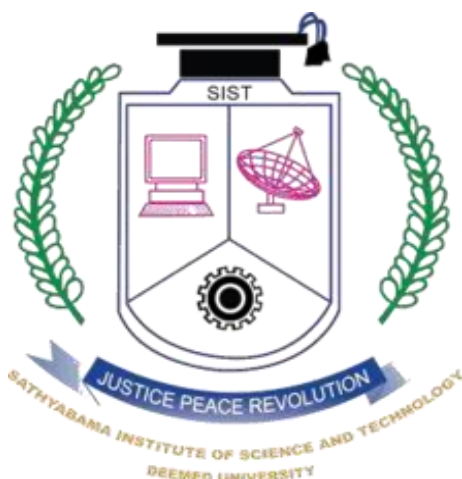


CHARACTERIZATION OF SALINE TOLERANT MULTIFUNCTIONAL BACTERIAL STRAINS ISOLATED FROM SOUTHERN OCEAN FOR PLANT GROWTH PROMOTION ON VEGETABLE CROPS

Submitted in partial fulfillment 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 **Miss. ARCHANA JEEVAN** (41810002) who carried out the project entitled **"CHARACTERIZATION OF SALINE TOLERANT MULTIFUNCTIONAL BACTERIAL STRAINS ISOLATED FROM SOUTHERN OCEAN FOR PLANT GROWTH PROMOTION ON VEGETABLE CROPS"** under our supervision from January 2023 to May 2023.

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ABSTRACT

Soil salinity threatens food security in South and Southeast Asia, where Solanaceous vegetable crops like tomato play an important role in human nutrition and helps to cope with malnutrition in our country India. Microbes that can tolerate salt may increase crop growth rather than increase crop tolerance to salt. This project intends to identify salt tolerant bacteria isolated from Indian sector of Southern Ocean that promote the growth of Solanaceous vegetable crops under saline conditions. Totally, 16 bacterial strains were isolated from Southern Ocean was screened for their saline tolerant ability in plate assay. All the bacterial strains grew well in 5% NaCl amended agar media. Similarly, out of 16 bacterial strains, 10 and 4 bacterial strains were able to tolerated 10% NaCl and 20% NaCl, respectively. All strains could produce IAA, but only Seaice-43 and Seaice-31 showed the maximum production in quantitative assay. Similarly, all the bacterial strains were further examined for the production of hydrolytic enzymes, where 11 bacterial strains produced amylase, 6 strains produced cellulase and none of the strains produced protease. Antagonistic activity of bacterial strains against *Colletotrichum gloeosporoides* showed the maximum percentage of inhibition by Seaice 16 (75%) followed by Seaice-43 (72%) and Seaice-37 (72%). All three PCR primers (BOX, REP and ERIC) confirmed the presence of high genetic diversity among the bacterial strains studied. The potential three bacterial strains (Seaice 16, Seaice-43 and Seaice-37) were chosen for in- planta and pot culture PGP study under saline condition. All three strains showed higher germination percentage, shoot length, root length and chlorophyll content than control seedlings in both in- planta and pot study. Finally, the production and identification of volatile compounds by potential strain, Seaice-43 in headspace GCMS showed some interesting volatile metabolites such as Hexadecane, 12 Methylaminolauric acid and Silanediol dimethyl, which maybe the reason for their potential PGP and antagonistic activity. The multi-potential strain Sea ice-43 identified as *Pseudomonas paracarnis* based on cultural, microscopic and molecular identification. From these results, we can conclude that, the strain Seaice 43 has potential

candidate to develop as a biofertilizer to enhance tomato plant growth and development under salt stress in future.

Keywords: Salinity, *Pseudomonas paracarnis*, indole acetic acid, REP-ERIC-BOX, Headspace- GCMS, tomato.

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CHAPTER 1

1. INTRODUCTION

The beginning of the 21st century is characterized by environmental pollution, water scarcity worldwide, and an increase in the salinization of soils and water. An increase in the number of people and a reduction in the amount of land that can be cultivated pose two threats to agricultural sustainability. Several environmental stresses have affected agricultural crops, including high winds, extreme temperatures, soil salinity, droughts, and floods (Shrivatsava *et al.*, 2015). The most devastating environmental stress, soil salinity, causes significant reductions in cultivated land area, quality, and crop yield (Egamberdieva *et al.*, 2019). As determined by the electrical conductivity (EC) of the saturation extract (EC) in the root zone, saline soils have an exchangeable sodium content of 15% and a conductivity (EC) of over four deciSiemens per metre (dS/m). Most crops exhibit yield reductions at this EC, while others at lower EC (Munns, 2005; Jamil *et al.*, 2011). A worldwide assessment has indicated that 33% of irrigated lands and 20% of all agricultural lands are affected by high salinity levels. There is an increase of 10% each year in salinized areas. This results from low precipitation, high surface evaporation, weathering of native rocks, irrigation with saline water, and poor cultural practices (Yadav *et al.*, 2020). According to Jamil *et al.*, (2011), more than half of all arable land will be salinized by 2050.

The processes of salinization might be primary (natural) as well as secondary (anthropogenic) (Ghassemi *et al.*, 1995). Salt water infiltration and salt deposition from the wind on land are the main contributors to naturally occurring salinity. Due to brackish water irrigation and seawater intrusion into aquifers, salt stress issues in the Mediterranean area are now getting worse (Rana and Katerji 2000). Irrigating crops with salt water, which substantially worsens and accelerates soil salinization, is one of the human contributors. Additional factors might include soil amendments, inorganic fertilizers, etc. (e.g. gypsum, composts and manures) (Kotuby Amacher *et al.*, 2000). Although the Earth is enveloped in water, just a small percentage (about 2.5% or 35 million km) of it is fresh or has a low salt concentration (less than 1 dS/m) and thus is

suitable for irrigation of crops. The majority, on the other hand, is salty and which means inappropriate for irrigation) (Ondrasek *et al.*, 2010; Shiklomanov and Rodda 2003).

It is anticipated that by 2050, nearly 50% of agricultural land would ultimately be saline impacted. Salinization of arable land occurs largely owing to the buildup of salt in land (Bharti *et al.*, 2016; Shi-Ying *et al.*, 2018), notably sodium (Na^+) and chloride (Cl^-) ions. High Na^+ buildup hinders soil porosity, aeration, and water conductivity. Additionally, the microbial diversity both within and around plant roots is adversely impacted by soil salt stress. Salinity induces a plant to go through a number of morphological, biological, and molecular modifications that obstruct its ability to grow and develop. Additionally, the rate of photosynthesis, stomatal conductance, and enzyme activity are all negatively affected by excessive salt concentrations. (Kumar and Verma, 2018).

Agricultural output on salinized soil has been facilitated by soil and water management techniques, but a further benefit from these strategies seems insufficient. (Zahir *et al.*, 2008). Every major crop in the globe experiences substantial yield limitations due to impacted soils. (Bacilio *et al.*, 2004, Shannon and Grieve, 1999). To adequately satisfy the estimated population by 2050's food supply requirements, a large increase (about 50%) in grain yields of significant agricultural crops including *Oryza sativa* L., rice, wheat (*Triticum aestivum* L.), and maize (*Zea mays* L.) must be achieved (Godfray *et al.*, 2010). The critical need to battle pollution in the soil, salinization, and desertification while simultaneously offering for a growing global population has made plant and agricultural productivity research of paramount importance. Given these conditions, it is necessary to use appropriate biotechnology to increase crop yield as well as improve the health of the soil through interactions between the roots of plants and soil microbes. (Lugtenberg *et al.*, 2002).

In an effort to foster sustainable agriculture, it is now necessary to use novel methods simultaneously, such as the use of plant-growth-promoting microbes (PGPR), as many salt-tolerant crop varieties are being produced through conventional breeding

and transgenic technologies, but each of these methods are insufficient, highly laborious, and time-consuming. Complex microbial diversity may be perceived as an outcome of natural interactions among microorganisms and plants in the rhizosphere.

According to a number of current studies, PGPB accelerates plant growth and enhances salt tolerance. (Vacheron *et al.*, 2013; Daliakopoulos *et al.*, 2016; Tiwari *et al.*, 2016). In saline soil, PGPB frequently reside in the root portion of plants. The generation of biofilms, extracellular polymer substance (EPS) synthesis, fixation of nitrogen, the phytohormone creation, and ACC-deaminase activity are only among the many PGPB-mediated methodologies. (Ansari *et al.*, 2019). Recent studies have implied that salt-tolerant plant growth-promoting rhizobacteria (ST-PGPR) may be employed in saline farming to increase production and promote soil fertility. (Grover *et al.*, 2011).

They engage in a variety of biotic activities that keep the soil ecosystem active for the cycling of nitrogen and robust for crop cultivation (Chandler *et al.*, 1986; Diby *et al.* 2005a, b). They boost plant growth by mobilizing soil nutrients, developing a variety of plant growth regulators, securing crops from phytopathogens by preventing or controlling them, strengthening soil structure, and bioremediating the contaminated soil by retaining toxic heavy metal species and breaking down xenobiotic compounds (Braud *et al.*, 2009; Hayat *et al.*, 2010).

The implementation of PGPR on multiple crops has been extremely effective. These crops include vegetables (Kumari *et al.*, 2018), pulses (Khare and Arora 2010), oil-producing plants (Shahid *et al.*, 2012; Tewari and Arora 2014; Sharifi 2017), and grains (Lucas *et al.*, 2009; Qaisrani *et al.*, 2014; Majeed *et al.*, 2015; Karnwal 2017). (Loganathan *et al.*, 2014; Moustaine *et al.*, 2017). In accordance with studies (Van Loon *et al.*, 1998; Spaepen *et al.*, 2009; Ahmad *et al.*, 2013; Korir *et al.*, 2017), PGPR increases seed germination, sapling vigor, seed emergence, root and shoot growth, the overall biomass of the plants, seed weight, flowering, and yields. In attempt to increase agricultural sustainability on saline soils, management strategies that promote biological

activity in the soil and therefore increase long-lasting productivity of soil and crop health are inevitably required.

Crop yields are negatively impacted by phytopathogens, which also cause serious diseases in vegetation. In accordance with Guo *et al.* (2013), phytopathogen invasion of food grains is also thought to adversely impact food quality and cause significant economic losses. The most effective approach to address diseases in plants in a manner that is environmentally friendly without endangering the environment is by the biocontrol of phytopathogens implementing natural organisms (Compant *et al.*, 2005; Fatima *et al.*, 2009). Due to their aggressive rhizosphere colonisation and broad-spectrum antagonistic activities, *Pseudomonas* and *Bacillus* are the most common (Weller *et al.*, 2002). As a result of competing for nutrients, antagonism by the formation of different metabolite (antibiotics, enzymes for hydrolysis, cyanide, siderophores), and development of systemic resistance, PGPR display an assortment of biocontrol activities against plant pathogens (Lugtenberg and Kamilova 2009).

CHAPTER 2

REVIEW OF LITERATURE

2.1. PROBLEM OF SOIL SALINIZATION:

One of the main causes of cultivated soils declining yield is soil salinization. Although it can be challenging to determine accurately, there are a rising number of salinized soils on the globe. Irrigated soils are particularly affected from this phenomenon. A major issue for irrigation-based cultivation is salinity of the soil. The soils tend to be saline and have poor agricultural potential in the hot, dry regions of the earth. The majority of crops are produced under irrigation in these regions, and to make matters worse, poor irrigation management contributes to secondary salinization, which impacts 20% of irrigated land globally (Glick *et al.*, 2007). Ions constitute the salts in the soil. (Electrically charged forms of atoms or compounds). Ions are produced by soil minerals that are weathering. In some cases, they migrate up in the soil from groundwater that is shallow or are administered as fertilizers or irrigation water. Salts build up in the soil and lead it to become salty when precipitation is inadequate to remove ions from the soil's surface (Blaylock *et al.*, 1994). Physical, chemical, as well as biological land degradation events have had significant negative effects on the world's natural resources over the past century (such as compaction, inorganic/organic pollution, and lowered microbial activity/diversity). Due to the installation of drainage in new places, the area beneath the affected soils expands every year (Patel *et al.*, 2011).

One-third of the world's arable area, or about 20 percent (45 million ha), appears to be salt-affected (Shrivastava *et al.*, 2015). About 200 106 hectares of land in the Southwest United States and Mexico are affected by salinity, according to Shahid (2013) in a thorough survey of the worldwide detection of salinity issues and the global salinity state. There is noticeable saltwater penetration into aquifers in Spain, Portugal, Greece, and Italy. In Spain, more than 20% of the land is desert or highly degraded and nonproductive (Shahid *et al.*, 2013). In India, it has been calculated that saline soil covers approximately seven million hectares of territory (Patel *et al.*, 2011). Salt stress leads to an assortment of reactions in crops and livestock. Salinity alters the area's ecological balance as well as the agricultural output of most crops. It also has an

influence on the physicochemical characteristics of the soil. Poor agricultural productivity, poor economic returns, and soil erosion are all effects of salinity (Hu *et al.*, 2004). The social effects of soil salinization involve decreased agricultural output, low revenue, altered choices for a living, and associated social restrictions. According to estimates, lost crop output from salt-induced degradation of soil in irrigated regions could cost the world \$27.3 billion annually (Qadir *et al.*, 2014). According to projections based on moving average data from 2012 to 2014, India loses 16.84 million tons of agricultural output (cereals, oil-seeds, pulses, and cash crops) every year, totaling Rs. 230.20 billion (Mandal *et al.*, 2018).

2.2 IMPACT OF SALINITY ON AGRICULTURAL PLANTS:

Salts interfere with plant nutrition and elevate the osmotic pressure in the soil, both of which have an impact on plant development. The osmotic or water-deficit impact of salinity is the decrease in plant water uptake caused by high levels of salt in soil solution. When the concentration becomes significant enough to start hindering crop development, damage occurs. Although there are few genotype differences, the osmotic impact of salinity causes metabolic alterations in the plant that are similar to those brought on by water stress-induced "wilting" (Munns *et al.*, 2002; Lauchli *et al.*, 2007). Effects of salinity result from the intricate interplay between morphological, physiological, and biochemical reactions like seed germination, plant development, and water and nutrient uptake (Akbarimoghaddam *et al.*, 2011; Singh *et al.*, 2001). Due to drought, high soil salinity levels, and harsh environmental conditions, salinity effects almost all facets of plant development, such as the germination process plant growth, and reproductive development (Machado *et al.*, 2017). Plants are hampered in their ability to absorb water from soil due to soil salinity, which also causes toxic ion concentrations, osmotic stress, a mineral (N, Ca, K, P, Fe, Zn) shortage, and oxidative stress. Plant phosphorus (P) uptake is greatly decreased by soil salinity because the phosphate ions condense with Ca ions (Bano *et al.*, 2009).

Sodic soils have increased Na⁺ absorption, but they also have decreased K⁺ absorption, which has a negative impact on the enzyme reactions that regulate

metabolic processes like protein synthesis and photosynthesis. This is problematic for plant development (Hauser *et al.*, 2010; Katiyar *et al.*, 2005). Salinity has a detrimental effect on photosynthesis by reducing CO₂ supply because of diffusional constraints and a decrease in the amount of photosynthetic pigments present (Delfine *et al.*, 1999). Salinity restricts a plant's ability to develop by lowering its total photosynthetic capacity through reduced growth of leaves and hindered photosynthesis (Yeo *et al.*, 1986). By obstructing the uptake of essential nutrients like K⁺, Ca²⁺, and NO₃, as well as causing an excess of Na⁺ and Cl, salt buildup in the root zone leads to the formation of osmotic pressure and affects cell ion homeostasis (Paranychianakis *et al.*, 2005).

Salinity affects reproductive development through the promotion of programmed cell death in some types of tissues, ovule abortion, and senescence of fertilized eggs. It also inhibits microsporogenesis and stamen filament elongation. Due to a low osmotic potential of the soil fluid (osmotic tension), specific ion effects (salt stress), nutrient imbalances, or any combination of these factors, the saline growth medium has many detrimental effects on plant development (Ashraf *et al.*, 2004). By decreasing the amount of expression and function of cyclins and cyclin-dependent kinases, salinity transiently arrests the cell cycle, leading to fewer cells in the meristem and thus hindering development. During salt stress, post-translational suppression also reduces the level of activity of cyclin-dependent kinase. Recent studies also suggest that salinity negatively impacts plant development and growth, as well as inhibiting the germination of seeds, development of seedlings, activity of enzymes, RNA, DNA, and protein production and mitosis (Seckin *et al.*, 2009; Tabur *et al.*, 2010).

There is a reduction in yields of different crops due to salinity and reduced flowering due to salinity. In turn, this affects pollination, resulting in a reduction in grain yields and seed production. According to Keating and Fisher (Keating and Fisher, 1985), many crops, such as cotton, barley, wheat, rice, and beans, are significantly affected by saline stress. Salt concentrations within the cell cause various physiological disorders, including reduced seed germination, flowering, fruiting, and seedling growth. A major component of the electron transport chain, photosystem 2, is inhibited by salinity stress.

Salinity stress affects several cellular enzymes, including RNase, DNase, protease, and enzymes involved in nitrogen metabolism and amino acid synthesis. Due to the high salt content in roots, the intracellular ionic equilibrium is disturbed (Rengasamy, 2010). Plant roots cannot absorb sufficient amounts of water for growth during these conditions. They must expend significant energy to adjust their osmotic balance by collecting compatible solutes (Robinson and Jones, 1986).

2.3 DIVERSITY OF SALINE TOLERANT BACTERIA FOR PLANT GROWTH

There is an extensive number of microbes, fungi, and archaea in soil, offering it an incredible amount of versatility. Some microorganisms are now well recognized for having an inherent ability to endure various salt concentrations and promote plant development. These helpful plant microbes that can withstand salinity are crucial to agribusiness. They exhibited their capacity to increase agricultural yield in arid and semi-arid areas (Niu *et al.*, 2018). Plant growth promoting microbes are soil and rhizosphere-dwelling microorganisms that have the capacity to thrive in plant roots in substantial quantities (105–107 CFU per gramme of fresh root) and positively affect the growth of plants. (Spaepen *et al.* 2009). Numerous PGPR species can be detected in the rhizospheric zone of plants. Specific prevalent PGPR families and species can vary among plants. A plant's root microbiome is influenced by a variety of environmental (biotic and abiotic) variables, including the species of root, species of plant, plant age, soil type, and plant species type, genotypes. (Lareen *et al.* 2016).

Several decades ago, bacteria were discovered, increasing plants' health and growth through vivid mechanisms. The bacteria are introduced into the soil on roots or seeds to enhance plants' health and growth (Raajimakers *et al.*, 2002). A well-known species of growth-promoting bacteria is the *Rhizobium* genus, which has successfully commercialized and developed symbiotic relationships with plants. Several bacterial species associated with plants were discovered at the beginning of this century, but these species were not symbiotically associated with their hosts (Bashan and Holguin, 1998).

Pseudomonas, *Rhizobium*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus*, and *Serratia* are only a few of the species of the PGPR that have been reported to promote growth and development of plants (Beneduzi *et al.* 2012; Ahemad and Kibret 2014). PGPR have been organized into many classes according to their roles and taxonomical classification. Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, and Proteobacteria are the five primary taxa that PGPR strains predominantly belong to, according to Tilak *et al.* (2005).

PGPR have been organized into four groups based on their degree of relationship with plant roots, according to Gray and Smith (2005): (i) organisms that live near the root, (ii) those residing on the surface of the root (rhizoplane-colonizing bacteria), (iii) those inhabiting the tissue of the root (spaces within cortical cells), and (iv) those living inside of the cells of specialised root structures or nodules. PGPR can also be categorized broadly into two kinds depending on these associations: (i) intracellular or endophytic (iPGPR) and (ii) extracellular (ePGPR) or rhizospheric (Vessey 2003; Gray and Smith 2005; Martinez-Viveros *et al.* 2010; Santoyo *et al.* 2016).

For increasing the cultivation of multiple crops in saline settings, genera such as *Pseudomonas*, *Bacillus*, *Enterobacter*, *Agrobacterium*, *Streptomyces*, *Klebsiella*, and *Ochromobacter* have the best results. (Sharma *et al.*, 2016; Sarkar *et al.*, 2018). In accordance with reports, the alkaliphilic bacteria *Planococcus rifietoensis* stimulates the growth and cultivation of wheat crops under salt stress conditions. (Rajput *et al.*, 2013). In accordance with Sharma *et al.*, the soil of Indian eastern Indo Gangetic plain has an array of salt-resistant bacilli. (2015). They found 95 distinct bacterial strains, of which 55 were salt-tolerant to more over 4% NaCl and revealed attributes that stimulated growth in plants. It is well-established that phosphate-solubilizing bacteria (PSB) and their application as inoculants enhance plants' intake of phosphate. (Zaidi *et al.* 2009). According to Rodriguez and Fraga (1999) and Bossis *et al.* (2000), the most common PSB utilized in commercial use are *Pseudomonas*, *Bacillus*, and *Rhizobium*.

Wheat rhizosphere-isolated ST-PGPR *Klebsiella* sp. IG 3 indicated up to 20% salt tolerance. Under salt-stress conditions, this strain significantly impacted the expression levels of the genes *rbcL*, that codes for the enzyme known as ribulose-1,5- bisphosphate carboxylase/oxygenase RuBisCo, and *WRKY1*. (Sapre *et al.*, 2018). There is significant agricultural importance with regard to these free-living rhizobacteria, which includes *Achromobacter*, *Acetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Azomonas*, *Bacillus*, *Beijerinckia*, *Clostridium*, *Corynebacterium*, *Derxia*, *Enterobacter*, *Herbaspirillum*, *Klebsiella*, *Pseudomonas*, *Rhodospir* (Prithiviraj *et al.* 2003; Bhattacharyya and Jha 2012). Gram-negative, aerobic, non-sporulating, rod- shaped Rhizobia bacteria are an extremely distinct class with extremely distinct phenotypes. (Tak *et al.*, 2017; Rao *et al.*, 2018).

