AMELIORATIVE EFFECT OF SALINITY STRESS USING PLANT GROWTH PROMOTING BACTERIA

A RESEARCH PROJECT

Submitted by

HARSHADA A. PANCHAL

RITIKA R. GHOSE

GAURAV J. PATIL

UNDER THE GUIDANCE OF

DR. K.K. BHISE

(Assistant Professor)

DEPARTMENT OF MICROBIOLOGY

VIVEKANAND COLLEGE, KOLHAPUR

(EMPOWERED AUTONOMOUS)

YEAR 2023-2024

"Dissemination of education for Knowledge, Science and culture"
- Shikshanmaharshi Dr. Bapuji Salunkhe

Shri Swami Vivekanand Shikshan Sanstha's

VIVEKANAND COLLEGE, KOLHAPUR (EMPOWERED AUTONOMOUS)

PG DEPARTMENT OF MICROBIOLOGY

CERTIFICATE OF RESEARCH PROJECT COMPLETION

This is to certify that **Ms. Harshada Anil Panchal** studying in M. Sc. part II Microbiology at Vivekanand College, Kolhapur (Empowered Autonomous) has sincerely completed research project work entitled "**AMELIORATIVE EFFECT OF SALINITY STRESS USING PGPB**" during academic year 2023-24.

Dr. Komal K. Bhise
Project supervisor

Dr. R. J. Bansode 20/5/24
Examiner

Dr. M. J. D.

Dr. G. K. Sontakke
Head of the Department

"Dissemination of education for Knowledge, Science and culture"
- Shikshanmaharshi Dr. Bapuji Salunkhe

Shri Swami Vivekanand Shikshan Sanstha's

VIVEKANAND COLLEGE, KOLHAPUR (EMPOWERED AUTONOMOUS)

PG DEPARTMENT OF MICROBIOLOGY

CERTIFICATE OF RESEARCH PROJECT COMPLETION

This is to certify that **Ms. Ritika Rajendra Ghose** studying in M. Sc. part II Microbiology at Vivekanand College, Kolhapur (Empowered Autonomous) has sincerely completed research project work entitled "AMELIORATIVE EFFECT OF SALINITY STRESS USING PGPB" during academic year 2023-24.

Dr. Komal K. Bhise
Project supervisor

Dr. R. S. Bansode
20/3/24
Examiner

Dr. G. K. Sontakke
Head of the Department

"Dissemination of education for Knowledge, Science and culture"
- Shikshanmaharshi Dr. Bapuji Salunkhe

Shri Swami Vivekanand Shikshan Sanstha`s

VIVEKANAND COLLEGE, KOLHAPUR (EMPOWERED AUTONOMOUS)

PG DEPARTMENT OF MICROBIOLOGY

CERTIFICATE OF RESEARCH PROJECT COMPLETION

This is to certify that **Mr. Gaurav Jitendra Patil** studying in M. Sc. part II Microbiology at Vivekanand College, Kolhapur (Empowered Autonomous) has sincerely completed research project work entitled "**AMELIORATIVE EFFECT OF SALINITY STRESS USING PGPB**" during academic year 2023-24.

Dr. Komal K. Bhise
Project supervisor

20/5/24
Examiner

Dr. G. K. Sontakke
Head of the Department

ACKNOWLEDGEMENT

First of all, we would like to thank Dr. Komal K. Bhise who is presently working as an Assistant Professor at PG Department of Microbiology, Vivekanand College, Kolhapur (Empowered Autonomous) for guiding us through this project work. We are extremely grateful to her for all her invaluable guidance and kind suggestions during all the phases of our project work. Her ever-encouraging attitude, guidance and whole hearted help were biggest motivation for us in completing this project work.

We are very grateful to Dr. R.R. Kumbhar (Principal) of Vivekanand College, Kolhapur (Empowered Autonomous) for motivating me for this project work. Also, we are thankful to Dr. G.K. Sontakke, Head of Department of Microbiology for providing necessary facilities for completion of this project work. We also thankful to whole team of the project for supporting each other in completion of this project work. Lastly, we would thank all the people who have guided and helped me directly or indirectly.

Place: Kolhapur.

Date: 15/05/2024.

Ms. Harshada Anil Panchal

Ms. Ritika Rajendra Ghose

Mr. Gaurav Jitendra Patil

INDEX

INTRODUCTION

1.0 INTRODUCTION

Agriculture is one of the main sources for livelihood of 86% rural households. It plays an important role for development of under developing countries (J.J. Dethier, 2011). It provides income and jobs which results in reduction of poverty rate (H.Alderman, 2007).The challenges regarding agriculture includes food productivity & production in developing countries as well as volatility of food prices. The integrated steps can ensure to cooperate the most vulnerable countries & people to obtain the nutrition they seek (Zoellick, 2011).

