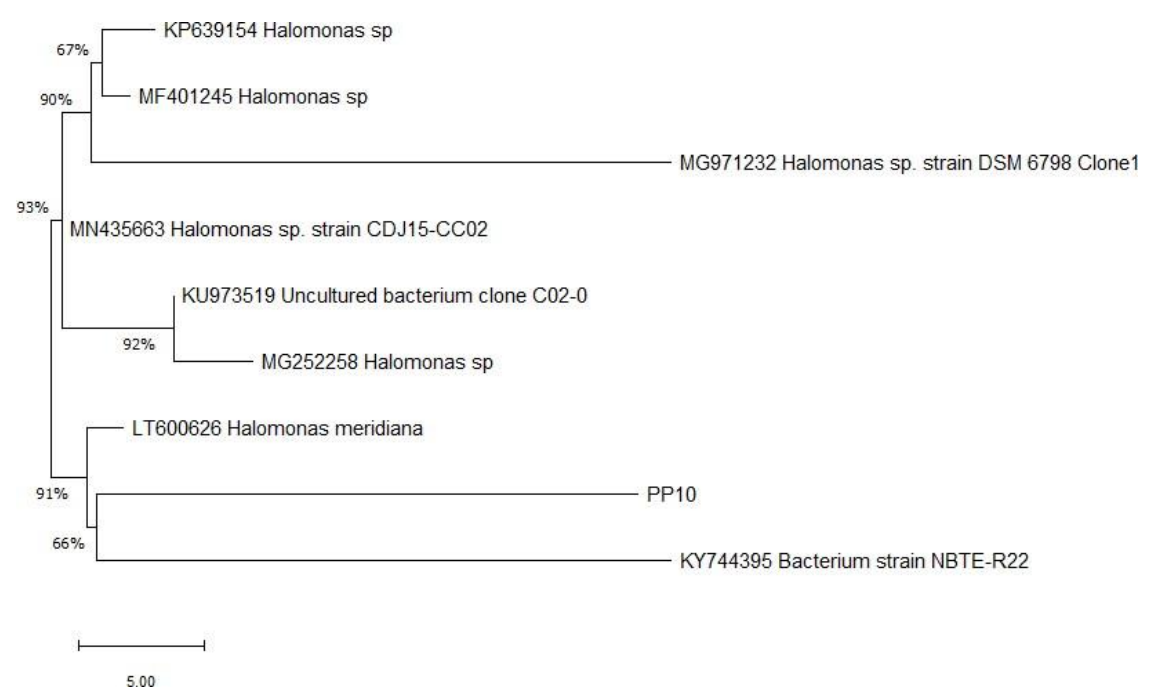
## 5.5. MOLECULAR CHARACTERIZATION GC-1 PP10

16 s RNA Sequence GC-1 PP10 Southern Ocean Culture for siderophore KU973519.1 The PCR amplification of Southern Ocean SOE11-GC1-PP10.16rRNA nucleotides base pair. NCBI BLAST analysis of SOE11-01-PP68 shows that unknown Culture. The Phylogenetic tree construction with southern ocean Actinobacteria culture SOE-11-GC1-PP10 designated as Unknown Culture (Figure 5.16).

TGCAGTCGAGCGGTAACAGATCCAGCTTGCTGGATGCTGACGAGCGGCGGA  
CGGGTGAGTAATGCATAGGAATCTGCCCGATAGTGGGGGATAACCTGGGGA  
AACCCAGGCTAATACCGCATACGTCCTACGGGAGAAAGGGGGCTCCGGCTC  
CCGCTATGGGATGAGCCTATGTCGGATTAGCTAGTTGGTGAGGTAACGGCTC  
ACCAAGGCCACGATCCGTAGCTGGTCTGAGAGGATGATCAGCCACATCGGG  
ACTGAGACACGGCCCGAACTCCTACGGGAGGCAGCAGTGGGGAATATTGGA  
CAATGGGGGAAACCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGCCCTC  
GGGTTGTAAAGCACTTTCAGCGAGGAAGAACGCCTAACGGTTAATACCCGCT  
AGGAAAGACATCACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGC  
CGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGC  
GCGCGTAGGTGGCTTGATAAGCCGGTTGTGAAAGCCCCGGGCTCAACCTGG  
GAACGGCATCCGGAAGTGTCAAGCTAGAGTGCAGGAGAGGAAGGTAGAATT  
CCCGGTGTAGCGGTGAAATGCGTAGAGATCGGGAGGAATACCAGTGGCGAA  
GGCGGCCTTCTGGACTGACACTGACACTGAGGTGCGAAAGCGTGGGTAGCA  
AACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTGACCGAGCCG  
TTGGGTGCCTAGCGCACTTTGTGGCGAAGTTAACGCGATAAGTCGACCGCCT  
GGGGAGTACGGCCGCAAGGTTAAACTCAAATGAATTGACGGGGGCCCCGCA  
CAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTA  
CTCTTGACATCCTGCGAATTTGGTAGAGATACCTTAGTGCCTTCGGGAACGC  
AGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTT  
AAGTCCCGTAACGAGCGCAACCCTTGTCCTTATTTGCCAGCGCGTAATGGCG  
GGAAGTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGACGAC  
GTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGTC  
GGTACAAAGGGTTGCCAACTCGCGAGAGTGAGCCAATCCCGAAAAGCCGAT  
CTCAGTCCGGATCGGAGTCTGCAACTCGACTCCGTGAAGTCGGAATCGCTAG  
TAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACAC

CGCCCGTCACACCATGGGAGTGGACTGCACCAGAAGTGGTTAGCCTAACGC  
AAGAGGGCGTCAC

5.6. PHYLOGENETIC TREE



**Figure 5.15. Phylogenetic tree PP10**

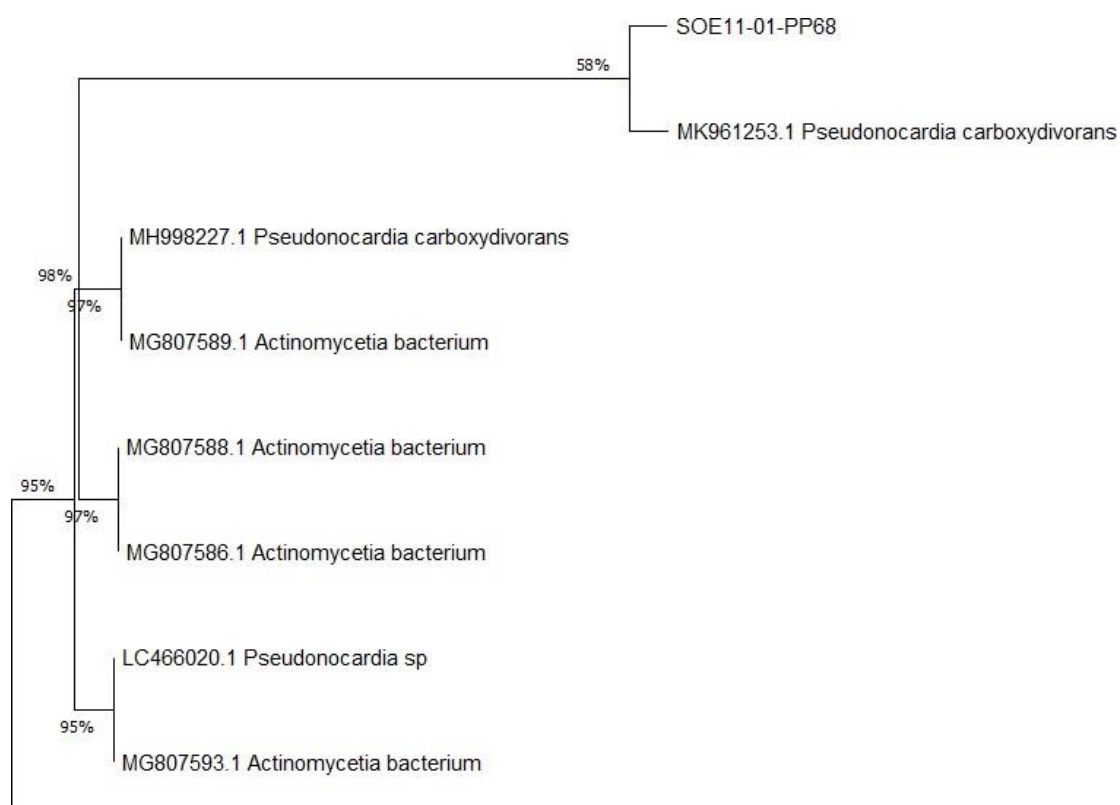
## 5.7. MOLECULAR CHARACTERIZATION SOE11-01-PP68

The PCR amplification of Southern Ocean SOE11-01-PP68 16 rRNA nucleotides base pair. NCBI BLAST analysis of SOE11-01-PP68 shows 100% Similarities to a sequence of *Pseudonocardia* Carboxydivorans MK961253. The evolutionary history was inferred by using the Maximum likelihood based on the Tamura-Nei model which confirms distinct Phylogenetic tree construction with southern ocean Actinobacteria culture SOE-11-01-PP68 designated as *Pseudonocardia* (Figure 5.16).