2.4 SALINE TOLERANT MICROORGANISMS AND THEIR MECHANISM

2.4.1. Plant growth regulators

Some vital inner stimuli stimulate organ genesis and development in plants. Small chemicals known as plant growth regulators or plant hormones may impact plant growth and development even at extremely low levels. One of the parameters used to control the effectiveness of bacteria is their capacity to support plant growth and the development of root systems (Fett *et al.*, 1987; Torres-Rubio *et al.*, 2000). Under salt stress, ST-PGPR-produced phytohormones play an important role in regulating plant metabolism. (Egamberdieva and Kucharova, 2009). These artificial compounds that mimic natural plant hormones are known as plant growth regulators or plant exogenous hormones. They are crucial tools for increasing agricultural output because they are employed to control plant development. It's also key to keep in mind that various hormones frequently work in conjunction to initiate every plant response. As a consequence of this, hormones, which stem from PGPR, are additionally recognized as plant developmental regulators since they may stimulate or impede plant development (Porcel *et al.*, 2014). Source-sink communications, biomass reallocation, and senescence of leaves are all modulated by an assortment of changes in the phytohormone status, which are thought to limit vegetative development throughout the osmotic phase of salinity (Pérez-Alfocea *et al.*, 2010). Auxins, ABA, cytokinin, and

gibberellic acid (GA), which are synthesized via PGPR, boost plant resilience to salt stresses (Kudoyarova *et al.*, 2019).

2.4.1.1. IAA

For the division and elongation of cells in plants enduring salt stress, the ST-PGPR generate IAA. The most widely acknowledged ST-PGPR that produces IAA during salt stress are *Rahnella*, *Arthrobacter*, *Azotobacter*, *Pseudomonas*, and *Azospirillum* (Piccoli *et al.*, 2011; Abd_Allah *et al.*, 2017). As demonstrated by Spaepen and Vacheron, exogenous IAA determines a wide range of processes that regulate plant growth and development. Low levels of IAA may foster initial root elongation, whereas high levels of IAA decreases primary root length, raise the growth of root hairs, and increase the development of lateral roots. IAA has been found to have beneficial effects on plant growth, as seen by the yucca's copious lateral roots and lengthy hypocotyls as well as the super root mutant, which produces excessive IAA. (Boerjan *et al.*, 1995; Zhao *et al.*, 2001). IAA produced by an ST-PGPR *Leclercia adecarboxylata* MO1 has recently been linked to sugar synthesis, the generation of organic acids, and an enhancement of chlorophyll fluorescence (Fv/Fm) in tomato, as reported by Kang *et al.* (2019). Rhizobacterial IAA unravels plant cell walls, thereby making it easier to allow additional root exudation to takes place and supply additional resources for rhizosphere bacteria to grow and prosper (Glick 2012). As an outcome, Spaepen and Vanderleyden (2011) uncovered rhizobacterial IAA as a molecule that acts in plant-microbe interactions associated with phytostimulation as well as disease control.

2.4.1.2. Cytokinins

Other phytohormones, in addition to auxins, have been suggested to assist plants adapt under salt stress. In particular, salt-tolerant *Arthrobacter*, *Bacillus*, *Halomonas*, *Azospirillum*, and *Pseudomonas* species have been reported to secrete cytokinins (CK), which are critical for cellular proliferation and differentiation (Garca de Salamone *et al.*, 2001; Karadeniz *et al.*, 2006; Naz *et al.*, 2009; TrParray *et al.*, 2016). In accordance with Liu [87], tropical Thuja seedlings administered with cytokinin-producing *Bacillus subtilis* isolates displayed greater resilience to draught-induced stress. A bacterial strain called *Bacillus megaterium* bacterium r has been indicated to have cytokinin receptors which have a role in stimulating plant growth. The *Phaseolus*

vulgaris L. plant's rhizosphere constituted the original source of the *B. megaterium* UMCV1 strain's isolation. By implementing this bacteria for inoculation, it has been discovered that the production of biomass of two plants, bean and *Arabidopsis thaliana*, is improved (López-Bucio *et al.*, 2007). According to Arkhipova *et al.* (2007), the cytokinin-producing PGPR *Bacillus subtilis* boosts the shoot biomass of lettuce plants developed in drying soil, demonstrating effective root-to-shoot cytokinin signalling. Three receptors—CRE1/AHK4/WOL, AHK2, and AHK3—are responsible for CK signalling in plants. Increased plant growth has a direct association with higher cytokinin levels. There aren't numerous research projects on the specific contribution of cytokinin-producing PGPB to mitigating salt stress in plants. (Kudoyarova *et al.*, 2019).

2.4.1.3. Gibberillic acid

Bacteria are also capable of producing gibberellins, assisting in encouraging plant development and output. Rhizobium-colonized rice roots produce greater auxins and gibberellins, thereby encouraging more substantial crop development and growth (Bottini *et al.*, 1989). Different PGPB are believed to be capable to synthesize numerous kinds of GA, which includes GA4, GA8, GA9, GA19, and GA20, that assist plants achieve physiological modifications for maximal development as well as enduring salt challenges. Significant quantities of GA1, GA19, GA20, and GA44 are synthesized by strains of bacteria with surged elongation development and such amounts can be identified via deuterated internal standards and GCMS analysis (Dobert *et al.*, 1992). Gibberellin produced by the bacteria *Bacillus* sp. and *Azospirillum* sp. following its inoculation in wheat roots has been related to increased N uptake (Kucey, 1988). Application of GA3 to the roots in a concentration equivalent to the amount produced by microorganisms stimulated maize root growth (Fulchieri *et al.*, 1993). Shoot elongation, however, is GA's most significant physiological outcome. In the words of Khan *et al.*, 2014, tomato plants afflicted with the gibberellin-producing *Sphingomonas* sp. LK11 variant showcase significant enhancements in an assortment of growth parameters.

2.4.1.5. ABA

Abscisic acid (ABA), widely referred to as a stress hormone, gets elevated in the root zone when there is a shortage of water due to saltwater stress. Under salinity, the amount of ABA rises again, and this helps the plant combat the detrimental effects of

stress. According to Numan *et al.*, (2018), ABA contributes in enhancing the accumulation of Ca^{2+} , K^{+} , and appropriate solutes like sugars and the hormone proline in the root vacuoles, which diminish the adverse impacts of elevated salinity. Furthermore, genomic assessments have revealed that while on drought and salinity stress, PGPB could influence the way genes are expressed of ABA-dependent stress communication networks in crops. For instance, *P. putida* switched the expression level of ABI5, an essential inhibitor of ABA transmission under abiotic circumstances including salt and drought (Skubacz *et al.*, 2016; Jatan *et al.*, 2019), in chickpea inoculation alongside PGPB. According to a 2009 study by Naz *et al.*, organisms obtained from Pakistan's Khewra Salt Range exhibited resilience when tested on salt medium generated by rhizosphere soil filter. Additionally, the isolates extracted ABA at an intensity that was significantly higher than in previous studies. Furthermore, under induced salt stress, soybean crops treated with these isolates analogously produced increased proline, shoot/root length, as well as dry weight. The regulation of many different plant phases of development, including as dormancy of seed, implantation and development, primary growth of roots, and the duration of flowering amid abiotic stresses, has recently been demonstrated to be governed by the antagonistic interactions among GA and ABA (Shu *et al.*, 2018).

2.4.2. ACC Deaminase activity

All higher plants produce ethylene, a gaseous regulator which can also be produced by certain microbes, fungi, and other inhabitants (Jordán and Casaretto, 2006). Since ethylene is a volatile vegetative hormone, it can be passively transported across plant tissues effortlessly without the need of targeted transporters. The enzyme referred to as SAM synthetase, formerly referred to as AdoMet-synthetase, stimulates the first procedure in the biosynthesis of ethylene in plants, which is the development of S-adenosyl-methionine (SAM), which is subsequently transformed to ACC by the enzyme known as ACC synthetase. Finally, the ACC is transformed into ethylene via an enzyme called ACC oxidase. This process causes the occurrence of an assortment of additional compounds, including the volatile chemicals cyanide (hydrogen) and carbon dioxide, in conjunction with ethylene. It is a frequent occurrence for ST-PGPR to carry

out ACC deaminase activity, particularly when relegated to substantial salt stress. ACC becomes broken down by the enzyme known as ACC deaminase into ammonia and -ketobutyrate, which the bacteria use as nitrogen, or N, and substrates for carbon (Glick, 2014). In addition to minimising the repression of down-regulated genes entailed in ethylene-induced stress in plants and up-regulating genes associated to plant growth, the presence of ST-PGPR substantially decreases the degree of stress-induced ethylene (Glick *et al.*, 2007). Ahmad *et al.*, 2013, has established that strains of *Rhizobium* and *Pseudomonas* that yield ACC-deaminase has the potential to improve the physiological processes, growth, and nutritional value of mung beans in salt-affected conditions.

Plant root lengthening, the enhancement of development of shoots, the enhancement in rhizobial nodulation and N, P, and K absorption, as well as mycorrhizal migration are among the primary outcomes of seed/root inoculating with ACC deaminase-producing bacterial species (Nadeem *et al.*, 2007; Shaharoona *et al.*, 2008; Nadeem *et al.*, 2009). In the research of Belimov *et al.* (2005), exacerbated root growth was significantly correlated with a surge in bacterium ACC deaminase action whilst cadmium amassed in plant tissues. Similar to the above, Rodriguez *et al.* (2008) noted that after *P. putida* HS-2 being inoculated tobacco plants grew promptly and that there was a significant buildup of minerals from nickel-contaminated ground. The growth and progression of fungi in the the extreme soils is significantly influenced by a collaboration of ACC deaminase bacterium. These bacteria also have the potential to disintegrate ACC, which gives plants gain access to more micronutrients (Nascimento *et al.*, 2014).

2.4.3 Exopolysaccharides

The acronym "EPS" describes to a class of bacterial polysaccharides exerted and discharged outside of the cell while biofilm formation by enzymes tethered to the cell wall. EPS have been separated into homo and a hetero polys based on the composition of their molecules (Nwodo *et al.*, 2012). Many PGPB have been reported to develop EPS, a substance that helps plants in resisting in salty conditions. When presented to salt stress, EPS made by PGPB are bound to Na⁺ cations, lessening their concentration in the root zone and assisting in the maintenance of ionic equilibrium. In

accordance with Upadhyay *et al.* (2011) and Kasotia *et al.* (2016), EPS generated via PGPB also aid in the aggregation of soil, retention of moisture, and neutralisation of metallic ions in plant cells under salinity. a further benefit is that EPS assists in the evolution of interactions between plants and microorganisms by bringing in a microenvironment in which microbes can survive in adverse circumstances (Ashraf *et al.*, 2004; Haggag, 2010). Moreover, it increases bacteria's penetration of the plant after interacting with root exudates. Under drought stress, rhizobacteria might generate additional exopolysaccharides than they can in a situation without stress. And as the length and intensity of the drought increased, so did the process of synthesis of exopolysaccharides. It implies that exopolysaccharide formation was a consequence to drought-related stress in microorganisms (Robertson *et al.*, 1992). Sunflower treated with the EPS-producing strain of bacteria YAAF34 under drought stress demonstrated an upsurge in root tissue density (Alami *et al.*, 2000).

According to Upadhyay *et al.*, salt-resistant strains of bacteria *Bacillus* sp. (SKU-3) as well as *Paenibacillus* sp. (SKU 11) released EPS and highlighted PGP traits including phosphate that are dissolved, auxin generation, sugar suppression, proline generation, and total soluble production of sugar, which had an impact on increasing the amount of biomass in inoculated crops when contrasted with uninoculated crops. When rice crops develop colonies under salt stress, the EPS-producing bacteria *Gluconacetobacter diazotrophicus* has been indicated to possess a the ability to protect PGPB from the effects of oxidative stress and to maximise microbial colonisation effectiveness (Meneses *et al.*, 2017). In a new investigation, Chu *et al.* (2019) hypothesised that the salt-resistant *Pseudomonas* PS01 strain might possess a role in the regulation of genes involved in resistance to stress in *Arabidopsis thaliana*. They uncovered that the LOX2 gene, which synthesises the lipoxygenase that is an indispensable component of the pathway that develops jasmonic acid (JA), significantly up-regulated. Bacterial EPS extend additional advantages in order for thriving under salty conditions given that JA itself serves as a beneficial regulator and gets accumulated swiftly in crops (under saline stress).

2.4.4. ISR Enzymes

Plants inoculated with ST-PGPR secrete antioxidative enzymes in them, which reduce the adverse impacts of oxidative stress on the plant (Manaf and Zayed, 2015). According to certain statistics, ST-PGPR produced a significant amount of antioxidant enzymes under salt exposure, including POD, SOD, CAT, nitrate reductase (NR), and glutathione reductase (GR) (Sen and Chandrasekhar, 2015; Ansari *et al.*, 2019). Antioxidants, a combination of enzymatic and non-enzymatic, play an integral part in the process of defence by lowering the amount of ROS. CAT, guaiacol per oxide, SOD, APX, monodehydro-ascorbate reduction, dehydrogenase ascorbate, and the enzyme glutathione reductase are a few of the prominent enzymatic antioxidants. The producers are able to capitalize and profit from the utilization of systemic acquired and systemic derived resistance as a pest mitigation technique. A zigzag model of the co-evolution amongst plants as well as potential microbial diseases has been presented (Jones and Dang 2006).

Induced resistance can also be categorized into induced systemic resistance (ISR) or systemic acquired resistance (SAR). ISR tends to be induced by rhizobacteria that promote plant growth (PGPR), is unrelated to tissue necrosis, and comprises the jasmonic acids (JA) and ethylene (ET) mechanisms (Henry *et al.*, 2012). Systemic acquired resistance can usually be distinguished by regional dying, the regulation of pathogenesis affiliated (PR) protein's genes, and encompasses the pathways of salicylic acid (SA) pathway. Rhizobacteria that encourage plant growth can prevent diseases by generating a systemic plant resistance against both rooted and foliage pathogens and by promoting antagonistic activity amongst bacteria and pathogens transmitted by the soil. When multiple bacteria are present naturally, the induced resistance results in an increase in basal resistance to a number of them at once, which is desirable. It has been reported that a number of certain *Pseudomonas* strains can cause systemic resistance in perennials such Arabidopsis, carnation, cucumber, radish, and tobacco. In addition to prolonging resistance to a wider spectrum of diseases versus ISR or SAR alone, an integrated approach of ISR and SAR may increase defence against infections that are opposed by both pathways (Van loon *et al.*, 2004).

Using a split rooted test, Dutta *et al.*, (2008) determined that the simultaneous administration of RRLJ04 and BS03 alongside the rhizobial bacterium RH2 was far more successful in establishing systemic resistance than either of the treatments individually. Furthermore, Berendsen *et al.* (2018) documented that a triple bacterial consortium provided systemic immunity against the *Hyaloperonospora arabidopsidis* and contributed to the development of the plant, despite the fact that the *Stenotrophomonas sp.*, as well as a *Microbacterium sp.* didn't independently have an impact on the plant. It has been verified that the Phytophthora blight on squash brought on by *Phytophthora capsici* can be efficiently suppressed by PGPR-mediated generated systemic resistance (Zhang *et al.*, 2010).

2.5 SALINE TOLERANT MICROBES FOR DEVELOPING CROP PRODUCTIVITY

It has become commonly accepted that ST-PGPR possess an innate capacity to successfully cope with high soil salt concentrations. Plants gain immediate advantages from their habitat in the environment. The inclusion of them in bioinoculants and bioformulations maximizes the production of crops while additionally easing plant survival in exceedingly salinated habitats (Table 2.1).

Table 2.1: Plant growth promoting saline tolerant bacteria

CROP	BACTERIAL STRAIN	PGP CHARACTERISTICS	REFERENCES
Sunflower	<i>P. fluorescens</i> <i>Pseudomonas aeruginosa</i>	IAA and siderophore production, phosphate solubilization, nitrogen fixation, HCN, chitinase and β -1-3 glucanase activity.	Shilev <i>et al.</i> , 2012; Tewari and Arora, 2014b; Tewari and Arora, 2016
Tomato	<i>Achromobacter piechaudii</i>	Increased plant growth and fewer	Mayak <i>et al.</i> , (2004)

		ethylene levels.	
Maize	<i>Rhizobium</i> and <i>Pseudomonas</i>	Lowers electrolyte leakage, stimulates proline production, and selectively absorbs ions while maintaining the water level.	Bano and Fatima (2009)
Peanuts	<i>Arachis hypogaea</i>	Siderophore and IAA production.	Shilev <i>et al.</i> , 2020
Soybean	<i>Bradyrhizobium japonicum</i> , <i>Bacillus subtilis</i> SU-12 and <i>Serratia proteamaculans</i> . <i>Pseudomonas simiae</i> <i>B. japonicum</i> and <i>B. subtilis</i> <i>P. fluorescens</i>	Gibberellins, salicylic acid, and ABA production.	Kang <i>et al.</i> , 2014 Egamberdieva <i>et al.</i> , 2017a El-Esawi <i>et al.</i> , 2018a Han and Lee, 2005 Vaishnav <i>et al.</i> , 2016 Han and Lee, 2005 Bhattacharyya and Jha, 2012
Lettuce	<i>Pseudomonas mendocina</i>	ACC deaminase activity.	Kohler <i>et al.</i> , (2009)
Cucumber	<i>Burkholderia cepacia</i> SE4, <i>Promicromonospora</i> sp. SE188 and <i>Acinetobacter calcoaceticus</i> SE370.	Reduced the osmotic pressure and ethylene production. catalase, oxidase, total phenolics, and total polyphenol production.	Kang <i>et al.</i> , (2014a)

Barley	<i>Hordeum vulgare</i>	Phosphate hydrolysis and IAA production	Cardinale <i>et al.</i> , (2015).
Groundnut	<i>P. aeruginosa</i> AMAAS57 and <i>P. aeruginosa</i> BM6 <i>B. saurashtrense</i> , <i>B. casei</i> , <i>Haererothalobacter</i> , <i>Klebsiella</i> , <i>Pseudomonas</i> , <i>Agrobacterium</i> , and <i>Ochrobactrum</i> .	Production of free amino acids, IAA, Hydrogen cyanide, ammonia, phosphate solubilization, phenol and ACC deaminase.	Ghorai <i>et al.</i> , 2015 Shukla <i>et al.</i> , 2012 Sharma <i>et al.</i> , 2016
Lettuce	<i>Azospirillum</i> <i>Pseudomonas</i>	Ascorbic acid production.	Fasciglione <i>et al.</i> , (2012) Kohler <i>et al.</i> , (2009)
Rice	<i>P. pseudoalcaligenes</i> and <i>B. pumilus</i> <i>Halobacillus dabanensis</i> strain SB-26, <i>Halobacillus</i> sp. GSP 34 <i>B. amyloliquefaciens</i> NBRISN13 (SN13)	Production of siderophore, IAA, HCN and calcium solubilization.	Jha and Subramanian, 2013 Sarkar <i>et al.</i> , 2018 Sultana <i>et al.</i> , 2018 Rima <i>et al.</i> , 2018 Misra <i>et al.</i> , 2017 Jha <i>et al.</i> , (2011)
Capsicum annum L	<i>Bacillus</i> sp.	Ethylene production.	Wang <i>et al.</i> , (2018)
Strawberry	<i>Bacillus</i> sp.	Fruit yield, leaf water content, ionic	Karlidag <i>et al.</i> , 2010

		makeup, and membrane permeability were enhanced	
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Increased length of roots, root surface area, and quantity as a consequence of PGPR synthesis of indole acetic acid, gibberellins, and other undetermined variables promote the uptake of nutrients and plant well-being under stressful circumstances (Egamberdieva and Kucharova, 2009). Under salinity, tomato, pepper, canola, bean, and lettuce have been revealed to grow more effectively when bacteria which encourage plant growth have been identified (Barassi *et al.*, 2006; Yildirim and Taylor, 2005). In comparison to non-inoculated control plants, rice cultivars treated with *Bacillus amyloliquefaciens* established substantial enhancements in their growth factors under salinity stress (Shahzad *et al.*, 2018).

The application of this bacterium into the soyabean crop elevated the length of the roots and shoots as well as dry mass during saline conditions of stress, representing that PGPR is an optimistic bio-fertilizer in the salt impacted soil. The halotolerant bacteria was obtained and distinguished by a high the amino acid proline production as well as other hormones produced by plants such as ABA, trans-zeatain riboside, the GA3 and IAA (Naz *et al.*, 2009). Plenty of microorganisms dwelling on high-salt-content crops illustrate modifications to salinity stress and do exceptionally well in these types of circumstances. These tend to be referred to as the halophyte microbiome. The propensity of halophilic bacteria to continue preserving structure of proteins and enzyme functioning for a wide range of reactions related to metabolism even when exposed to elevated levels of salt is one of their most fundamental adaptations (Ruppel *et al.*, 2013).

In the agro-farming system, PGPB can be successfully assigned as a replacement approach to control a vast majority of the aforementioned abiotic stresses encountered by crops, to improve their yields, and to produce the least engagement of

artificial fertilisers (Majeed *et al.*, 2018). With the application of PGPB, improved development of plants has been pulled off in saline soils.

2.6 SALINE TOLERANT MICROBES: FUTURE PERSPECTIVES

In order to mitigate salt-related stress and increase the production of crops in salty soils, PGPB has evolved into a potent strategy (Arora *et al.*, 2021). Although great work has been accomplished in comprehending how salinity damages vegetation, there hasn't been much success in addressing productivity losses in an ecologically sound manner. The long-standing goal of increasing crop yield in saline agro-ecosystems has been capitalised on, and this has led to endeavours to clarify the underlying processes of osmo-adaptation through ST-PGPR (Paul, 2013). Conventional methods of integrating PGPR as fertilisers are fruitless given that 90% of them are lost to the atmosphere over implementation, they are sensitive of environmental conditions (heat, ultraviolet (UV) rays, etc.), and they elevate application costs for the farmer considering run-off. Employing nanoencapsulation technology, PGPR could be insured while also having their lifespan extended and dispersion in fertiliser formulation optimised. This would make it possible for the PGPR to be discharged in a controlled environment (Pravin *et al.*, 2016).

A research investigation of the root system microbiota may also shed more insight into well-known PGPB taxonomic classification that show elevated PGP activity when subjected to high salt concentrations (Sapre *et al.*, 2019). In addition, it may provide insightful data on novel PGPB strains with a high salt tolerance that might be adopted as viable commercial inoculation agents to help plants cope with salt stresses (Yuan *et al.*, 2019; Goswami *et al.*, 2020). Consortia-based bioinoculants are currently becoming more significant because they enable concurrently processed consumption of nutrients, suppression of phytopathogens, as well as management of salinity stress (Woo and Pepe, 2018). Additionally, it has been postulated that using a variety of microbes in consortial formulations, such as PGPR, mycorrhiza, and endosymbionts, can be a very successful method to help crops cope against stress (Ilangumaran and Smith, 2017).

Additionally, scientific techniques like genetic modification could be used in order to regulate the expression of genes in PGPB for enhanced characteristics that promote plant development as well as greater tolerance to salt (Kashyap *et al.*, 2019). The discovery and production of salt-tolerant, halophilic PGPBs could help us realize our goals for food security and the averting of agro-ecosystem desertification while additionally improving the productivity of saline soils.

CHAPTER-3

AIM AND OBJECTIVE OF THE STUDY

1. To characterize the saline tolerant ability of Southern Ocean (SO) bacterial strains for their plant growth promoting and antagonistic activity.
2. Molecular diversity of SO bacterial strains based on REP-ERIC-BOX PCR analysis.
3. *In-planta* and pot culture based plant growth promotion of potential SO bacterial strains under saline condition.
4. Cultural, microscopic and molecular identification of potential plant growth promoting bacteria.
5. Identification of volatile organic compounds from potential bacterial strain involved in pathogenic antagonism and plant growth promotion.

CHAPTER 4

MATERIALS AND METHODS

4.1. COLLECTION OF SAMPLE

A total of 16 bacterial strains were already isolated from Southern Ocean Sea Ice samples collected during 11th Indian sector of Southern Ocean Expedition (ISOE) by our Centre for Drug Discovery and Development Scientists. They were cultured in Nutrient agar, ISP2 agar and SCA agar medium by standard microbiological techniques. All 16 bacterial strains were sub-cultured and preserved as glycerol stock at -80C in the Bioprospecting division, Centre for Drug Discovery and Development, Sathyabama Institute of Science and Technology, Chennai.

4.2. SCREENING OF SALT TOLERANCE

The resistance of the bacterial isolates to salinity was performed by observing the growth on Nutrient agar medium amended with various concentrations of NaCl (5%, 10% and 20%). The plates were then incubated for 96 hours at $28 \pm 2^\circ\text{C}$, after which the growth on NaCl amended media was recorded.

4.3. SCREENING OF BACTERIAL ISOLATES FOR PLANT GROWTH PROMOTING ACTIVITIES

4.3.1. IAA production

The production of IAA by salt tolerant bacterial strains was evaluated spectrophotometrically. The bacterial strains were inoculated into 50 ml centrifuge tubes containing 10ml of czapek-dox broth. After incubation for 24 hours, the inoculated broth was centrifuged. The supernatant was mixed with 2ml of Salkowski's reagent (1 ml of 0.5 M FeCl_3 in 50 mL of 35% HClO_4). This mixture was incubated at room temperature for 20 minutes and the absorbance was recorded at 535 nm.

4.3.2. Siderophore production

The bacterial strains were spot inoculated on Nutrient agar medium amended with the tertiary complex chrome azural S (CAS) as an indicator and was incubated at

28 ± 2°C for 96 hours. Change of fluorescent yellow or orange colour surrounding the bacterial colonies were to be observed which indicated the production of siderophore.

4.4. SCREENING FOR HYDROLYTIC ENZYME PRODUCTION ACTIVITY

4.4.1. Cellulase Production Activity

CMC agar medium was used for the Qualitative screening of cellulose activity. The CMC medium was made by the addition of Carboxymethyl cellulose (1.5g), Monopotassium phosphate (0.15g), Dipotassium phosphate (0.15g), Magnesium sulphate (0.06g), Sodium chloride (0.007g) Ferrous sulphate (0.0001g) and Agar agar (3.4g). This mixture was then autoclaved and poured onto plates. The isolates were spot inoculated on the medium and incubated for 2 days at 28°C. After cell growth was observed, the presence of extracellular cellulase was detected by flooding the plates with 1% Congo red solution for 15 minutes. Followed by, destaining of the plates with 1 M NaCl for 15 minutes. They were then visualized for halo zone which indicates cellulase production.

4.4.2. Amylase production activity

For screening of Amylase producing bacterial strains, starch hydrolysis test was done. All the bacterial isolates were spot inoculated on Nutrient agar medium plates amended with 1% starch. The plates were then incubated for 7 days at 28°C. After incubation period, the plates were flooded with Iodine solution. The plates were kept undisturbed in room temperature for 10-15 minutes after which the iodine solution was discarded. The emergence of a distinct halo surrounding the bacterial colonies indicates that the bacterial isolates have produced amylase.

4.4.3. Protease production activity

Skim milk agar medium was used for quantification of protease production by bacterial isolates. All the bacterial isolates were spot inoculated on Nutrient agar medium amended with 1% skim milk powder. The plates were incubated at 30°C for 4 days. The development of a halo zone encompassing the bacterial colonies was thought to be a sign indicating protease was being produced.

4.5. SCREENING OF ANTAGONISTIC ACTIVITY OF BACTERIAL ISOLATES

4.5.1. Antibacterial activity against *Ralstonia solanacearum*

The antagonistic activity against the bacterial pathogen *Ralstonia solanacearum* was carried out by agar well diffusion method on Mueller hinton agar medium for all 16 bacterial isolates. In this assay, 48-hour grown *Ralstonia solanacearum* strain was inoculated in 1ml of freshly prepared mueller hinton broth. The mueller hinton agar was prepared, sterilized by placing in autoclave and poured into plates, which were then allowed to solidify. After solidification, the agar plate surface was inoculated by spreading a volume of the microbial inoculum over the entire surface of the plate using a cotton swab. After which wells were punched out on this agar surface aseptically with a sterile cork bearer. Each bacterial isolate was inoculated in 500 distilled water separately. 100µl of this mixture was then dispensed into the wells made before hand on the agar plate surface. The agar plates were then incubated for 24 hours at 37°C. After 24 hours the plates were observed for their zone of inhibition.

4.5.2. Antifungal activity against *Colletotrichum gloeosporioides*

A dual culture test was used to assess the antagonist action of salt-resistant strains of bacteria against the fungal pathogen *Colletotrichum gloeosporioides*. On potato dextrose agar, the fungi pathogens were grown and incubated for 72 hours. After incubation, the fungal pathogen agar plug was placed at the centre of the freshly prepared petri dish containing potato dextrose agar. The bacterial isolates were inoculated 4 cm away from the plug in the corner of each plate respectively. Plates without the test isolate served as control and these test plates were incubated at $30 \pm 2^{\circ}\text{C}$ for 7 days. After this incubation period, the zone of inhibition was measured and percentage of inhibition was calculated by using the formula:

$$\text{PI} = [(C-T)/C] \times 100$$

Where, PI is the percentage of inhibition, C is the growth of fungal pathogen on control and T is the growth of fungal pathogen towards the bacterial isolate.

4.6. MOLECULAR DIVERSITY OF SALT TOLERANT BACTERIAL STRAINS

4.6.1. Isolation of genomic DNA

The chelex-100 microwave method was employed for extraction of genomic DNA from all 16 bacterial isolates. Each strain was sub-cultured on freshly prepared Nutrient agar medium plates, a day prior to isolation of genomic DNA. Three to four loopful of each culture strain was inoculated in 1.5ml of sterile distilled water in respective centrifuge tubes. The samples were centrifuged at 10,000rpm for 5 minutes at 4°C. The supernatant was discarded and the pellets were washed with 500µL of TE buffer (10 mM TRIS-Base, pH 7.5; 1 mM EDTA, pH 8.0). This mixture was centrifuged again at 10,000rpm for 5 minutes at 4°C. After centrifugation the supernatant was discarded and the pellets were re-suspended in 50µL TES buffer (10 mM TRIS-Base, pH 7.5; 1 mM EDTA, pH 8.0; 0.5% SDS). These tubes were then placed in microwave oven for 15 seconds at 625 W (Power Level 6). Immediately after removing the samples from microwave 150µg proteinase K and 20 µg RNase A were added to each of the suspension respectively. These suspensions were then microwaved again under the same power conditions. Following lysis, the suspensions were incubated at room temperature for 2 minutes. A mixture of 25mg of chelex 100 and 150 µL of TE buffer was added to each of the suspensions. The suspensions were then microwaved again under the aforementioned conditions and centrifuged at 10,000rpm for 10 minutes at 4°C. After centrifugation the supernatant was recovered for further proceedings.

4.6.2. Agarose gel electrophoresis

The agarose gel electrophoresis was performed in order to evaluate the band integrity and for crude visualization to ensure the presence of DNA. The gel trough was cleaned with ethanol and the ends were sealed with cellophane tape and the combs were placed in the respective positions to form wells. 0.24g of agarose was weighed and dissolved in 30ml of 1× TAE buffer (0.8% agarose gel). The agarose was melted in microwave oven and 1 µl of ethidium bromide was added to the molten agarose. This was mixed well and poured on to the sealed trough and allowed to set in the dark. After 20 minutes, when the gel gets solidified, the cellophane tape and combs were removed and the trough was placed in electrophoresis tank containing 250ml of 1× TAE buffer.

8µl of resuspended DNA was mixed with 2µl of 0.1% bromophenol blue and loaded into the wells. 1µl of molecular weight marker diluted with 4µl of 1× TAE buffer and mixed with 3µl of bromophenol blue and loaded into the wells. The electrophoresis was run at 120V for 25 minutes. Once the run was over, the gel was placed under UV and the DNA bands were visualized, where a single band without smear was considered as integral gDNA. The gel was then discarded following safety regulations for carcinogenic material in a separate disposal.

4.6.3. PCR amplification

One bacterial strain was chosen as potential strain since it showed maximum range of activity against fungal pathogen and broad range of plant growth promoting traits. 16S rDNA analysis was done for molecular identification of this potential isolate using a PCR Thermal cycler. The PCR amplification was done using the universal primers 27F (5-AGAGTTTGATCTTGGCTCAG-3) and 1492R (5-GGT TAC CTT GTT ACG ACT T-3). To decrease the pipetting errors a 25µL PCR master mix was prepared by mixing 10x buffer (2.5µL), 10mM dNTPs (0.5µL), 10 pM forward primer (0.5µL), 10pM reverse primer (0.5µL), DNA polymerase (0.125µL), Magnesium chloride (0.5µL), template DNA (1µL) and distilled water (18.125µL). The PCR mastermix was gently vortexed prior to running PCR reaction. The PCR conditions were adjusted with an initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation (94°C for 10 seconds), annealing (55°C for 30 seconds) and extension (72°C for 3 minutes). 2% agarose gel was prepared by dissolving 2g of agarose in 100ml 1× TAE buffer. The agarose gel electrophoresis was run and the bands were observed under UV transilluminator.

4.6.4. REP-ERIC-BOX PCR

After identification of sub-species level through multiplex-PCR, genetic variability analysis was performed for all 16 isolates using Rep-PCR approach. The BER primers (BOX, ERIC and REP) were used in repetitive sequence based PCR fingerprint method. For DNA amplification, 25µL PCR master mix was prepared by mixing 5x buffer (5µL), 400µM of deoxynucleoside triphosphate mix, 0.4µM of primer, 2mM of magnesium

chloride, 2.5 U of Taq DNA polymerase and 5µL of DNA template. The PCR conditions were adjusted with an initial denaturation at 95°C for 7 minutes, followed by 30 cycles of denaturation (94°C for 1 minute). Annealing was done at 53°C (1min) for BOX, 40°C (1min) for REP and 52°C (1min) for ERIC primers. For all three primers extension was done at 65°C for 15 minutes before cooling at 4°C. A 10 µL aliquot of the generated products of PCR was resolved for 4 hours at 80 V in 2% agarose in 1% Tris acetate EDTA buffer. Ethidium bromide was used to stain the gel, and a Gel documentation equipment was used to take pictures. For the purpose of comparison of the molecule sizes of the electrophoresis-generated fragments, a 1kb (1–3,000 kb) DNA ladder was utilized. Each strain's presence or absence depending on presence/absence of a band was given a score of 1 (positive) and 0 (negative) respectively. The Unweighted Pair Group Approach with Arithmetic Averages was employed for cluster analysis. Similarities were determined using the Jaccard's coefficient in the (UPGMA) utilising the NTSYS-PC software, version 2.1.

4.7. *IN-PLANTA* EVALUATION OF POTENTIAL STRAINS FOR PLANT GROWTH PROMOTION

4.7.1. *Seeds bio-priming*

The tomato seeds were surface sterilized prior to bio-priming. The seeds were first washed in sterile distilled water, followed by washing with 70% ethanol. The seeds were then disinfected with 2% sodium hypochloride and washed again with autoclaved distilled water. These surface sterilized seeds were then allowed to air-dry. Nutrient broth was prepared in separate Erlenmeyer flasks and sterilized by autoclaving prior to seeds bio-priming. Five bacterial strains were selected as potential strains based on their antagonistic activity and plant growth promoting traits. Each of the five strains were inoculated in 50ml Nutrient broth separately and was placed in rotating shaker at 150rpm for 48 hours. After growth was observed the broth was centrifuged at 10,000rpm for 10 minutes and the supernatant was discarded. To each of the pellet, 0.5% salt treatment (0.5g NaCl in 100ml distilled water) was given. The surface sterilized seeds were then added to the salt-treated pellets. They were placed in rotatory shaker for 24 hours. Seeds which were not treated with bacteria and salt

solution was used as control. After 24 hours incubation in rotary shaker, the treated seeds were placed in separate petri dishes and were allowed to air-dry before sowing.

4.7.2. Seed germination

The efficacy of 5 selected strains in promoting plant growth were evaluated in seed germination assay. 100ml of agar medium was prepared in Erlenmeyer flask and autoclaved. 5ml of this medium was poured in each of the sterilized test tubes and allowed to solidify. In each of the tubes, 2 bio-primed seeds were placed using a forcep. The uninoculated seeds were used as control. The tubes were kept in a sterile tissue culture space at 26°C with regulated lighting for 7 days. The tubes were initially kept in darkness for first three days due to water inhibition period. The tubes were then placed in light for next four days due to radicle protrusion period. After 20 days, the root and shoot length can be measured and the total chlorophyll content can be detected.

4.7.3. Measurement of total chlorophyll

The tomato seedlings grown in test tubes were removed carefully along with their roots intact with the help of forceps. 200 ml of 80% acetone solution was prepared by adding 140ml acetone with 60ml distilled water. 1g of the seedling was measured and placed in a clean mortar and pestle. It was grinded to a fine pulp after the addition of 20ml of freshly prepared acetone. Before grinding each seedling, the mortar and pestle were cleaned using water. This mixture was then centrifuged at 10,000rpm for 5 minutes and the supernatant was retrieved. The absorbance of the solutions were measured at 645 and 663nm in BioTek Epoch 2 Microplate Spectrophotometer and the solvent (80% acetone) was used as the blank. The chlorophyll content of each seedling was calculated by using the formula:

$$\text{Total chlorophyll} = (\text{Absorbance at 645}) 20.2 + (\text{Absorbance at 663}) 8.02$$

4.8. MOLECULAR IDENTIFICATION OF POTENTIAL STRAIN

The bacterial strain which showed prominent plant growth promoting traits, antagonistic activity and good germination was selected as potential strain. This strain was subjected to morphological characterization.

4.8.1. Grams staining

The potential bacterial isolate was sub-cultured on a freshly prepared Nutrient agar medium plate, prior to gram's staining. A glass slide was cleaned with ethanol and loopful of water was placed in the middle of the glass slide. Using a loop, the bacterial colony on the plate was touched and was mixed with the water on the slide until there is a thin, uniform film of microorganism on the slide. The slide was allowed to air dry. It was then heat fixed and allowed to cool. The slide was flood with crystal violet for 1 minute and slide was washed with water. Iodine solution was applied by flooding the slide with iodone for a minute and was rinsed with water immediately after. Flood the slide with Gram Stain Decolorizer to decolorize it for 3 seconds. Rinse with water immediately afterward. The slide was flood with Safranin counterstain and was allowed to sit for 30 seconds before rinsing with water. The slide was then air dried and the organism was observed under the oil immersion objective of the microscope.

4.8.2. KOH test

The potential bacterial strain was sub-cultured on a freshly prepared Nutrient agar medium plate prior to KOH test. 3% sodium hydroxide solution was prepared by addition of 3g KOH in 100 ml distilled water. This solution was sterilized by placing in autoclave. A glass slide was taken and cleaned with ethanol prior to use. One drop of KOH solution was placed in the centre of the sterilized glass slide. A loopful of the bacterial strain was taken from the plate and stirred onto the KOH solution. With KOH positive isolates, within the first 30 seconds, a viscous coating of a mixture composed up of microbial cells and dissolved components would attach to the loop and create a string 1–5 cm long.

4.8.3. Sequencing of 16S rRNA

Sanger sequencing was done (Sanger dideoxy chain terminator sequencing) in an automated DNA sequence. In this technique of sequencing four reaction mixtures were prepared, each consisting of only one ddNTP and was labelled with four different fluorescent dyes so that only one reaction mixture was required. On application of electric current to the gel, the negatively charged 16S rRNA fragments mitigated

towards the positive end which resulted in the illumination of the rRNA fragments with a laser beam. A special recorder was used for capturing the light emitted from different bases of specified wavelengths on the excitation of the fluorescent dyes. By determining the order of the colours emitted from specific peaks as they crossed the detector, the sequences were read. This sequence was analyzed after the information was fed directly to the computer systems. The computer then generates a tracing electrogram of the light emitted by the four dyes and hence the computer converts the data into nucleotide sequences.

4.8.3.1. 16S rRNA sequence analysis

Using the DNA baser v5.2, partial sequences gotten with reverse and forward primers were assembled to produce full-length sequences (1400–1500 bp). The sequence's homology was identified using the BLAST search tool from the NCBI database which was used for comparing for similarity level. Following BLAST analysis, a dataset with potential orthologs was generated by taking into account database sequences that shared more than 99% of the query sequence's sequence identity.

4.8.3.2. Phylogenetic analysis

Using software from the MEGA version 4 package, a phylogenetic evaluation of a potential strain was performed. The potential strain 16S rRNA sequence was matched with the appropriate nucleotide sequence obtained from GenBank. The neighbour joining approach was used to create evolutionary distance matrices and construct a phylogenetic tree. By using a bootstrap methodology with a sample size of 1000 neighbours, tree topologies were assessed.

4.8.3.3. Nucleotide accession number

The partial 16S rRNA nucleotide sequence of the potential bacterial strain was deposited at GenBank database by using NCBI submission portal.

4.9. VOLATILE COMPOUNDS IDENTIFICATION FROM POTENTIAL STRAIN

The instrument used for headspace GC-MS was Gas chromatography (Agilent:GC:(G3440A) 5975 MS equipped with Mass spectrometry detector with Headspace. 1 gm of the homogenized sample (Seaice-43) was weighed to the nearest 0.01 g, vial with PTFE-faced septa and seal, to screen the bacterial sample by GC-MS

with headspace. By inserting the vial into the HS auto sampler, analysis was done using the headspace technique with column - VF 624 ms 60m 0.25mm, 1.8 μ m. Helium (99.999%) was used as carrier gas at a constant flow of 1.0 ml/min. A linear velocity mode with a constant flow of 1.0 mL/min was used to operate the injection port. The oven's temperature was scheduled to rise from 50°C (isothermal at 0.0 min) to 240 °C (isothermal for 1.0 min) with a 6°C/min increase. After holding the temperature at 50 °C for one minute, temperature was ramped up to 240°C (with an increase of 6°C/min). The temperature of the ion source was 280°C, the tuning file or 70e, was related to the detector voltage.

4.9.1. Statistical analysis

From the data received through Headspace GC-MS, the statistical analysis was carried out. The volatile compounds produced by the potential strain was interpreted and analyzed with existing data to intercept whether or not the volatile compounds provide additional support for the plant growth promoting capacity of the potential strain.

CHAPTER 5

RESULTS AND DISCUSSION

5.1. SCREENING OF SALT TOLERANCE

On nutrient-agar medium plates supplemented with various amounts of NaCl (5%, 10%, 20%), an overall of 16 isolates of bacteria were assessed for their capacity to survive under the salt. It was found that all Sixteen strains had the ability to grow at 5% salt concentrations after 96 hours of incubation at 28^oC, based on their growth in the streak line (Fig 5.1). Ten isolates-Seaice-37, Seaice-45, Seaice-31, Seaice-13, Seaice- 5, Seaice-16, Seaice-39, Seaice-35, and Seaice-14, out of 16 were able to grow at 10% salt concentration (Fig 5.2). Only the Seaice strains 43, 45, and 12 exhibited a wide range of growth at a 20% concentration (Fig 5.3). The common ice plant *Mesembryanthemum crystallinum* L. was used as the source for the rhizosphere bacteria that Mahmood and colleagues (2019) identified and tested. Eighty of the 152 isolated strains that were used in the study were tolerant of the soil salinity. *Bacillus* sp. PR-6 and *Streptomyces* sp. PR-3 bacterial strains were effective at combating the salinity of the soil (1250 mM NaCl). *Pseudomonas plecoglossicida* strain Pp20's ability to reduce the harm caused by salt and aluminium to maize roots was investigated by Zerrouk *et al.* These investigators revealed that Pp20 might broaden at levels of sodium chloride that varied from 50 – 600 mM.