>SOE11-01-PP68

```
CTTTCGGGGGTACACGAGCGGCGAACGGGTGAGTAACACGTGGGTGACCTG
CCCTCCACTCTGGGATAAGCCCGGGAACTGGGTCTAATACCGGATAGGAC
CACTGATCGCATGGTTGGTGGTGGAAAGTTTTTCGGTGGGGGATGGGCCC
GCGGCCTATCAGCTTGTTGGTGGGGTGATGGCCTACCAAGGCGGTGACGGG
TAGCCGGCCTGAGAGGGGCGACCGGCCACACTGGGACTGAGACACGGCCCA
GACTCCTACGGGAGGCAGCAGTGGGGAATATTGCGCAATGGGCGGAAGCCT
GACGCAGCGACGCCGCGTGGGGGATGACGGCCTTCGGGTTGTAAACCTCTT
TCGCCAGGGACGAAGAGCAATTGACGGTACCTGGAGAAGAAGCACCGGCCA
ACTACGTGCCAGCAGCCGCGGTAACACGTAGGGTGCGAGCGTTGTCCGGAA
TTATTGGGCGTAAAGAGCTCGTAGGCGGTGTGTGCGGTCGGCCGTGAAAAC
TGGGGCTTAACCTCTGAGCGTGCGGTGATACGGGCATCACTTGAGTTCGGCA
GGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGG
AACACCGGTGGCGAAGGCGGGTCTCTGGGCCGATACTGACGCTGAGGAGC
GAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAA
ACGTTGGGCGCTAGGTGTGGGGACCATTCCACGGTTTCTGTGCCGCAGCTA
ACGCATTAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACCTCAAG
GAA
```

## 5.8. PHYLOGENETIC TREE



**Figure 5.16: Phylogenetic tree PP68**

## CHAPTER-6

### CONCLUSION

Actinobacteria are one of the largest phyla in the Bacteria domain and can be found in a variety of terrestrial and aquatic environments. They successfully produce novel secondary metabolites that have a range of biological effects, including antimicrobial and anticancer capabilities. These compounds are also crucial from an industrial standpoint since they help degrade heavy metals, produce siderophores, and produce chromium. The current research aimed to understand the bacteria that had been isolated from the southern ocean's Indian region. For the suggested investigations from the Centre for Drug Discovery and Development, 25 bacterial cultures are used. In the medical field, some Actinobacteria have been demonstrated to be less successful at having antibacterial and antifungal capabilities. While in non-medical fields, siderophore and chromium showed signs of heavy metals degrading in a favourable manner. On CAS agar, actinobacteria create a siderophore for 23 bacteria samples, of which 5 bacterial cultures do so. The siderophore detection test was conducted using these 5 bacterial cultures. According to the results of the Micro broth siderophore assay for mesophilic and psychrophilic conditions, PP10 culture showed about 80% and 69% the Production of siderophore in both Psychrophilic and Mesophilic Conditions. Chromium is a highly hazardous heavy metal that has been subject to extensive microorganism use in an effort to decrease it. On ISP2 and AIA media, 25 bacterial samples were utilised to resist chromium at various concentrations of 1000 ppm, 500 ppm, 250 ppm, 100 ppm, and 50 ppm, with 1000 ppm and 500 ppm showing resistance to the heavy metal. Chromium resistance test for four cultures in a mesophile environment, where the pp68 culture degraded the chromium at a concentration of 1000 ppm. The mesophile and psychrophilic conditions required PP68 culture; mesophiles required a 500ppm concentration of chromium, while psychrophilic required a 250ppm concentration. Among that PP68 strain was able to degrade the potassium dichromate in Mesophilic and Psychrophilic condition for about 63% at 1000PPM. Based on their Phenotypic and molecular Characteristics, the potential strain PP10 and PP68 were identified as *Pseudonocardia* and PP10 known as Unknow culture.

## CHAPTER-7

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