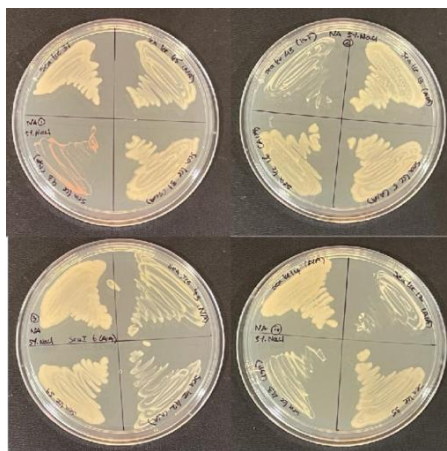


Fig 5.1: Growth of SO bacterial isolates under 5% salt concentration.

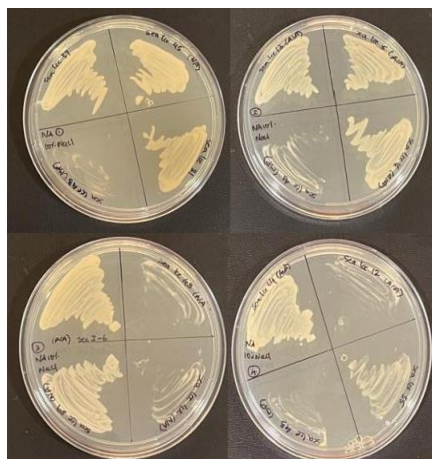


Fig 5.2: Growth of SO bacterial isolates under 10% salt concentration.



Fig 5.3: Growth of SO bacterial isolates under 20% salt concentration.

Table 5.1: Growth of 16 bacterial isolates on Nutrient agar media amended with 5%, 10% and 20% NaCl

S.NO	SAMPLE	5% NaCl	10% NaCl	20% NaCl
1	Sea ice 37	+	+	-
2	sea ice 45	+	+	+
3	Sea ice 31	+	+	-
4	Sea ice 43	+	-	+
5	Sea ice 3	+	-	+
6	Sea ice 13	+	+	-
7	Sea ice 5	+	+	-

8	Sea ice 16	+	+	-
9	Sea ice 42	+	-	-
10	Sea ice 6	+	+	-
11	Sea ice 4	+	-	-
12	Sea ice 39	+	+	-
13	Sea ice 12	-	-	+
14	Sea ice 35	+	+	-
15	Sea ice 7	-	-	-
16	Sea ice 14	+	+	-

5.2. SCREENING OF BACTERIAL ISOLATES FOR PLANT GROWTH PROMOTING ACTIVITIES

5.2.1. IAA production

The potential to synthesize IAA is one of the features of microorganisms that promote plant growth. For the division and elongation of cells in plants enduring salt stress, the ST-PGPR generate IAA (Vejan *et al.*, 2016). In our study, all 16 isolates were tested for their ability to produce IAA (Fig 5.4). The SO bacterial strains were observed to produce the phytohormones in a range between 40.19µg/ml to 118.46µg/ml. Out of 16 strains, seaice-37 showed the highest production of IAA with 118.46µg/ml followed by Seaice-43 which showed second highest production value of IAA with 107.31µg/ml. The third highest production of IAA, 76.92µg/ml was observed in the isolate Seaice-14. The lowest IAA production was seen in the isolate Seaice-13 with the production value of 40.19 µg/ml (Table 5.2). According to a recent study by Nutaratat *et al.*, 2017, under optimum culture conditions, *Enterobacter* sp. could secrete IAA up to 5561.7 g/mL. In a different study, Samayoa *et al.*, 2020, researchers took samples from onion bulbs, the rhizosphere, and endophytic roots and extracted and identified 23 strains having PGP attributes. The strains of bacteria that developed the highest concentrations of IAA were derived from the rhizosphere soil (111.8 mg mL⁻¹) and the root endophyte (103.8 mg mL⁻¹ on average), whereas none were isolated from the

bulbs (6.8 mg mL⁻¹ on average). The most IAA, 527 and 462 mg mL⁻¹, were generated by *Bacillus megaterium* (ORE8) and *Pantoea* sp. (ORTB2), which enhanced the radicle and hypocotyl lengths in onions and indicated to be the most promising biofertilizers of onions. In a different study, *Kocuria flava* FA10 was investigated for various PGP characteristics by Marchawala *et al.* in 2015. It produced Indole-3-acetic acid (IAA) with a concentration as high as 102 g/ml, the highest level of any PGP characteristic studied. It was also adept at inhibiting an array of fungal phytopathogens, making it a promising PGP bacterium as well as biocontrol agent.

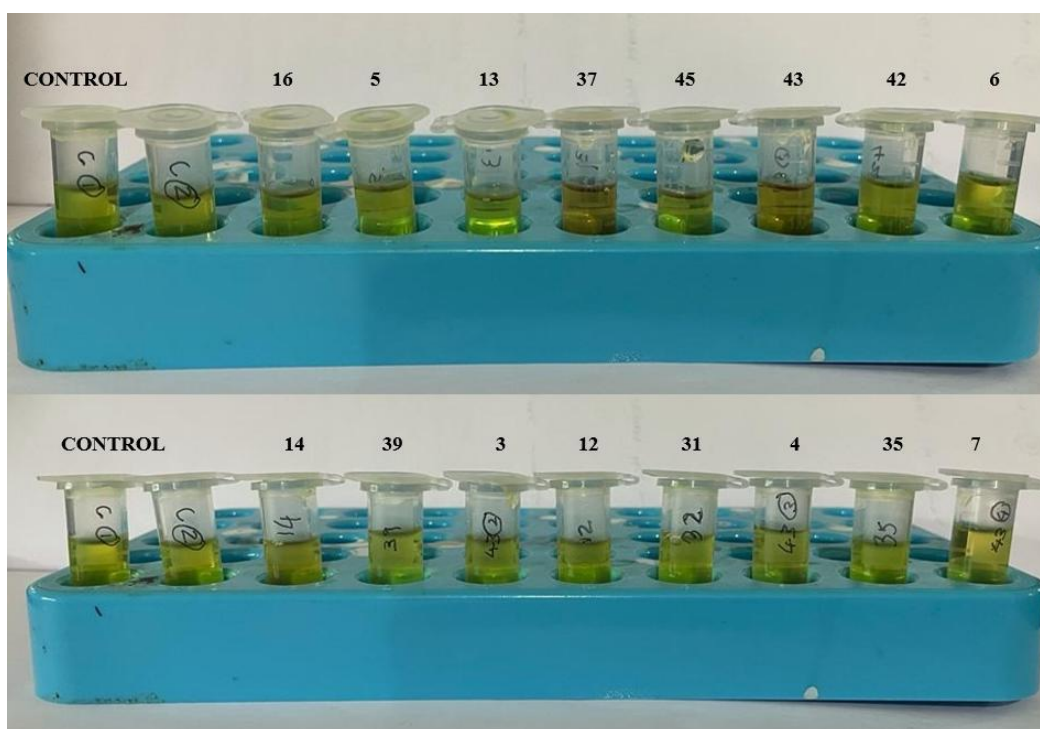


Fig 5.4: Qualitative testing of IAA production by observing colour change

Table 5.2: Indole acetic activity assay

S.NO	SAMPLE	R1	R2	AVERAGE	µg/µl
1.	Sea ice 16	0.176	0.145	0.1605	61.73
2.	Sea ice 5	0.138	0.143	0.1405	54.04
3.	Sea ice 13	0.105	0.104	0.1045	40.19
4.	Sea ice 37	0.298	0.318	0.308	118.46

5.	Sea ice 45	0.151	0.150	0.1505	57.88
6.	Sea ice 43	0.284	0.274	0.279	107.31
7.	Sea ice 42	0.153	0.160	0.1565	60.19
8.	Sea ice 6	0.130	0.130	0.13	50.00
9.	Sea ice 14	0.196	0.204	0.2	76.92
10.	Sea ice 39	0.127	0.127	0.127	48.85
11.	Sea ice 3	0.153	0.152	0.1525	58.65
12.	Sea ice 12	0.149	0.153	0.151	58.08
13.	Sea ice 31	0.155	0.148	0.1515	58.27
14.	Sea ice 4	0.153	0.159	0.156	60.00
15.17	Sea ice 35	0.141	0.138	0.1395	53.65
16.18	Sea ice 7	0.156	0.180	0.168	64.62

5.2.2. Siderophore production

One of the biocontrol features against pathogenic microorganisms under iron-limiting settings is the siderophore produced via PGPR. A relatively small quantity of siderophore with a strong affinity for iron can be produced through PGPRs. The colour shift of the CAS reagent around the region where the bacterial isolate was previously spot-inoculated in CAS agar plates, from blue to yellow / orange was employed for screening the siderophore positive isolates (Fig 5.5). Out of 16 isolates, 10 bacterial isolates were observed to show siderophore production. The two strains with highest siderophore productivity were Seaice-43 and Seaice-6 with predominant colour change from blue to yellow. In total, four siderophore-producing bacteria were detected in rhizospheric soil samples from various locations in Odisha, by Pahari et al., 2017, and BGBA-1 was shown to be the most productive of these (76.67% SU) which was identified as genus *Bacillus* using biochemical and enzymatic analysis. The prospective isolate were then tested with various vegetables to measure the root length, shoot length, and germination percentage which displayed a noticeable rise that was statistically significant. Studies have demonstrated the purpose of siderophores as iron chelators. For example, Saha et al., 2016, evaluated that siderophores from

Chryseobacterium sp. C138 were successful in supplying iron to tomato plants when they were transported to the roots. Sultana et al., 2021, evaluated the siderophore- producing capacity of four salt-tolerant PGPR isolated from the root zone of salt-prone rice fields in Bangladesh. The results of this study revealed that *B. aryabhatai* MS3 was the best PGPR at producing siderophores (60%) under non-saline conditions. This ability, which is able to function even in salinity-stressed conditions, can be used as a biofertilizer to aid the development of plants in response to changing climatic conditions, particularly in coastal regions.

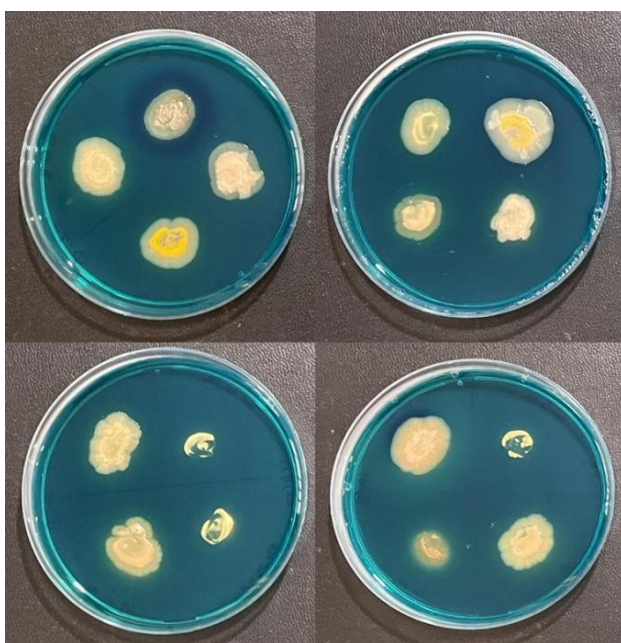


Fig 5.5: Siderophore production tested on 16 bacterial isolates

Table 5.3: Siderophore production potentiality of bacterial isolates

S.NO	SAMPLE	SIDEROPHORE
1	Sea ice 37	+
2	sea ice 45	+
3	Sea ice 31	+
4	Sea ice 43	+
5	Sea ice 3	-

6	Sea ice 13	+
7	Sea ice 5	+
8	Sea ice 16	+
9	Sea ice 42	-
10	Sea ice 6	+
11	Sea ice 4	-
12	Sea ice 39	+
13	Sea ice 12	-
14	Sea ice 35	-
15	Sea ice 7	-
16	Sea ice 14	+

5.3. SCREENING FOR HYDROLYTIC ENZYME PRODUCTION

5.3.1. Cellulase Activity

Out of 16 isolates, only four isolates including, Seaice-43, Seaice-42, Seaice-14 and Seaice-35 showed moderate production of cellulase. Similarly, cellulolytic bacteria were collected from peat soils in Ogan Komering Ilir, South Sumatera, Indonesia for a study by Lisdiyanti et al. in 2012, and their ability to break down cellulose was tested using 1% CMC. *Paenibacillus elgii* S3B40, one of four bacterial isolates chosen for their high cellulase activities, has the highest cellulase activity; its optimum activity at pH 8 and temperature 60°C was 14.5 U/mL (Lisdiyanti *et al.*, 2012). A total of 159 soil cellulolytic bacterial strains were isolated by Shamshitov *et al.*, (2020) and Cellulolytic activity was estimated using qualitative assays such as Congo red, Gram's iodine solution, and sugar utilisation pattern. According to the results of all three screening assays, sixty-four, thirty-eight and fifty-one isolates were able to degrade at some level of cellulose, respectively.

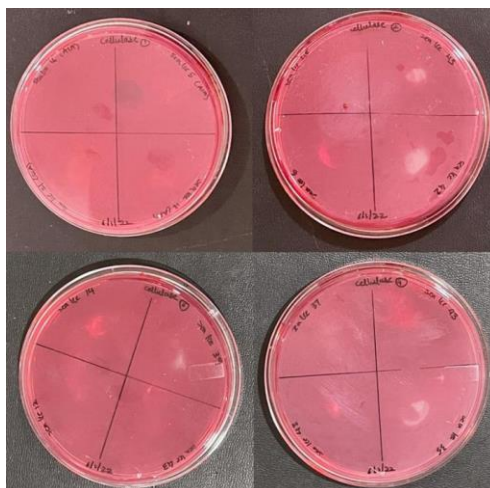


Fig 5.6: Congo red clearing zone assay

Table 5.4: Cellulase Production Activity

S.NO	SAMPLE	CELLULASE
1	Sea ice 37	-
2	sea ice 45	-
3	Sea ice 31	-
4	Sea ice 43	+
5	Sea ice 3	-
6	Sea ice 13	-
7	Sea ice 5	-
8	Sea ice 16	-
9	Sea ice 42	+
10	Sea ice 6	-
11	Sea ice 4	-
12	Sea ice 39	-
13	Sea ice 12	-
14	Sea ice 35	+
15	Sea ice 7	-
16	Sea ice 14	+

5.3.2. Amylase production activity

In this present study, out of 16 isolates 9 isolates were observed to produce the amylase enzyme. Among them, Seaice-43, Seaice-16 and Seaice-14 displayed distinct zone therefore showing higher amylase production comparatively (Table 5.5). Similarly, Vaidya *et al.*, 2015 isolated 25 bacteria and characterized for amylase production. Out of which five bacterial isolates showed zones of clearance in a test for starch hydrolysis. Also, Islam *et al.*, 2018, isolated a total of eight bacterial isolates, all of which having the capacity to produce amylase.

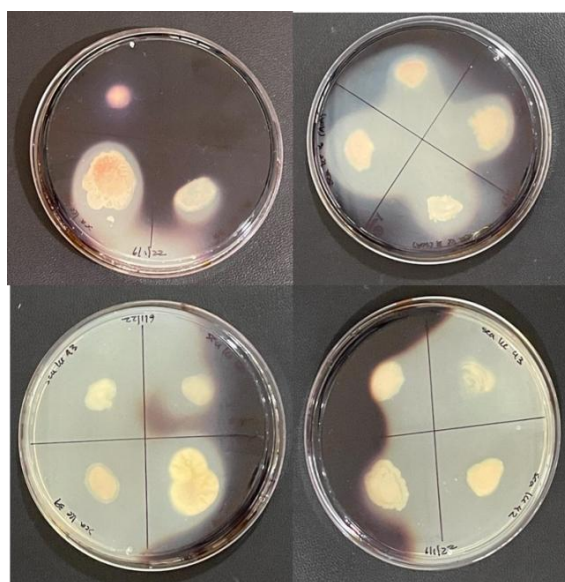


Fig 5.7: Starch hydrolysis test to determine the production of Amylase enzyme

Table 5.5: Amylase production activity

S.NO	SAMPLE	AMYLASE
1	Sea ice 37	-
2	sea ice 45	+
3	Sea ice 31	+
4	Sea ice 43	+
5	Sea ice 3	-
6	Sea ice 13	+

7	Sea ice 5	+
8	Sea ice 16	+
9	Sea ice 42	-
10	Sea ice 6	+
11	Sea ice 4	-
12	Sea ice 39	-
13	Sea ice 12	-
14	Sea ice 35	+
15	Sea ice 7	-
16	Sea ice 14	+

5.4. SCREENING OF ANTAGONISTIC ACTIVITY OF BACTERIAL ISOLATES

5.4.1. Anti fungal activity against *Colletotrichum gloeosporoides*

A vital feature of bacterial inoculants is PGPR's antifungal activity. The soil-born phytopathogen *Colletotrichum gloeosporoides* that causes the economically significant disease anthracnose has been found to be effectively inhibited by the current isolates. A dual culture test was used to assess the antagonist action of salt-resistant strains of bacteria against the fungal pathogen *Colletotrichum sp.* (Fig 5.8). Out of the 16 bacterial isolates test, 9 isolates showed inhibition against *C. gloeosporoides* with percentage of inhibition higher than that observed in control. Among these 9 bacterial isolates, only three isolates showed the maximum percentage of inhibition, which were, Seaice 16 (75%) followed by Seaice-43 (72%) and Seaice-37 (72%) (Table 5.6). Cruz *et al.*, 2020 identified a total of 41 actinobacterial isolates from the rhizosphere of avocado trees (*Persea americana*); 15% of the isolates inhibited *C. gloeosporioides* growth, 22% inhibited *Xanthomonas sp.* growth, and 7% inhibited the growth of both phytopathogens. According to Svetlana *et.al.*, 2010, the dual culture assay results demonstrated that all antagonistic bacteria, to varied degrees, reduced the mycelial growth of *C. acutatum* and *C. gloeosporioides*. The pathogens' radial growth was

decreased by more than 38% and 43%, respectively, by *T. harzianum* and *G. roseum*. In a separate study by Kejela et al. (2017), forty *Pseudomonas* strains were collected and their antagonistic activity was assessed using dual culture methods. The isolate *Pseudomonas* PT11 demonstrated the highest levels of suppression of *Fusarium oxysporum* (72%) and *Colletotrichum gloeosporioides* (70%) which indicates that *Pseudomonas* PT11 can be utilised in order to improve coffee germination.

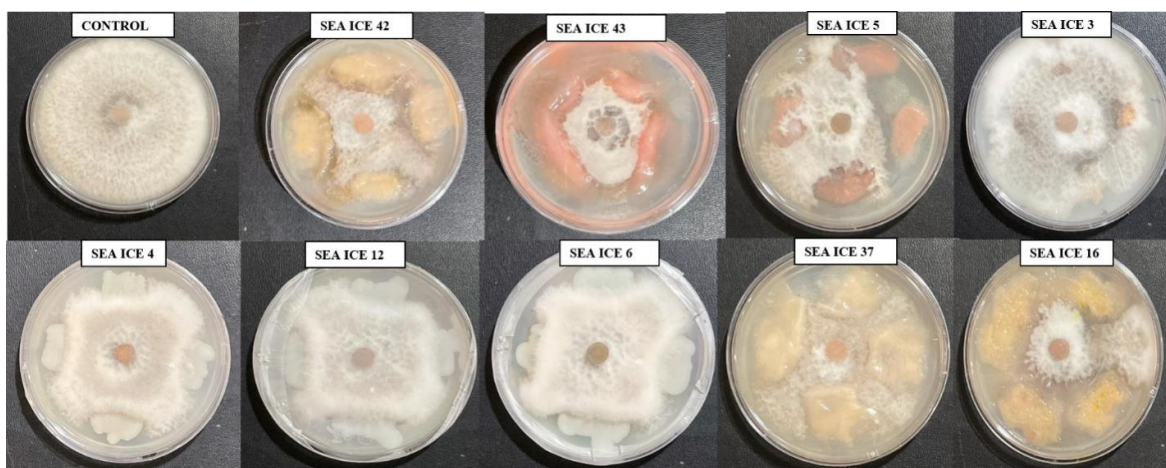


Fig 5.8: In vitro Screening for growth inhibition of phytopathogen *Colletotrichum gloeosporioides*

Table 5.6: Antifungal Activity against phytopathogen *Colletotrichum gloeosporioides*

S.NO	SAMPLE	% OF INHIBITION
1	Sea ice 6	65
2	Sea ice 37	72
3	Sea ice 43	72
4	Sea ice 12	41.2
5	Sea ice 4	40
6	Sea ice 16	75
7	Sea ice 5	57.5
8	Sea ice 42	61.25
9	Sea ice 3	60

5.5. MOLECULAR DIVERSITY OF SALT-TOLERANT BACTERIAL STRAINS WITH PGPR TRAITS

The Genomic DNA was isolated from all 16 bacterial isolates through the chelex-100 microwave method. The modified DNA extraction method using Chelex 100 by HwangBo *et al.*, 2010, was able to successfully amplify transgenes in transgenic tobacco, tomato, potato, Arabidopsis, rice, strawberry and Chlamydomonas. The total time required to extract the DNA is lesser making it comparable to methods employed with bacteria, Chlamydomonas, and cells from animals lines. The DNA was observed to be good due to the presence of a single, high molecular weight DNA band exhibiting minimal shearing and no RNA contamination, detected by 2% gel electrophoresis (Fig 5.9). This DNA was amplified using PCR which was done using the universal primers 27F and 1492R. In accordance with Frank *et al.*, 2008, examined and reevaluated the conventional primers for replicating the DNA from bacterial 16S rRNA genes within positions 27 and 1492. Particularly under strict amplification conditions in gene studies of DNA from human vaginal samples, this proposed formulation performed more effectively in retaining the initial rRNA genetic proportion between *Lactobacillus* spp. to *Gardnerella* spp. The amplification of 16 strains was achieved with good integrity, its product size being 1500, was confirmed by the presence of distinct bands about 1500bp long, as seen on a 2% agarose gel (Fig 5.10). Following 16S rDNA amplification, genetic variability analysis was performed for all 16 isolates using Rep-PCR approach. The BER primers (BOX, ERIC and REP) were used in repetitive sequence based PCR fingerprint method.

In a study by Manigundan *et al.* (2017), 32 strains of *Xanthomonas oryzae* (Xoo), representing 12 pathotypes, were assessed using Rep-PCR and *IS1112*-based PCR methods from the Andaman Islands and important rice-growing Indian mainland states. The REP primers generated more PCR fragments than other sets of primers and combined dendrogram constructed using Rep-PCR analysis was effective in distinguishing Islands Xoo strains from Indian mainland strains with the similarity index of Islands clusters of about 53%. The Rep-PCR technique was shown to be more exact and thus suitable for discriminating the variability across bacterial strains.

DNA fingerprint approaches have been established to evaluate and distinguish between different strains of organisms since 16S rRNA gene sequences tend to be limited for recognizing bacteria at the species or strain levels (Weisburg *et al.* 1991; Janda and Abbott 2007). Therefore, in the current study, REP-PCR, genotypic fingerprinting method using BER primers (Rep, Eric and Box primers), were utilised to detect diversity across and within bacterial strains. Using this all sixteen strains generated a number of bands in the present study, showing variability in the band pattern. In ERIC and REP primers, the banding pattern ranged from 300-300 basepairs as seen in figures 5.11 and 5.13, whereas in the case of BOX primers the banding pattern ranged from 200-2000 basepairs as seen in figure 5.12. The dendrogram constructed based on REP-PCR profile, all 16 isolates were clustered into two main clusters. Where the first cluster was separated into clads and it was observed that Seaice-43 and Seaice-42 were grouped together in the same clad (Fig 5.14). The cluster analysis of BOX-PCR showed that the 16 isolates were diversified into two clusters. The first cluster was separated into two clads. Seaice-37, Seaice-31, Seaice- 13, Seaice-5, Seaice-16 were grouped into one clad and Seaice-42, Seaice-7, Seaice- 35, Seaice-4, Seaice-39, Seaice-6 were grouped into the second clad of first cluster (Fig 5.15). The dendrogram constructed based on ERIC-PCR profile showed that the 16 isolated were clustered into two major clusters, where each cluster was separated into two clads specifically. Seaice-37 and Seaice-43 was observed to be grouped into the same clad as observed in cluster analysis of REP-PCR. Seaice-4 and Seaice31 were not grouped to any other isolates. Seaice-45 and Seaice-6 were grouped together under the same clad (Fig 5.16). Dendrogram constructed with combined REP-PCR analysis (Box, Rep and Eric primers- BER primers) showed high variation among the 16 isolates which were seen to be separated into two large clusters. Seaice43 and Seaice-42 were grouped together under the same clad, similar to ERIC-PCR and REP-PCR cluster analysis. The first cluster was subdivided into three clads, where Seaice-45 remained ungrouped. The second major cluster was subdivided into two clads. The first clad was divided into three groups. Seaice-13, Seaice-5 and Seaice-16 belonged to one group, Seaice-42, Seaice-39, Seaice-31 and Seaice-7 belonged to second group and Seaice- 12 remained ungrouped under the same clad (Fig 5.17).

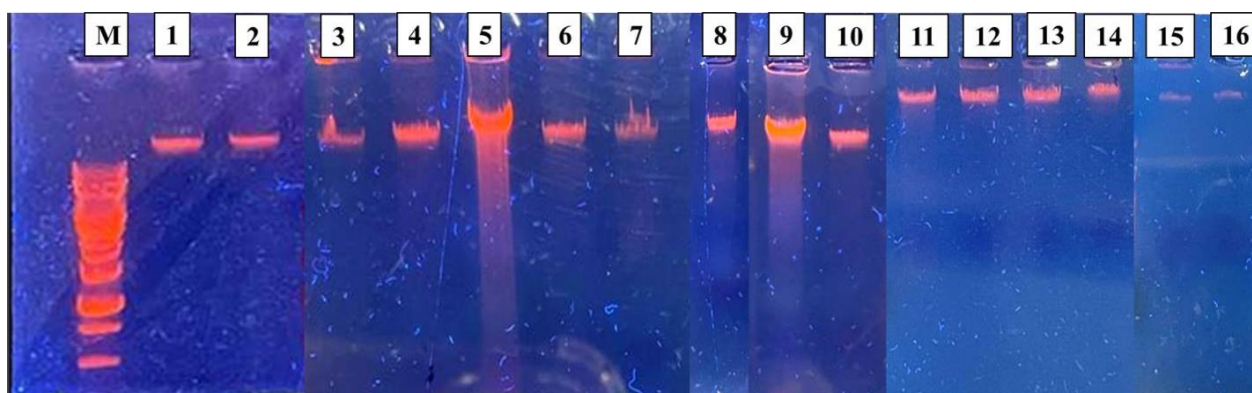


Fig 5.9: Agarose gel electrophoresis of genomic DNA isolated by chelex-100 microwave method.

(Lane 1: 1kb Marker, 2: Seaice-43 , 3: Seaice-3 , 4: Seaice-16 , 5: Seaice- 5, 6: Seaice-13, 7: Seaice-31, 8: Seaice-45, 9: Seaice-39, 10: Seaice- 37, 11: Seaice-35, 12: Seaice- 4, 13: Seaice-42, 14: Seaice-6, 15: Seaice-14, 16: Seaice-7, 17: Seaice-12)

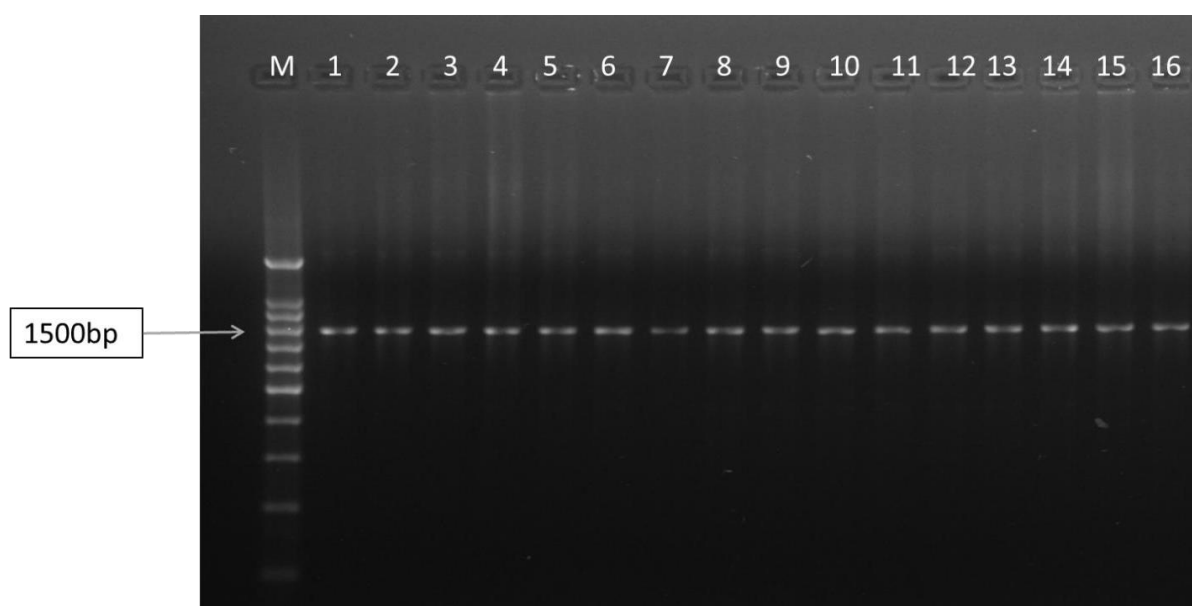


Fig 5.10: 2% agarose gel electrophoreses of amplified PCR products.

(Lane 1: 1kb Marker, 2: Seaice-43 , 3: Seaice-3 , 4: Seaice-16 , 5: Seaice- 5, 6: Seaice-13, 7: Seaice-31, 8: Seaice-45, 9: Seaice-39, 10: Seaice- 37, 11: Seaice-35, 12: Seaice- 4, 13: Seaice-42, 14: Seaice-6, 15: Seaice-14, 16: Seaice-7, 17: Seaice-12)

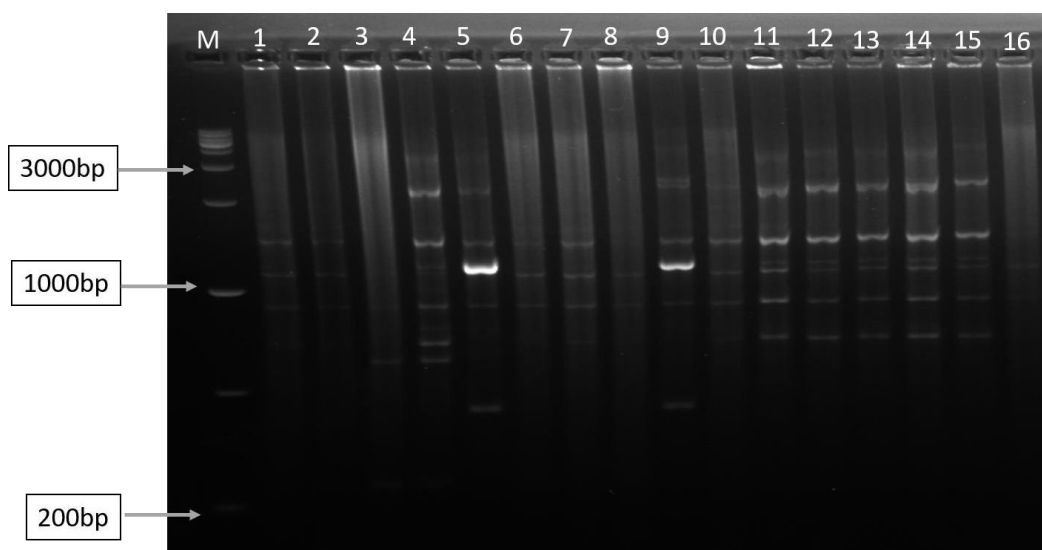


Fig 5.11: REP-PCR profile of 16 bacterial isolates.

(Lane 1: 1kb ladder, 2: Seaice-43 , 3: Seaice-3 , 4: Seaice-16 , 5: Seaice- 5, 6: Seaice-13, 7: Seaice-31, 8: Seaice-45, 9: Seaice-39, 10: Seaice- 37, 11: Seaice-35, 12: Seaice- 4, 13: Seaice-42, 14: Seaice-6, 15: Seaice-14, 16: Seaice-7, 17: Seaice-12)

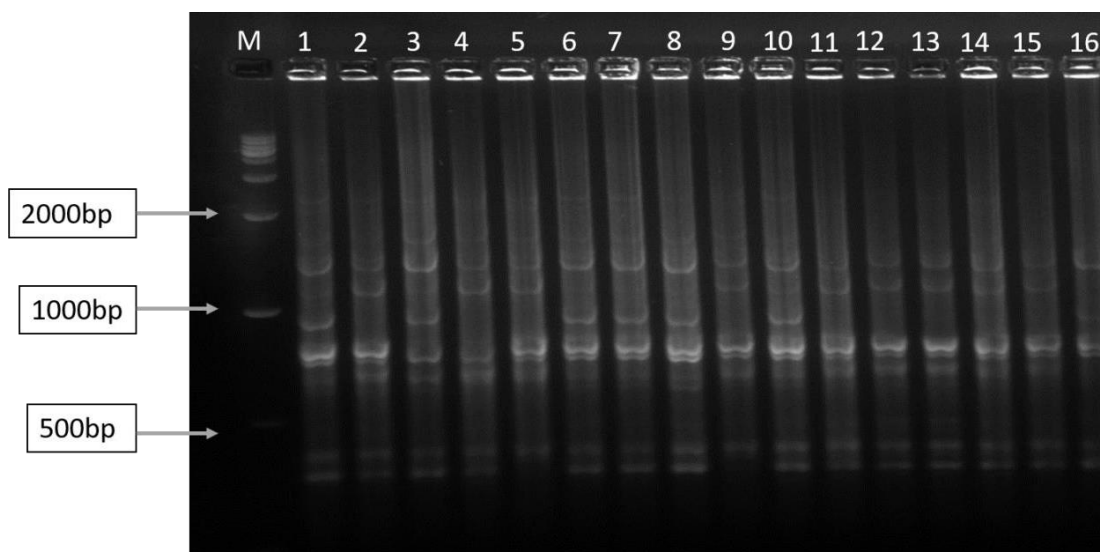


Fig 5.12: BOX-PCR profile of 16 bacterial isolates

(Lane 1: 1kb ladder, 2: Seaice-43 , 3: Seaice-3 , 4: Seaice-16 , 5: Seaice- 5, 6: Seaice-13, 7: Seaice-31, 8: Seaice-45, 9: Seaice-39, 10: Seaice- 37, 11: Seaice-35, 12: Seaice- 4, 13: Seaice-42, 14: Seaice-6, 15: Seaice-14, 16: Seaice-7, 17: Seaice-12)

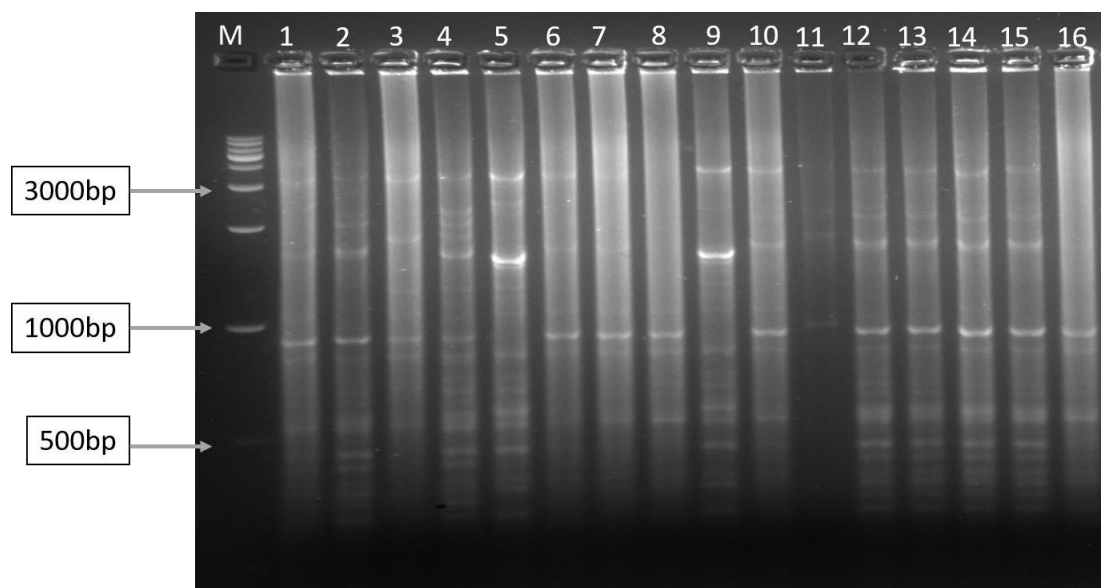


Fig 5.13: ERIC-PCR profile of 16 bacterial isolates

(Lane 1: 1kb ladder, 2: Seaice-43 , 3: Seaice-3 , 4: Seaice-16 , 5: Seaice- 5, 6: Seaice-13, 7: Seaice-31, 8: Seaice-45, 9: Seaice-39, 10: Seaice- 37, 11: Seaice-35, 12: Seaice- 4, 13: Seaice-42, 14: Seaice-6, 15: Seaice-14, 16: Seaice-7, 17: Seaice-12)

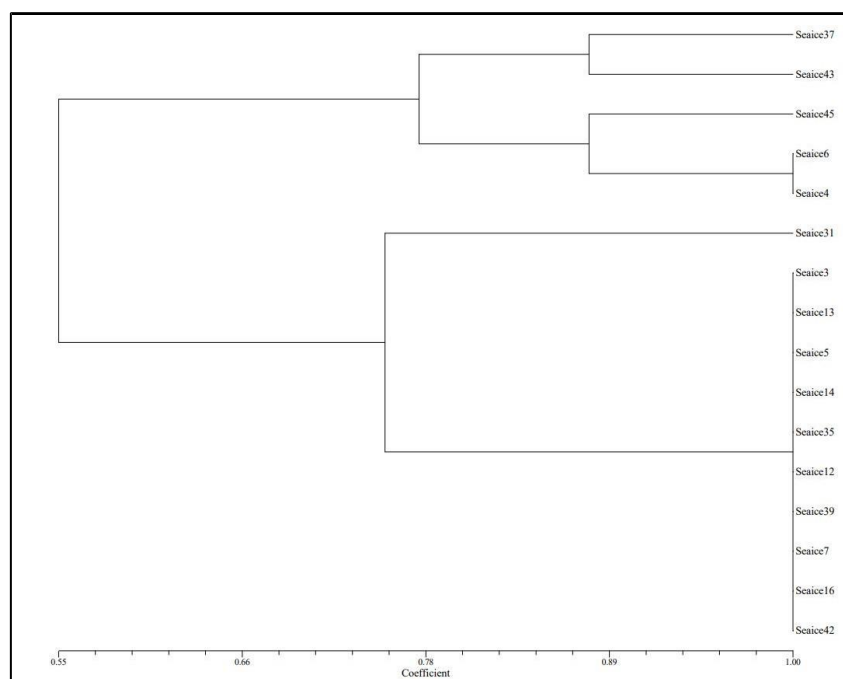


Fig 5.14: Dendrogram constructed based on REP-PCR of 16 bacterial isolates

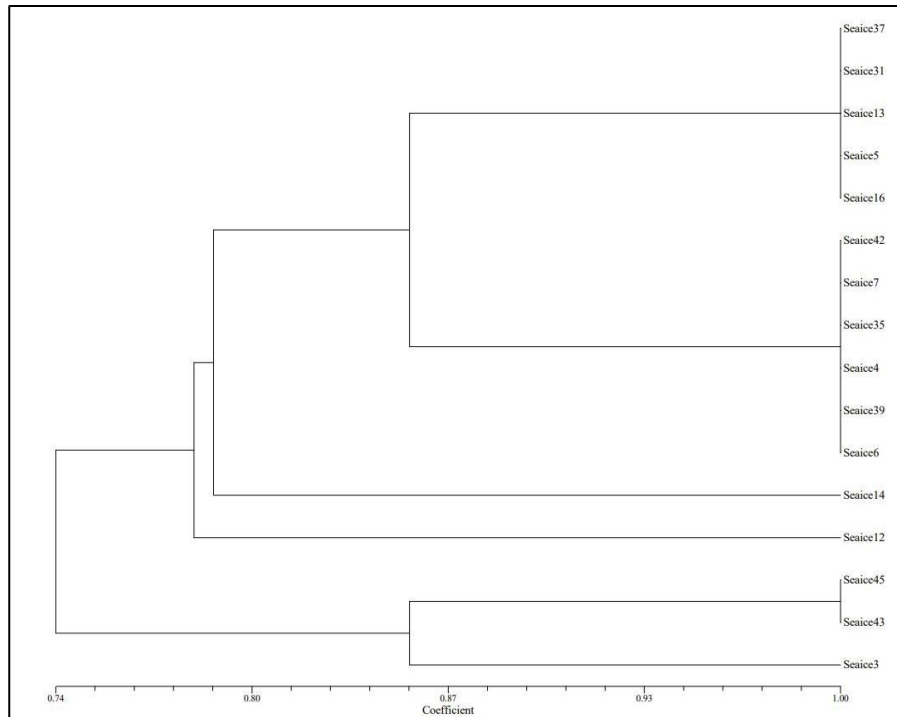


Fig 5.15: Dendrogram constructed based on BOX-PCR of 16 bacterial isolates

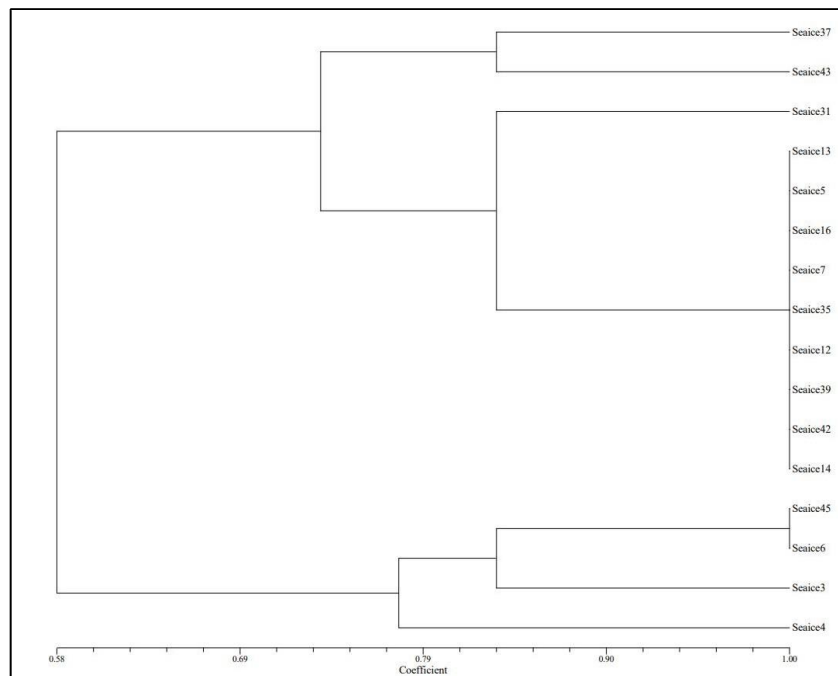


Fig 5.16: Dendrogram constructed based on ERIC-PCR of 16 bacterial isolates

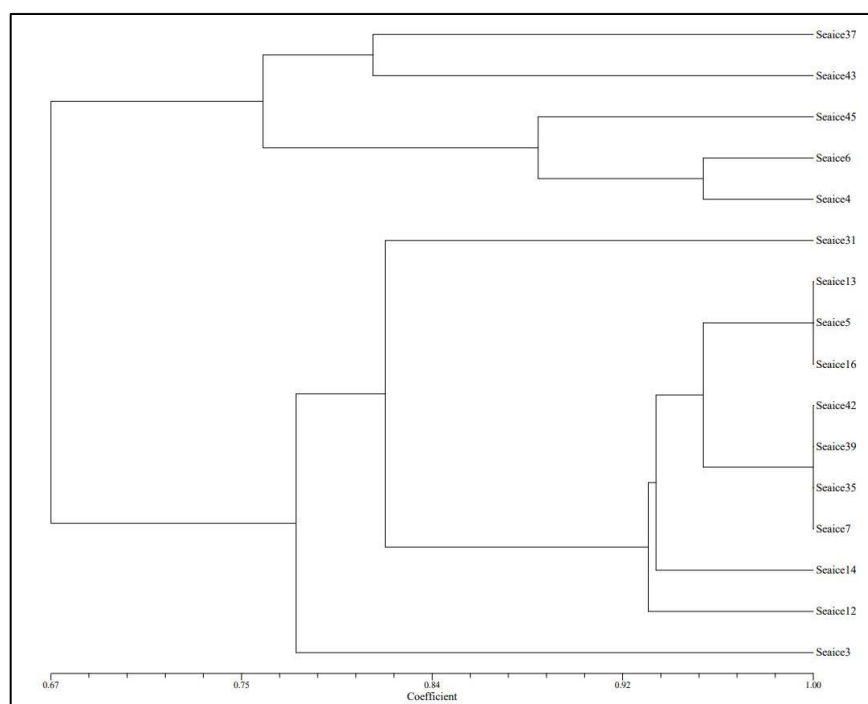


Fig 5.17: Dendrogram constructed with combined REP-PCR analysis (Box, Rep and Eric primers- BER primers).

5.6. *In-planta* EVALUATION OF POTENTIAL STRAINS FOR PLANT GROWTH PROMOTION

5.6.1. Seed germination

The efficacy of 5 selected strains in promoting plant growth such as Seaice-6, Seaice-16, Seaice42, Seaice-43 and Seaice-37 were evaluated in seed germination assay (Fig 5.19). The tomato seeds were bio-primed by inoculating with mixture of 5% NaCl and potential bacterial strains before conducted seed germination assay and pot study (Fig5.18). In a 2014 study, Fatemeh *et al.*, studied the germination of seeds of *Crataegus pseudoheterophylla* Pojark using various combinations of rhizobacteria that promote growth under regulated environments. These results suggest that PGPRs effectively supported *C. pseudoheterophylla* germination of seeds.

In the current study, the tomato seedlings grown in test tubes were measured for their chlorophyll content, root and shoot. The tomato seedling growth was not highly influenced under the bacterial strain and saline conditions. The root and shoot length was significantly increased than control (untreated). the highest shoot and root length was observed in tomato seedling treated with bacterial isolate Seaice-6 (Shoot length- $9.2 \pm 0.1\text{cm}$; root length- $7.0 \pm 0.5\text{cm}$), followed by Seaice-42 (Shoot length- $8.1 \pm 0.5\text{cm}$; root length- $5.4 \pm 0.1\text{cm}$). All 5 seedlings were subjected to chlorophyll measurement spectrophotometrically.

The chlorophyll content of treated seedlings were observed to be higher than control (untreated) as observed in table 5.8. The enhanced photosynthetic pigment was recorded in tomato seedlings with treatment Seaice-16 (36.2 mg g^{-1}) followed by Seaice-6 (13.79 mg g^{-1}) and Seaice-43 (2.06 mg g^{-1}) respectively. The lowest chlorophyll content was observed in control untreated tomato seedling when compared to treated seedlings which was 1.61 mg g^{-1} . Similarly, Photosynthetic pigments like chlorophyll and carotenoids of tomato were found in higher concentrations in *Bacillus safensis*-inoculated treatment (T6) under normal conditions (T1) as compared to control treatment (C) in a study by Mukhtar *et al.*, 2022.

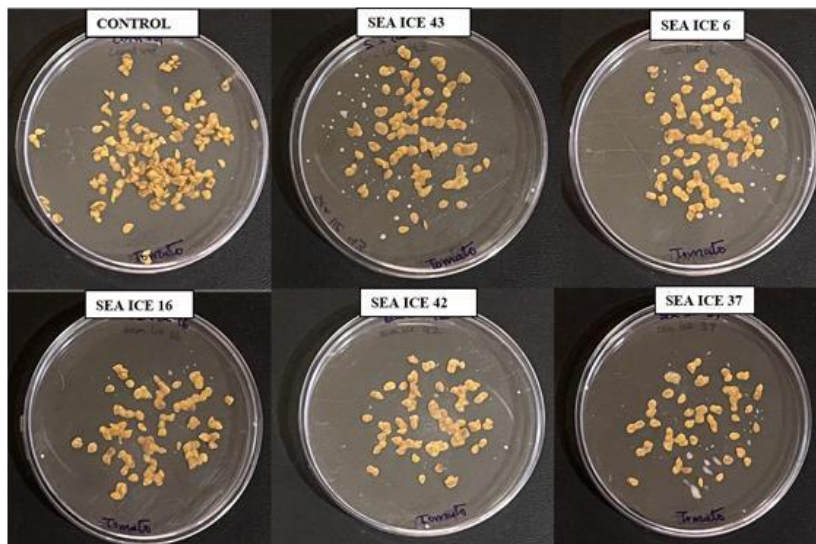
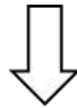
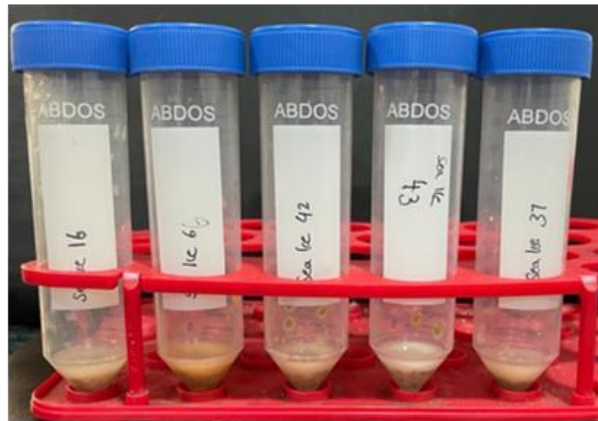
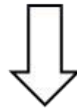


Fig 5.18: Bio-priming of tomato seeds.

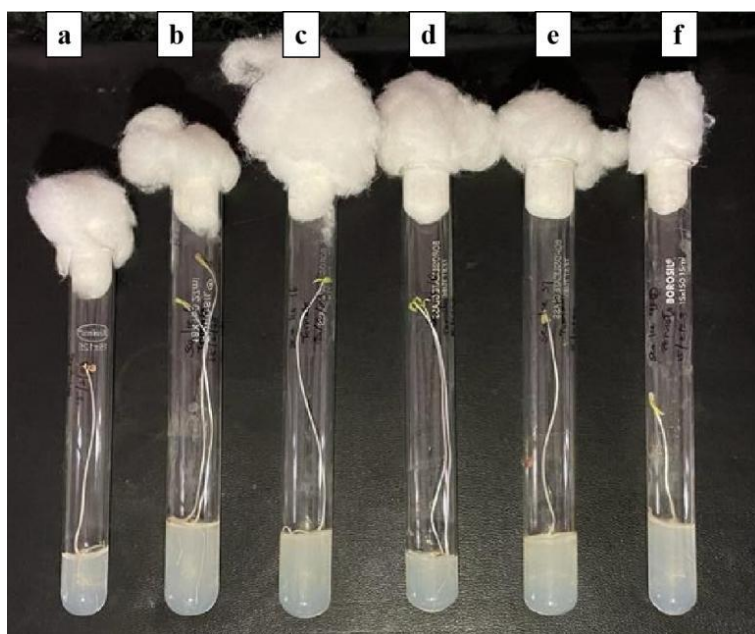


Fig 5.19: Germination test of PGPR treatments in Tomato
(a. Seaice-43, b. Seaice-6, c.Seaice-16, d. Seaice-42, e. Seaice-37, f. Control)

Table 5.7: Effect of potential bacterial isolates on tomato seed germination

S.NO	SAMPLE	SHOOT LENGTH (cm)	ROOT LENGTH (cm)
1	Control	3.2 ± 0.15	2.2 ± 0.1
2	Sea ice 6	9.2 ± 0.1	7.0 ± 0.5
3	Sea ice 16	8.1 ± 0.3	3.3 ± 0.1
4	Sea ice 42	8.1 ± 0.5	5.4 ± 0.1
5	Sea ice 37	9.1 ± 0.6	3.5 ± 0.2
6	Sea ice 43	4.0 ± 0.2	2.8 ± 0.2

Table 5.8: Measurement of total chlorophyll on tomato seedlings

S.NO	SAMPLE	TOTAL CHLOROPHYLL (mg g ⁻¹)
1	Control	1.61
2	Sea ice 6	13.79
3	Sea ice 16	36.42
4	Sea ice 37	1.58
5	Sea ice 42	1.89
6	Sea ice 43	2.06

5.6.2. Pot study

Based on the seed germination assay results, three isolates were selected further to test in the pot experiments, which were Seaice-43, Seaice-16 and Seaice-6. The results showed that the tomato seedlings can tolerate salt stress in pot culture more efficiently when inoculated with saline tolerant bacterial isolates compared with control (Fig 5.20). The strain Seaice-43 showed significantly better results of shoot and root length enhancement compared with other strains. Plants inoculated with Seaice-16 and Seaice-6 did not show significantly greater growth than Seaice-43, but it was greater in comparison to control plants. Maximum shoot and root length were recorded in treatment Seaice-43 (21.3 ± 0.9 cm and 5.1 ± 0.9 cm, respectively) followed by Seaice-16 (19 ± 0.8 cm and 4.5 ± 0.4 cm, respectively). In the present study, these plants were then subjected to chlorophyll measurement spectrophotometrically. The chlorophyll content of each plant was calculated by using the formula:

$$\text{Total chlorophyll} = (\text{Absorbance at 645}) 20.2 + (\text{Absorbance at 663}) 8.02.$$

The chlorophyll content of treated plants were observed to be higher than control (untreated) as seen in table 5.10. The enhanced photosynthetic pigment was recorded in tomato seedlings with treatment Seaice-43 (3.24 mg g^{-1}) followed by Seaice-6 (3.15 mg g^{-1}) and Seaice-16 (2.86 mg g^{-1}) respectively. Similarly, 18 regionally isolated *Enterobacter* spp. were examined in a 2020 investigation by Roslan *et al.* When in

comparison to the uninoculated control crops, the bioprimes okra seedlings in the pot experiment demonstrated significantly enhanced plant growth ($> 28\%$) and elevated P and K uptakes ($> 89\%$). The chlorophyll index of bioprimes plants rose by up to 29% . Multiple plant-beneficial characteristics of *Enterobacter hormaechei* sp. offer an excellent potential sustainable method for improving soil fertility. In an investigation conducted by Ramavath *et al.*, 2019, 4 isolates of bacteria were chosen from 200 acquired from different source materials, and their potential for promoting plant growth (PGP) were determined. These isolates promoted tomato plant development during seed germination ($14\text{--}19\%$) and pot culture (biomass increase $47\text{--}76\%$). *Bacillus siamensis* was shown to be responsible for the most significant growth in tomato plant (71%).



Fig 5.20: Pot study by inoculation with potential plant growth promoting bacterial isolates. (a. Control, b. Seaice-43, c. Seaice-6, d. Seaice-16)

Table 5.9: Effect of potential bacterial isolates on tomato plants

S.NO	SAMPLE	SHOOT LENGTH (cm)	ROOT LENGTH (cm)
1	Control	14.5 $\pm$ 1.4	2.5 $\pm$ 0.2
2	Sea ice 43	21.3 $\pm$ 0.9	5.1 $\pm$ 0.9
3	Sea ice 16	19 $\pm$ 0.8	4.5 $\pm$ 0.4
4	Sea ice 6	18.1 $\pm$ 0.3	2.5 $\pm$ 0.2

Table 5.10: Measurement of total chlorophyll on tomato plants

S.NO	SAMPLE	TOTAL CHLOROPHYLL (mg g ⁻¹)
1	Control	2.69
2	Sea ice 43	3.24
3	Sea ice 16	2.86
4	Sea ice 6	3.15

5.7 MOLECULAR IDENTIFICATION OF POTENTIAL STRAIN

5.7.1. *Gram's staining*

The most widely used staining method for identifying Gram positive and Gram negative microorganisms in both clinical settings and research labs is called a gram staining. Gram-negative bacteria are reddish or pink in colour because they have a thinner lining of peptidoglycan (ten percent of their cell walls) and loses the Crystal violet-iodine mixture during the decolorization process with the alcohol rinse. When the potential isolate Seaice-43 was subjected to gram's staining, the resultant slide was air dried, and the organism was viewed under the microscope's oil immersion objective, which demonstrated that the organism was Gram negative.

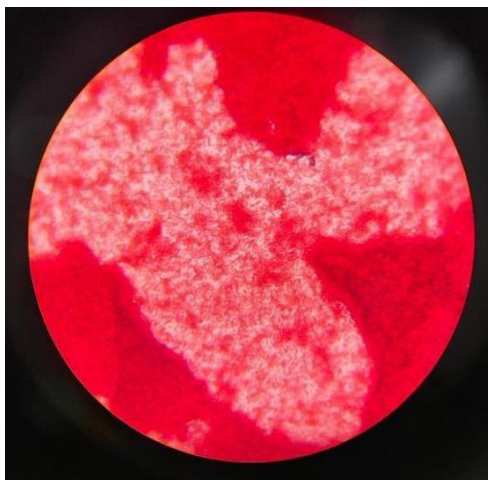


Fig 5.21: Microscopic observation of Gram's staining

5.7.2. KOH test

The KOH String Test, which involves mixing a portion of a colony with 3% potassium hydroxide, depends on the difference in resistance to the chemical between gram-positive and gram-negative cells. When cells lyse, the DNA from the released cells makes the resulting mixture viscous or "stringy". When a loopful of the potential isolate Seaice-43 was mixed with a drop of 3%KOH on a slide, within the first 30 seconds, a viscous coating attached along with loop and create a string 1–5 cm long resulting in positive KOH test as seen in figure 5.22. Since the test was positive, the potential isolate Seaice-43 was determined as Gram negative organism. Similarly, 495 food-borne, water-borne, and yeast-borne bacteria and yeasts were examined with 3% potassium hydroxide to evaluate their Gram responses in the Power *et al.*, 1995 study. Gram-negative bacterial suspensions in KOH thicken and string out. Bacteria that are gram-positive were unaffected. The KOH string test results and both gram-positive and gram-negative bacteria correlated perfectly. In the Arthi *et al.*, 2003 study, 218 bacterial isolates from diverse clinical samples were tested using the potassium hydroxide (KOH) string test. String test results for gram-positive bacteria were entirely negative. 98.85% of the gram-negative bacteria tested positive for the string test, which is a quick, low- cost alternative to Gram staining for identifying bacterial cultures.

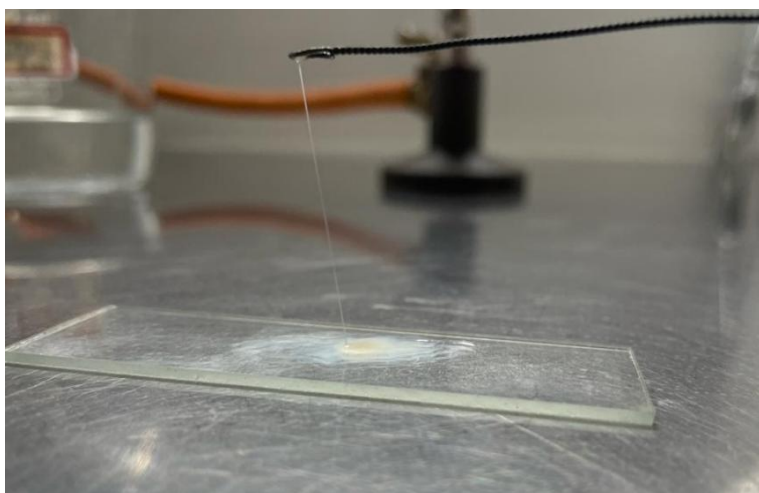


Fig 5.22: KOH string test

5.7.3. 16S rRNA sequence analysis

The bacterial strain, Seaice-43 was chosen as potential strain since it showed maximum range of activity against fungal pathogen and broad range of plant growth promoting traits along with good growth in pot study. Following 16S rDNA amplification, the PCR product had been sequenced using the Sanger method. To get full coverage, purified products have been sequenced in both directions. Contigs were constructed in DNA baser after the sequences had been revised, and the BLAST analysis was used to compare them to GenBank sequences. Using software from the MEGA version 4 package, a phylogenetic evaluation of a potential strain was performed. The potential strain's 16S rRNA sequence was matched with the appropriate nucleotide sequence obtained from GenBank. The neighbour joining approach was used to create evolutionary distance matrices and construct a phylogenetic tree. By using a bootstrap methodology with a sample size of 1000 neighbours, tree topologies were assessed. In the phylogenetic tree, the strain Seaice-43 and other closest *Pseudomonas Paracarnis* strains were grouped together at a 99 % similarity (Fig 5.23). Sequence analysis of the 16S rDNA gene revealed that the potential isolate, Seaice-43 was identified as *Pseudomonas Paracarnis*. Similarly, In the research carried out by Sharma *et al.*, 2021, 16S rDNA sequencing was used to characterise the molecular characteristics of robust isolates with tolerance to salt and PGPR activity. The strain HB6J2 shared 99.87% of its

similarities with *Bacillus paramycoides* M9a1a according to a BLAST-N comparison of the sequences searched in the NCBI nucleotide database. Using the neighbor-joining approach, the phylogenetic tree of the identified bacterial isolates was constructed. Strain NFB12 developed the longest shoots in a study by Yavarian *et al.*, 2021, and was recognised for having the PGPB characteristic and subjected to molecular analysis. The phylogenetic study of the 16S rDNA gene sequence revealed the strongest similarity with *Bacillus megaterium* strain ATCC 14581.

5.7.3.1. Nucleotide accession number

The partial 16S rRNA nucleotide sequence of the potential bacterial strain was deposited at GenBank database by using NCBI submission portal. The provided Genbank accession number for the Seaice-43 (*Pseudomonas paracarnis*) sequence was OQ874062.

Table 5.11: Top 23 strains closest to potential strain

S no	Name	Strain	Accession	Pairwise Similarity(%)	Mismatch /Total nt	Completeness(%)
1	<i>Pseudomonas paracarnis</i>	V5/DAB/2/5	KX186983	99.78463747	3/1393	98.08
2	<i>Pseudomonas lactis</i>	DSM 29167	JYLO01000038	99.71305595	4/1394	100.00
3	<i>Pseudomonas azotoformans</i>	DSM 18862	MNPV01000020	99.64131994	5/1394	100.00
4	<i>Pseudomonas carnis</i>	B4-1	CABIVL01000053	99.64131994	5/1394	100.00
5	<i>Pseudomonas</i>	DSM 29164	KP756921	99.49640288	7/1390	97.33

	<i>paralactis</i>					
6	<i>Pseudomonas synxantha</i>	DSM 18928	JYLJ01000042	99.3543759	9/1394	100.00
7	<i>Pseudomonas gessardii</i>	DSM 17152	MNPU01000117	99.3543759	9/1394	100.00
8	<i>Pseudomonas libanensis</i>	CIP 105460	AF057645	99.3543759	9/1394	100.00
9	<i>Pseudomonas mucidolensis</i>	LMG 2223	LT629802	99.28263989	10/1394	100.00
10	<i>Pseudomonas cedrina subsp. cedrina</i>	CFML 96-198	AF064461	99.13916786	12/1394	100.00
11	<i>Pseudomonas edaphica</i>	RD25	LC272923	99.13916786	12/1394	100.00
12	<i>Pseudomonas cedrina subsp. fulgida</i>	P515/12	AJ492830	99.06743185	13/1394	100.00
13	<i>Pseudomonas brenneri</i>	CFML 97-391	AF268968	98.92318737	15/1393	99.45
14	<i>Pseudomonas costantinii</i>	CFBP 5705	AF374472	98.85222382	16/1394	100.00
15	<i>Pseudomonas lurida</i>	LMG 21995	PDJB01000001	98.85222382	16/1394	100.00

16	<i>Pseudomonas canadensis</i>	2-92	AYTD01000015	98.7804878	17/1394	100.00
17	<i>Pseudomonas pisciculae</i>	P115	LR797573	98.74804382	16/1278	87.59
18	<i>Pseudomonas simiae</i>	OLi	AJ936933	98.73417722	17/1343	92.05
19	<i>Pseudomonas poae</i>	DSM 14936	JYLI01000039	98.70875179	18/1394	100.00
20	<i>Pseudomonas proteolytica</i>	CMS 64	AJ537603	98.70875179	18/1394	100.00
21	<i>Pseudomonas allii</i>	MAFF 301514	LC548100	98.70875179	18/1394	100.00
22	<i>Pseudomonas spelaei</i>	SJ/9/1	HQ844525	98.70875179	18/1394	99.31

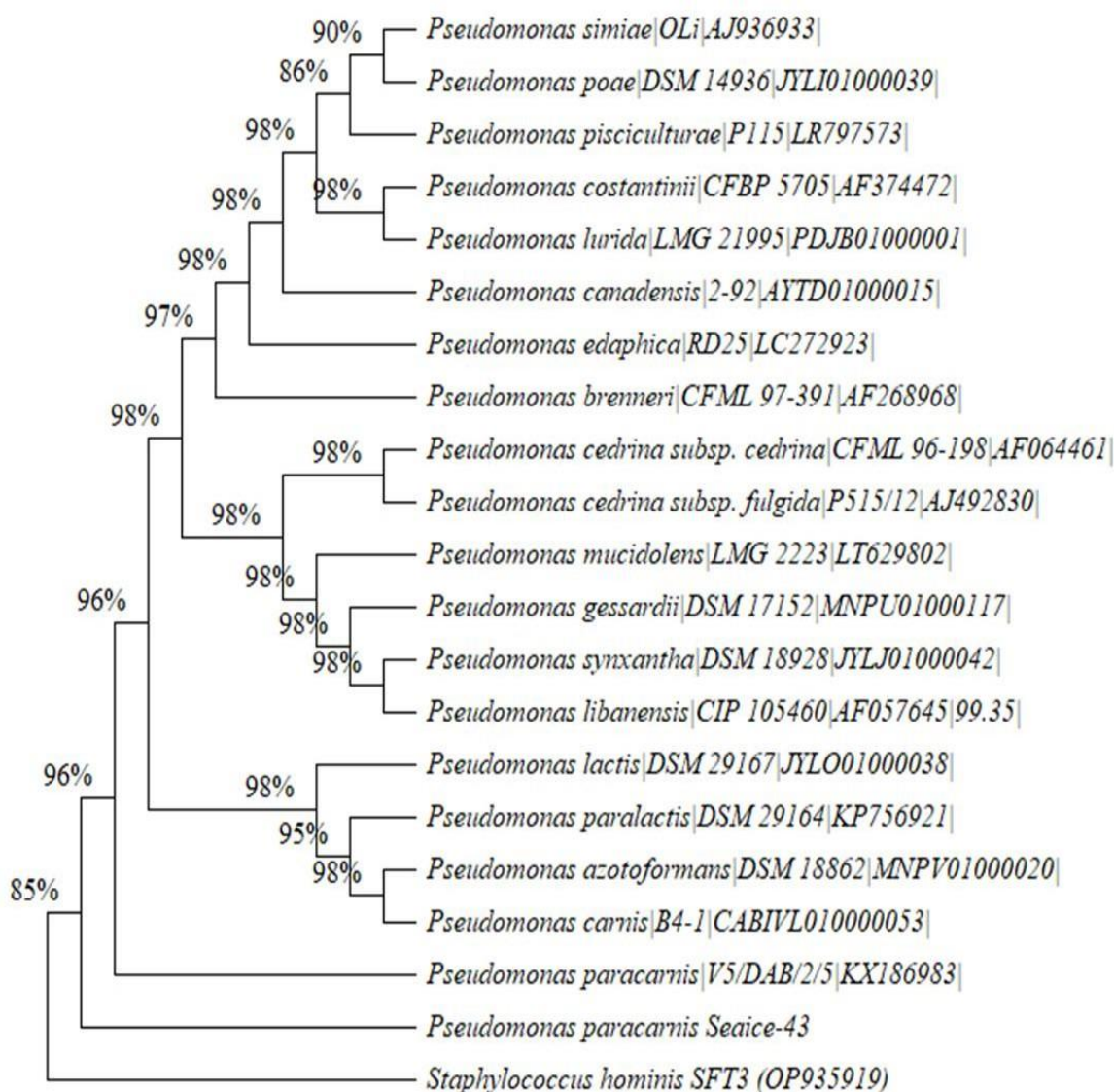


Fig 5.23: Phylogenetic tree of potential isolate (Seiace-43) constructed by the neighbor-joining method derived from analysis of the 16S rRNA gene sequence

5.7.4. Volatile compounds identification from potential isolate

Bacterial volatile compounds control the production or processing of phytohormones, which has a substantial impact on promoting the development of plants (Tahir *et al.*, 2017). Headspace GC-MS was conducted to investigate the presence of volatile organic compounds (VOCs) produced by the plant growth promoting rhizobacterium *Pseudomonas paracarnis* strain Seiace-43, to understand the impact it

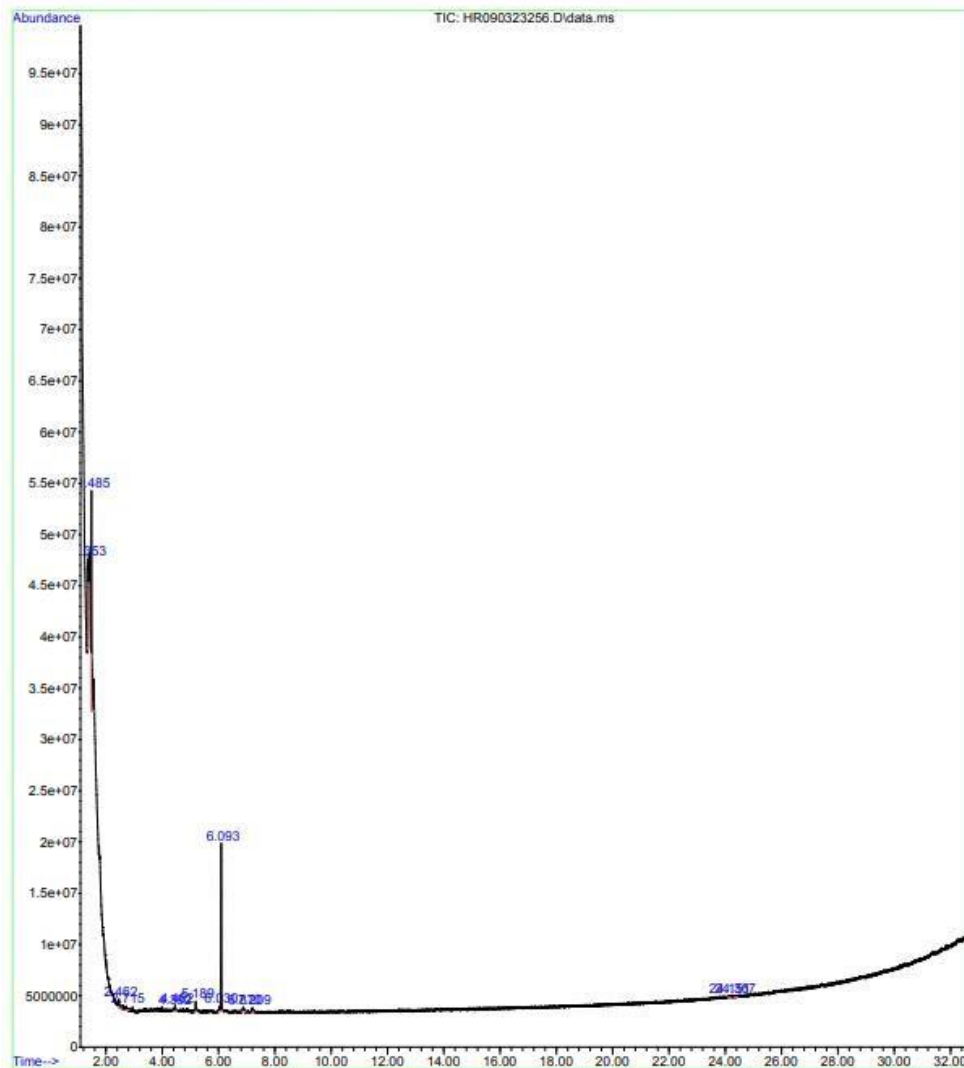
has on hormone regulation and growth promotion in tomato plants (Fig 5.25). 27 volatile compounds were identified in the headspace of the sample Seaice-43 via GC-MS. Silanediol dimethyl (R.T:1.485min, 29.22%), hexadecane (R.T: 6.093 min, 28.32%) and methyl amino lauric acid (R.T:1.353 min, 32.52%) were identified as the major components in this bacterial isolate sample. Similarly, Silanediol dimethyl has been observed to be present in the CZ-6 strain with broad-spectrum antagonistic activity isolated from saline-alkali land, identified as (Shehzad *et al.*, 2021). In another study, Silanediol dimethyl was also present in *B. subtilis* SYST2 which have shown to promote plant growth by triggering growth hormone activity (Hafiz *et al.*, 2017). Moreover, Silanediol dimethyl-was the predominant compound present in *P. aeruginosa* B1-SQU, effectively reduced the incidence of cucumber damping-off under greenhouse conditions under soil application (Dhuha *et al.*, 2023). In one study, Hexadecane has been proven to confer protection to *Arabidopsis* plants from infection of a biotrophic pathogen, *P. syringae* *pv* *maculicola* and a necrotrophic pathogen, *Pectobacterium carotovorum* (Hyo *et al.*, 2013). The unexpected stimulating effects in *Shiraia hypocrellins* have been attributed to hexadecane's substantial improvement of fungal HA accumulation. These findings also showed that the fungus may increase its antibacterial effectiveness in response to the microbial volatile-mediated antagonism by amassing PQs. According to Binbin *et al.*, 2014 research, the acid lauric acid has been seen to increase the development of peach seedling under drought stress.

Data Path : D:\GC MS\Data\2023\April\05-04-2023 SCREENING SAMPLE\
 Data File : HR090323256.D
 Acq On : 5 Apr 2023 15:14
 Operator :
 Sample : Miscellaneous
 Misc :
 ALS Vial : 2 Sample Multiplier: 1

Search Libraries: C:\Database\NIST11.L Minimum Quality: 0

Unknown Spectrum: Apex

Integration Events: RTE Integrator - rteint.p



VOC Sea Water.M Wed Apr 05 17:07:38 2023 LAB

Page: 1

Fig 5.24: Headspace GC-MS chromatogram showing profiles of VOCs of potential bacterial strain (Seaice-43)

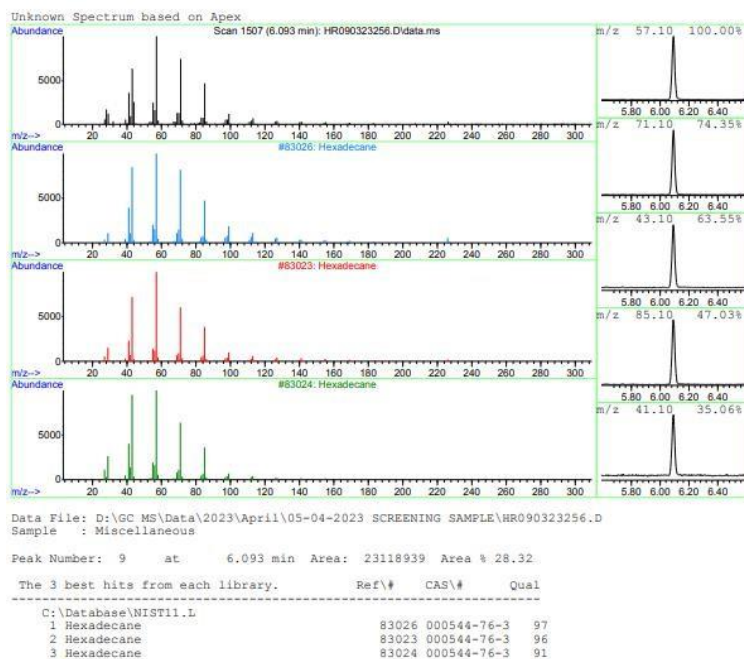


Fig 5.25: Best hits from third highest peak (R.T: 6.093 min; Area%: 28.32)

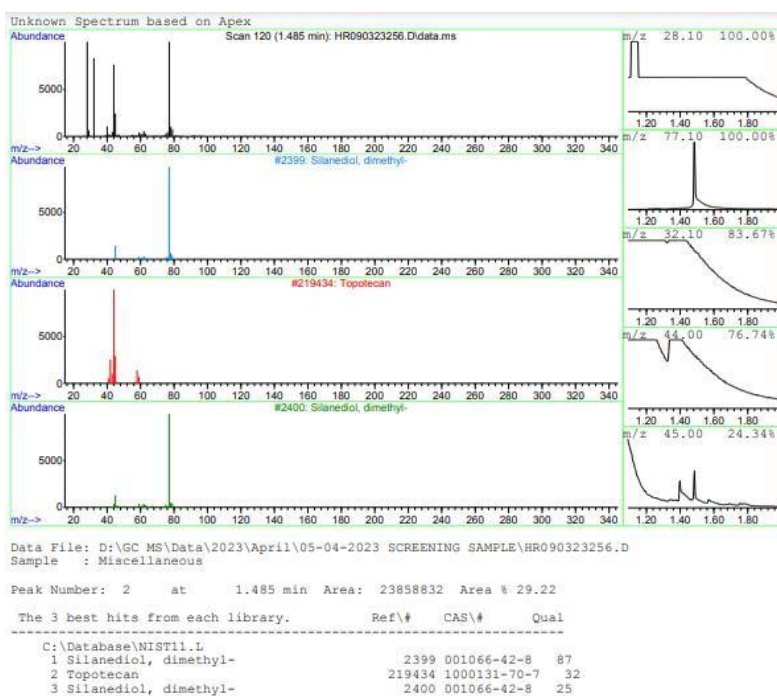


Fig 5.26: Best hits from first highest peak (R.T: 1.485 min; Area%: 29.22)

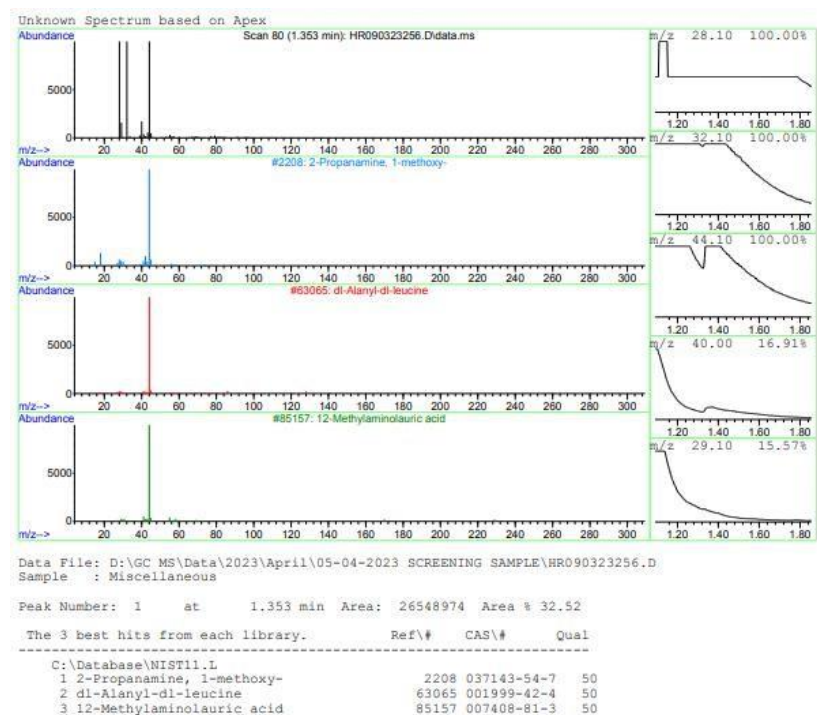


Fig 5.27: Best hits from second highest peak (R.T: 1.353min; Area%: 32.52)

CHAPTER 6

SUMMARY AND CONCLUSION

Food security is a social necessity. In 50 years, there will be 10 billion people on the planet. Agriculture must increase its yields by 50% in order to meet the rising demand for food. Agricultural soils in areas that are semi-arid are deteriorating by 1% to 2% annually due to elevated salinity. High salinity, caused by low precipitation and high temperatures, inhibits the growth of salt-sensitive plants, including several halophytes. Wheat, maize, rice, barley, and vegetable crop yields are all adversely affected by salt stress by up to 70%. Microbes that are salt-tolerant could boost the development of crops rather than increase the crop's ability to withstand salt. Plant growth in soils affected by salt is enhanced by soil salinity-tolerant microorganisms, indicating that this approach may be successful when salt-tolerant germplasm development has failed. This project intends to identify salt-tolerant bacteria isolated from Indian sector of Southern Ocean that promote the growth of Solanaceous vegetable crops under saline conditions. Totally, 16 bacterial strains were isolated from Southern Ocean was screened for their saline tolerant ability in plate assay. All the bacterial strains grew well in 5% NaCl amended agar media. Similarly, out of 16 bacterial strains, 10 and 4 bacterial strains were able to tolerated 10% NaCl and 20% NaCl, respectively. The potential to synthesize IAA is one of the features of microorganisms that promote plant growth. For the division and elongation of cells in plants enduring salt stress, the ST- PGPR generate IAA. All 16 isolates were tested for their ability to produce IAA. All isolates could produce IAA, but only Seaice-43 and Seaice-31 showed the maximum production in quantitative assay. They were further examined for the production of hydrolytic enzymes, where 11 bacterial strains produced amylase, 6 strains produced cellulase and none of the strains produced protease.

One of the biocontrol features against pathogenic microorganisms under iron-limiting settings is the siderophore produced via PGPR. Out of 16 isolates, 10 bacterial isolates were observed to show siderophore production. The two strains with highest siderophore productivity were Seaice-43 and Seaice-6 with predominant colour change

from blue to yellow. A vital feature of bacterial inoculants is PGPR's antifungal activity. The soil-born phytopathogen *Colletotrichum gloeosporoides* that causes the economically significant disease anthracnose has been found to be effectively inhibited by the current isolates. Antagonistic activity of bacterial strains against *Colletotrichum gloeosporoides* showed the maximum percentage of inhibition by Seaice-16 (75%) followed by Seaice-43 (72%) and Seaice-37 (72%). In this study, REP-PCR, genotypic fingerprinting method using BER primers (Rep, Eric and Box primers), were utilized to detect diversity across and within bacterial strains. Using this all sixteen strains generated a number of bands in the present study, showing variability in the band pattern.

The efficacy of 5 selected strains in promoting plant growth such as Seaice-6, Seaice-16, Seaice-42, Seaice-43 and Seaice-37 were evaluated in seed germination assay. The tomato seedlings grown in test tubes were measured for their chlorophyll content, root and shoot. The tomato seedling growth was not highly influenced under the bacterial strain and saline conditions. The enhanced photosynthetic pigment, chlorophyll, was recorded in tomato seedlings with treatment Seaice-16 (36.2 mg g⁻¹) followed by Seaice-6 (13.79 mg g⁻¹) and Seaice-43 (2.06 mg g⁻¹) respectively. Based on the seed germination assay results, three isolates were selected further to test in the pot experiments, which were Seaice-43, Seaice-16 and Seaice-6. The results showed that the tomato seedlings can tolerate salt stress in pot culture more efficiently when inoculated with saline tolerant bacterial isolates compared with control. The strain Seaice-43 showed significantly better results of shoot and root length enhancement compared with other strains (21.3 ± 0.9 cm and 5.1 ± 0.9 cm, respectively). The chlorophyll content in plants from pot study was conducted which showed enhanced presence in tomato seedlings with treatment Seaice-43 (3.24 mg g⁻¹) followed by Seaice-6 (3.15 mg g⁻¹) and Seaice-16 (2.86 mg g⁻¹) respectively.

Finally, the production and identification of volatile compound by potential strain, Seaice-43 in headspace GCMS showed some interesting volatile metabolites such as Hexadecane, 12-Methylaminolauric acid and Silanediol dimethyl, which maybe the

reason for their potential PGP and antagonistic activity. The multi-potential strain Sea ice-43 identified as *Pseudomonas paracarnis* based on cultural, microscopic and molecular identification. From these results, we can conclude that, the strain Seaice-43 has potential candidate to develop as a bio-fertilizer to enhance tomato plant growth and development under salt stress in future.

CHAPTER 7

REFERENCES

1. Abd_Allah EF, Alqarawi AA, Hashem A, Radhakrishnan R, Al-Huqail AA, Al-Otibi FO, Malik JA, Alharbi RI, Egamberdieva D. Endophytic bacterium *Bacillus subtilis* (BERA 71) improves salt tolerance in chickpea plants by regulating the plant defense mechanisms. *Journal of Plant Interactions*. 2018 Jan 1;13(1):37-44.
2. Arthi K, Appalaraju B, Parvathi S. Vancomycin sensitivity and KOH string test as an alternative to gram staining of bacteria. *Indian Journal of Medical Microbiology*. 2003 Apr 1;21(2):121-3.
3. Arora NK, Egamberdieva D, Mehnaz S, Li WJ, Mishra I. Salt tolerant Rhizobacteria: for better productivity and remediation of saline soils. *Frontiers in Microbiology*. 2021 Apr 22;12:660075.
4. Ansari, F. A., Ahmad, I., and Pichtel, J. (2019). Growth stimulation and alleviation of salinity stress to wheat by the biofilm forming *Bacillus pumilus* strain FAB10. *Appl. Soil Ecol.* 3, 45–54. doi: 10.1016/j.apsoil.2019.05.023
5. Ahmad M, Zahir ZA, Asghar HN, Asghar M. Inducing salt tolerance in mung bean through coinoculation with rhizobia and plant-growth-promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate deaminase. *Canadian Journal of Microbiology*. 2011 Jul;57(7):578-89.
6. Alami Y, Champolivier L, Merrien A, Heulin T. 2000. The role of *Rhizobium* sp. rhizobacterium that produces exopolysaccharide in the aggregation of the rhizospheric soil of the sunflower: Effects on plant growth and resistance to hydric constraint. *OCL – Oleagineux Corps Gras Lipides*. 6:524–528.
7. Ashraf, M., Hasnain, S., Berge, O., and Mahmood, T. (2004). Inoculating wheat seedlings with exopolysaccharide-producing bacteria restricts sodium uptake and stimulates plant growth under salt stress. *Biol. Fertil. Soils* 40, 157–162. doi: 10.1007/s00374-004-0766-y
8. Ahmad M., Zahir Z.A., Khalid M. Efficacy of *Rhizobium* and *Pseudomonas* strains to improve physiology, ionic balance and quality of mung bean under salt-affected conditions on farmer's fields. *Plant Physiol. Biochem.* 2013;63:170–176. doi: 10.1016/j.plaphy.2012.11.024
9. Barassi CA, Ayrault G, Creus CM, Sueldo RJ, Sobrero MT. Seed inoculation with *Azospirillum* mitigates NaCl effects on lettuce. *Scientia Horticulturae*. 2006 Jun 9;109(1):8-14.

10. Belimov A. A., Hontzeas N., Safronova V. I., Demchinskaya S. V., Piluzza G., Bullitta S., *et al.* (2005). Cadmium tolerant plant growth-promoting bacteria associated with roots of Indian mustard (*Brassica juncea* L Czern.). *Soil Biol. Biochem.* 37, 241–250. 10.1016/j.soilbio.2004.07.033
11. Berendsen R.L., Vismans G., Yu K., Song Y., de Jonge R., Burgman W.P., Burmølle M., Herschend J., Bakker P., Pieterse C.M.J. Disease-induced assemblage of a plant-beneficial bacterial consortium. *ISME J.* 2018;12:1496–1507. doi: 10.1038/s41396-018-0093-1
12. Boerjan W, Cervera MT, Delarue M, Beeckman T, Dewitte W, Bellini C, Caboche M, Van Onckelen H, Van Montagu M, Inzé D. Superroot, a recessive mutation in *Arabidopsis*, confers auxin overproduction. *The Plant Cell.* 1995 Sep;7(9):1405-19.
13. Bottini R, Fulchieri M, Pearce D, Pharis RP. Identification of gibberellins A1, A3, and iso-A3 in cultures of *Azospirillum lipoferum*. *Plant physiology.* 1989 May;90(1):45-7.
14. Chu TN, Tran BT, Van Bui L, Hoang MT. Plant growth-promoting rhizobacterium *Pseudomonas* PS01 induces salt tolerance in *Arabidopsis thaliana*. *BMC research notes.* 2019 Dec;12(1):1-7.
15. Dobert RC, Rood SB, Blevins DG. Gibberellins and the legume-rhizobium symbiosis: I. Endogenous gibberellins of lima bean (*Phaseolus lunatus* L.) stems and nodules. *Plant Physiology.* 1992 Jan;98(1):221-4.
16. Delfine S, Alvino A, Villani MC, Loreto F. Restrictions to carbon dioxide conductance and photosynthesis in spinach leaves recovering from salt stress. *Plant Physiology.* 1999 Mar 1;119(3):1101-6.
17. Egamberdieva D, Kucharova Z. Selection for root colonising bacteria stimulating wheat growth in saline soils. *Biology and fertility of soils.* 2009 Jul;45:563-71.
18. Fatemeh A, Masoud T, Pejman A, Aidin H. Effect of plant growth promoting rhizobacteria (PGPRs) and stratification on germination traits of *Crataegus pseudoheterophylla* Pojark. *Seeds. Scientia Horticulturae.* 2014 Jun 9;172:61-7.
19. Fett WF, Osman SF, Dunn MF. Auxin production by plant-pathogenic pseudomonads and xanthomonads. *Applied and environmental microbiology.* 1987 Aug;53(8):1839-45.
20. Frank JA, Reich CI, Sharma S, Weisbaum JS, Wilson BA, Olsen GJ. Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Applied and environmental microbiology.* 2008 Apr 15;74(8):2461-70.

21. Fulchieri M, Lucangeli C, Bottini R. Inoculation with *Azospirillum lipoferum* affects growth and gibberellin status of corn seedling roots. *Plant and Cell physiology*. 1993 Dec 1;34(8):1305-9.
22. Glick BR, Todorovic B, Czarny J, Cheng Z, Duan J, McConkey B. Promotion of plant growth by bacterial ACC deaminase. *Critical Reviews in Plant Sciences*. 2007 Oct 23;26(5-6):227-42.
23. Goswami M, Malakar C, Deka S. Rhizosphere microbes for sustainable maintenance of plant health and soil fertility. *Rhizosphere Microbes: Soil and Plant Functions*. 2020:35-72.
24. García de Salamone, I. E., Hynes, R. K., and Nelson, L. M. (2001). Cytokinin production by plant growth promoting rhizobacteria and selected mutants. *Can. J. Microbiol.* 47, 404–411. doi: 10.1139/w01-029
25. HwangBo, K., Son, S.H., Lee, J.S. et al. Rapid and simple method for DNA extraction from plant and algal species suitable for PCR amplification using a chelating resin Chelex 100. *Plant Biotechnol Rep* 4, 49–52 (2010).
26. Haggag, W. M. (2010). The role of biofilm exopolysaccharides on biocontrol of plant diseases. *Biopolymers* 14, 271–284. doi: 10.5772/10266
27. Henry G, Thonart P, Ongena M (2012) PAMPs, MAMPs, DAMPs and others: an update on the diversity of plant immunity elicitors. *Biotechnol Agron Soc Environ* 16:257–268.
28. Jones JD, Dangl JL. The plant immune system. *nature*. 2006 Nov 16;444(7117):323-9.
29. Ilangumaran, G., and Smith, D. L. (2017). Plant growth promoting rhizobacteria in amelioration of Salinity stress: a systems biology perspective. *Front. Plant Sci.* 8:1768. doi: 10.3389/fpls.2017.01768
30. Jatan R, Chauhan PS, Lata C. *Pseudomonas putida* modulates the expression of miRNAs and their target genes in response to drought and salt stresses in chickpea (*Cicer arietinum* L.). *Genomics*. 2019 Jul 1;111(4):509-19.
31. Kudoyarova G, Arkhipova T, Korshunova T, Bakaeva M, Loginov O, Dodd IC. Phytohormone mediation of interactions between plants and non-symbiotic growth promoting bacteria under edaphic stresses. *Frontiers in plant science*. 2019 Oct 29;10:1368.

32. Karadeniz AS, Topcuoğlu ŞF, İnan SÜ. Auxin, gibberellin, cytokinin and abscisic acid production in some bacteria. *World Journal of Microbiology and Biotechnology*. 2006 Oct;22:1061-4.
33. Kang, S. M., Shahzad, R., Bilal, S., Khan, A. L., Park, Y. G., Lee, K. E., *et al.* (2019). Indole-3-acetic-acid and ACC deaminase producing *Leclercia adecarboxylata* MO1 improves *Solanum lycopersicum* L. growth and salinity stress tolerance by endogenous secondary metabolites regulation. *BMC Microbiol.* 19:80. doi: 10.1186/s12866-019-1450-6
34. Kudoyarova G, Arkhipova T, Korshunova T, Bakaeva M, Loginov O, Dodd IC. Phytohormone mediation of interactions between plants and non-symbiotic growth promoting bacteria under edaphic stresses. *Frontiers in plant science*. 2019 Oct 29;10:1368.
35. Kasotia A, Varma A, Tuteja N, Choudhary DK. Amelioration of soybean plant from saline-induced condition by exopolysaccharide producing *Pseudomonas*-mediated expression of high affinity K⁺-transporter (HKT1) gene. *Current Science*. 2016 Dec 25:1961-7.
36. Karlidag, H.; Esitken, A.; Yildirim, E.; Donmez, M.F.; Turan, M. Effects of plant growth promoting bacteria on yield, growth, leaf water content, membrane permeability, and ionic composition of strawberry under saline conditions. *J. Plant Nutr.* 2010, 34, 34–45
37. Khan A.L., Waqas M., Kang S.M. Bacterial endophyte *Sphingomonas* sp. LK11 produces gibberellins and IAA and promotes tomato plant growth. *J. Microbiol.* 2014;52:689–695. doi: 10.1007/s12275-014-4002-7.
38. Kashyap BK, Ara R, Singh A, Kastwar M, Aaysha S, Mathew J, Solanki MK. Halotolerant PGPR Bacteria: Amelioration for Salinity Stress. *Microbial Interventions in Agriculture and Environment: Volume 1: Research Trends, Priorities and Prospects*. 2019:509-30.
39. Kejela T, Thakkar VR, Patel RR. A novel strain of *Pseudomonas* inhibits *Colletotrichum gloeosporioides* and *Fusarium oxysporum* infections and promotes germination of coffee. *Rhizosphere*. 2017 Dec 1;4:9-15.
40. Lisdiyanti P, Suyanto E, Gusmawati NF, Rahayu W. Isolation and characterization of cellulase produced by cellulolytic bacteria from peat soil of Ogan Komering Ilir, South Sumatera. *International journal of environment and bioenergy*. 2012;3(3):145- 53.

41. López-Bucio J, Campos-Cuevas JC, Hernández-Calderón E, Velásquez-Becerra C, Farías-Rodríguez R, Macías-Rodríguez LI, Valencia-Cantero E. *Bacillus megaterium* rhizobacteria promote growth and alter root-system architecture through an auxin- and ethylene-independent signaling mechanism in *Arabidopsis thaliana*. *Molecular Plant-Microbe Interactions*. 2007 Feb;20(2):207-17.
42. Liu F., Xing S., Ma H. Cytokinin-producing, plant growth-promoting rhizobacteria that confer resistance to drought stress in *Platycladus orientalis* container seedlings. *Appl. Microbiol. Biotechnol.* 2013;97:9155–9164. doi: 10.1007/s00253-013-5193-2.
43. Lareen, A., Burton, F., & Schäfer, P. (2016). Plant root-microbe communication in shaping root microbiomes. *Plant Molecular Biology*, 90, 575–587.
44. Manigundan K, Kumar PP, Singh R, Sakthivel K, Gautam RK, Singh PK, Laha GS. Rep-PCR distinguishes rice bacterial blight pathogen (*Xanthomonas oryzae* pv. *oryzae*) strains of Indian mainland and bay islands. *Journal of Plant Pathology*. 2017 Nov 1:773-8.
45. Mukhtar T, Sultan T, Hussain Munis F, Chaudhary HJ. Induction of heat tolerance in tomato cultivar with heat tolerant bacteria under field condition. *Asian Journal of Agriculture and Biology*. 2021(Online).
46. Manaf, H. H., and Zayed, M. S. (2015). Productivity of cowpea as affected by salt stress in presence of endomycorrhizae and *Pseudomonas fluorescens*. *Ann. Agri. Sci.* 60, 219–226. doi: 10.1016/j.aoas.2015.10.013
47. Martinez-Viveros, O., Jorquera, M. A., Crowley, D. E., Gajardo, G. M. L. M., & Mora, M. L. (2010). Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. *Journal of Soil Science and Plant Nutrition*, 10(3), 293–319.
48. Marchawala FZ, Amin SR. Elucidating IAA producing *Kocuria flava* FA10 as a Potent PGPB and Biocontrol Agent. *International Journal of Biotechnology and Biochemistry*. 2018;14(3):185-91.
49. Majeed, A.; Muhammad, Z.; Ahmad, H. Plant growth promoting bacteria: Role in soil improvement, abiotic and biotic stress management of crops. *Plant Cell Rep.* 2018, 37, 1599–1609.
50. Meneses C, Gonçalves T, Alquéres S, Rouws L, Serrato R, Vidal M, Baldani JI. *Gluconacetobacter diazotrophicus* exopolysaccharide protects bacterial cells against oxidative stress in vitro and during rice plant colonization. *Plant and Soil*. 2017 Jul;416:133-47.

51. Numan, M., Bashir, S., Khan, Y., Mumtaz, R., Shinwari, Z. K., Khan, A. L., *et al.* (2018). Plant growth promoting bacteria as an alternative strategy for salt tolerance in plants: a review. *Microbiol. Res.* 209, 21–32. doi: 10.1016/j.micres.2018.02.003
52. Naz I, Bano A, Ul-Hassan T. Isolation of phytohormones producing plant growth promoting rhizobacteria from weeds growing in Khewra salt range, Pakistan and their implication in providing salt tolerance to *Glycine max* L. *African Journal of Biotechnology*. 2009;8(21).
53. Nadeem SM, Zahir ZA, Naveed M, Arshad M (2007) Preliminary investigations on inducing salt tolerance in maize through inoculation with rhizobacteria containing ACC deaminase activity. *Can J Microbiol* 53:1141–1149
54. Nascimento F. X., Rossi M. J., Soares C. R. F. S., McConkey B. J., Glick B. R. (2014). New insights into 1-aminocyclopropane-1-carboxylate (ACC) deaminase phylogeny, evolution and ecological significance. *PLoS ONE* 9:e99168. 10.1371/journal.pone.0099168
55. Nwodo UU, Green E, Okoh AI. Bacterial exopolysaccharides: functionality and prospects. *International journal of molecular sciences*. 2012 Oct 30;13(11):14002- 15.
56. Paul, D. (2013). Osmotic stress adaptations in rhizobacteria. *J. Basic Microbiol.* 53, 101–110. doi: 10.1002/jobm.201100288.
57. Powers EM. Efficacy of the Ryu nonstaining KOH technique for rapidly determining gram reactions of food-borne and waterborne bacteria and yeasts. *Applied and environmental microbiology*. 1995 Oct;61(10):3756-8.
58. Paranychanakis NV, Chartzoulakis KS. Irrigation of Mediterranean crops with saline water: from physiology to management practices. *Agriculture, ecosystems & environment*. 2005 Apr 2;106(2-3):171-87.
59. Pahari A, Mishra BB. Characterization of siderophore producing rhizobacteria and its effect on growth performance of different vegetables. *Int J Curr Microbiol App Sci*. 2017;6(5):1398-405.
60. Porcel R, Zamarreño ÁM, García-Mina JM, Aroca R. Involvement of plant endogenous ABA in *Bacillus megaterium* PGPR activity in tomato plants. *BMC plant biology*. 2014 Dec;14(1):1-2.

61. Piccoli, P., Travaglia, C., Cohen, A., Sosa, L., Cornejo, P., Masuelli, R., *et al.* (2011). An endophytic bacterium isolated from roots of the halophyte *Prosopis strombulifera* produces ABA, IAA, gibberellins A 1 and A 3 and jasmonic acid in chemically-defined culture medium. *Plant Growth Regul.* 64, 207–210. doi: 10.1007/s10725-010-9536-z
62. Pérez-Alfocea F, Albacete A, Ghanem ME, Dodd IC. Hormonal regulation of source–sink relations to maintain crop productivity under salinity: a case study of root-to-shoot signalling in tomato. *Functional Plant Biology.* 2010 Jul 2;37(7):592- 603.
63. Rodriguez H., Vessely S., Shah S., Glick B. R. (2008). Effect of a Nickel-tolerant ACC deaminase-producing *Pseudomonas* strain on growth of nontransformed and transgenic canola plants. *Curr. Microbiol.* 57, 170–174. 10.1007/s00284-008-9181-1.
64. Roslan MA, Zulkifli NN, Sobri ZM, Zuan AT, Cheak SC, Abdul Rahman NA. Seed biopriming with P-and K-solubilizing *Enterobacter hormaechei* sp. improves the early vegetative growth and the P and K uptake of okra (*Abelmoschus esculentus*) seedling. *PloS one.* 2020 Jul 9;15(7):e0232860.
65. Roberson EB, Firestone MK. Relationship between desiccation and exopolysaccharide production in a soil *Pseudomonas* sp. *Appl Environ Microbiol* 1992;58:1284–91
66. Ruppel S, Franken P, Witzel K. Properties of the halophyte microbiome and their implications for plant salt tolerance. *Functional Plant Biology.* 2013 Apr 9;40(9):940-51.
67. Samayoa BE, Shen FT, Lai WA, Chen WC. Screening and Assessment of Potential Plant Growth-promoting Bacteria Associated with *Allium cepa* Linn. *Microbes and environments.* 2020;35(2):ME19147.
68. Sharma A, Dev K, Sourirajan A, Choudhary M. Isolation and characterization of salt-tolerant bacteria with plant growth-promoting activities from saline agricultural fields of Haryana, India. *Journal of Genetic Engineering and Biotechnology.* 2021 Dec;19(1):1-0.
69. Sapre S, Gontia-Mishra I, Tiwari S. ACC deaminase-producing bacteria: a key player in alleviating abiotic stresses in plants. *Plant growth promoting rhizobacteria for agricultural sustainability: from theory to practices.* 2019:267-91.
70. Shamshitov A, Decorosi F, Viti C, Fornasier F, Kadžienė G, Supronienė S. Characterisation of Cellulolytic Bacteria Isolated from Agricultural Soil in Central Lithuania. *Sustainability.* 2022 Dec 29;15(1):598.

71. Shahzad, R.; Khan, A.L.; Bilal, S.; Waqas, M.; Kang, S.M.; Lee, I.J. Inoculation of abscisic acid-producing endophytic bacteria enhances salinity stress tolerance in *Oryza sativa*. *Environ. Exp. Bot.* 2017, 136, 68–77.
72. Sultana S, Alam S, Karim MM. Screening of siderophore-producing salt-tolerant rhizobacteria suitable for supporting plant growth in saline soils with iron limitation. *Journal of Agriculture and Food Research*. 2021 Jun 1;4:100150.
73. Spaepen S., Vanderleyden J., Remans R. Indole-3-acetic acid in microbial and microorganism-plant signaling. In: Uden F., editor. *FEMS Microbiology Reviews*. Blackwell Publishing Ltd.; New York, NY, USA: 2007. pp. 425–448
74. Spaepen S, Vanderleyden J. Auxin and plant-microbe interactions. *Cold Spring Harbor perspectives in biology*. 2011 Apr 1;3(4):a001438.
75. Sarkar A, et al. A halotolerant *Enterobacter* sp. displaying ACC deaminase activity promotes rice seedling growth under salt stress. *Res Microbiol*. 2018;169:20e32. doi: 10.1016/j.resmic.2017.08.005.
76. Seckin B, Sekmen AH, Türkan I. An enhancing effect of exogenous mannitol on the antioxidant enzyme activities in roots of wheat under salt stress. *Journal of Plant Growth Regulation*. 2009 Mar;28:12-20.
77. Shaharoona B, Naveed M, Arshad M, Zahir ZA (2008) Fertilizer-dependent efficiency of *Pseudomonads* for improving growth, yield, and nutrient use efficiency of wheat (*Triticum aestivum* L.). *Appl Microbiol Biotechnol* 79:147–155
78. Skubacz A, Daszkowska-Golec A, Szarejko I. The role and regulation of ABI5 (ABA-Insensitive 5) in plant development, abiotic stress responses and phytohormone crosstalk. *Frontiers in plant science*. 2016 Dec 16;7:1884.
79. Shu K, Zhou W, Chen F, Luo X, Yang W. Abscisic acid and gibberellins antagonistically mediate plant development and abiotic stress responses. *Frontiers in Plant Science*. 2018 Mar 27;9:416.
80. Torres-Rubio MG, Valencia-Plata SA, Bernal-Castillo J, Martínez-Nieto P. Isolation of *Enterobacteria*, *Azotobacter* sp. and *Pseudomonas* sp., producers of indole-3-acetic acid and siderophores, from Colombian rice rhizosphere. *Revista Latinoamericana de Microbiología*. 2000;42(4):171-6.

81. Tilak, K. V. B. R., Ranganayaki, N., Pal, K. K., De, R., Saxena, A. K., Nautiyal, C. S., Mittal, S., Tripathi, A. K., & Johri, B. N. (2005). Diversity of plant growth and soil health supporting bacteria. *Current Science*, 89, 136–150.
82. TrParray, A. P., Jan, S., Kamili, A. N., Qadri, R. A., Egamberdieva, D., and Ahmad, P. (2016). Current perspectives on plant growth promoting rhizobacteria. *Plant Growth Regul.* 35, 877–902.
83. Upadhyay SK, Singh JS, Singh DP. Exopolysaccharide-producing plant growth-promoting rhizobacteria under salinity condition. *Pedosphere*. 2011 Apr 1;21(2):214-22.
84. Upadhyay SK, Singh JS, Saxena AK, Singh DP. 2012. Impact of PGPR inoculation on growth and antioxidant status of wheat under saline conditions. *Plant Biol.* 14: 605-611.
85. Vacheron J., Desbrosses G., Bouffaud M.-L., Touraine B., Moënne-Loccoz Y., Muller D. Plant growth-promoting rhizobacteria and root system functioning. *Front. Plant Sci.* 2013;4 doi: 10.3389/fpls.2013.00356.
86. Van Loon LC & Glick BR (2004) Increased plant fitness by rhizobacteria. In: *Molecular ecotoxicology of plants* (Sander mann H eds.) Springer-Verlag, Berlin: Heidelberg, pp 177–205.
87. Vaidya S, Rathore P. Isolation, screening and characterization of amylase producing bacteria from soil of potato dump sites from different regions of madhya pradesh. In *Conference Paper 2015*.
88. Woo, S. L., and Pepe, O. (2018). Microbial consortia: promising probiotics as plant biostimulants for sustainable agriculture. *Front. Plant Sci.* 9:1801. doi: 10.3389/fpls.2018.01801
89. Zhao Y, Christensen SK, Fankhauser C, Cashman JR, Cohen JD, Weigel D, Chory J. A role for flavin monooxygenase-like enzymes in auxin biosynthesis. *Science*. 2001 Jan 12;291(5502):306-9.
90. Yeo AR, Flowers TJ. Salinity resistance in rice (*Oryza sativa* L.) and a pyramiding approach to breeding varieties for saline soils. *Functional Plant Biology*. 1986;13(1):161-73.
91. Yildirim E, Taylor AG. Effect of biological treatments on growth of bean plants under salt stress. *Science*. 2005;123(1).

92. Yuan Y, Brunel C, van Kleunen M, Li J, Jin Z. Salinity-induced changes in the rhizosphere microbiome improve salt tolerance of *Hibiscus hamabo*. *Plant and Soil*. 2019 Oct;443:525-37.
93. Yavarian S, Jafari P, Akbari N, Feizabadi MM. Selective screening and characterization of plant growth promoting bacteria for growth enhancement of tomato, *Lycopersicon esculentum*. *Iranian Journal of Microbiology*. 2021 Feb;13(1):121.
94. Zhang, S., Thomas L. White, T.L., Martinez, M.C., McInroy, J.A., Kloepper, J.W., Klassen, W. Evaluation of plant growth-promoting rhizobacteria for control of *Phytophthora* blight on squash under greenhouse conditions. *Biol Control*. 2010; 53: 129–135.
95. Zerrouk IZ, Rahmoune B, Khelifi L, Mounir K, Baluska F, Ludwig-Müller J. Algerian Sahara PGPR confers maize root tolerance to salt and aluminum toxicity via ACC deaminase and IAA. *Acta Physiol Plant*. 2019;41:91. doi: 10.1007/s11738-019- 2881-2.