One of the agricultural systems in world is Indian agriculture. It involves different agro systems based on the united effects of climate, soil, geological vegetations & other natural features which concludes variations within crops. Along with physical diversification depends upon economic , cultural , religious & survival factors .Due to its 50% total geographical area utilization for cultivation, India is top user of land for agriculture. It is the essential factor of economy for sustainable & economical growth of country. Throughout the world, India is the largest producer of milk, pulses and jute; second largest rice, wheat, cotton, fruits and vegetables & leading producer of spices, fish, poultry, livestock and plantation crops. (T Mohapatra, 2022)

Although being self sufficient for food, production came at risk due to famine between 1947 & 1960. Hence to alleviate the extreme poverty & malnourishment 'Green Revolution' was initiated in 1960. One of the greatest achievements enlisted of 20th century highlights 'Green Revolution' which helped India triple food grain production in between 1968-2000 (Singh 2014) .The Green Revolution technology involves utilization of new highly yielding seeds & chemical fertilizers (Newman, 2007). Hence the Green Revolution was pivotal moment in India's history due to agricultural transformation which was driven by introduction of High Yield Variety seeds, fertilizers, pesticides & irrigation infrastructure. It was a time which aimed

at addressing chronic food security & propels country for being self sufficient (Singh S. S., 2019).

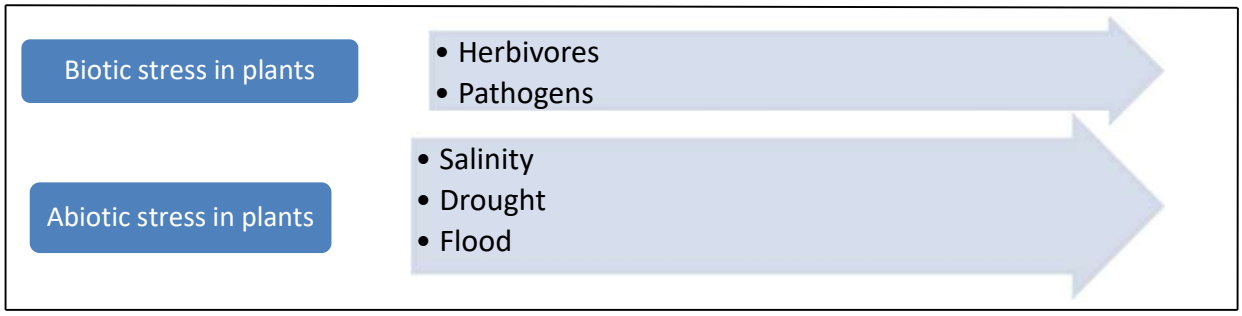
Traditionally India dominated at farming of rice & wheat. But awareness regarding benefits of pulses led to increased production & consumption of pulses (P. K.Joshi, 2020). The late 2010 brought up 'Pulse Revolution' after efforts of around three decades (O.P. Yadav, 2019). In India 12 pulses crops which mainly includes Cicer arietunum (chickpea), Cajanus cajan(pigeon pea), Vigna radiata (mungbean), Vigna mungo(urdbean), Lens culinaris(lentil) & Pisum sativum (fieldpeas).India contributes 25.7% in global pulses production (FAOSTAT, 2015).

Vigna radiata which is commonly known as mung bean or green gram holds significant positions in Indian agriculture& is believed to be belonging to India (M.Kalyani, 2015). It is one of the important pulse crops which are cultivated throughout the country. It is a rich source of readily digestible plant based protein with essential amino acids (V.Jawahar, 2021). Furthermore, mungbean is good source of vitamins (vitamins A, B, C) & minerals (iron) contributes to dietary needs (G. M. Naik, 2020).One of the research emphasizes its adaptability to diverse agro- economical zones within India (W.Walelign, 2021).

Stress in plants refers to the physiological and biochemical responses elicited when plants are exposed to adverse environmental conditions, which can include biotic and abiotic factors such as drought, salinity, extreme temperatures, pathogens, and pollutants (Mittler, Abiotic stress, the field environment and stress combination., 2006). The stress causing factors can be of two types: Biotic stress & Abiotic stress. Biotic stress is originated from living entities including herbivores or pathogens (J.Bailey-Serres, 2019).Abiotic stress arises usually from non living factors like drought, extreme temperatures, or nutrient deficiencies (M. Hasanuzzaman, 2017). Abiotic factors such as temperature extremes, water scarcity, salinity, nutrient deficiency,

and heavy metal toxicity can directly impact plant growth and development by disrupting physiological processes (M. Farooq, 2009). Biotic factors including pest infestations, pathogen attacks, and allelopathic interactions further exacerbate stress conditions by compromising plant defense mechanisms and resource allocation (P.Pandey, 2017).

Figure 1 Types of stress



Stress in plants can also lead to the generation of reactive oxygen species (ROS), which are highly reactive molecules that can cause oxidative damage to cellular components such as DNA, proteins, and lipids (Mittler, Oxidative stress, antioxidants and stress tolerance., 2002). To mitigate the harmful effects of ROS, plants have evolved antioxidant defense mechanisms, including enzymes such as superoxide dismutase, catalase, and peroxidases, as well as non-enzymatic antioxidants like glutathione and ascorbate (R.Mittler S. M., 2004).Similarly, under high-salinity conditions, plants may accumulate salt ions in vacuoles or excrete them through specialized structures like salt glands or trichomes (R.Munns & M.Tester, 2008).

Stress in plants, particularly salinity stress, poses a significant challenge to agricultural productivity and food security worldwide. According to studies, stress in plants refers to any adverse environmental condition that disrupts normal physiological processes, leading to impaired growth, development, and ultimately, reduced yield. (R.Munns., & M. Tester, 2008). One of the most prevalent forms of stress in plants is salinity stress, which occurs when soil or irrigation water contains high levels of soluble salts, primarily sodium chloride (NaCl) (R. Gilliham, 2015). Salinity stress, in particular, poses a significant threat to crop

production in many regions, especially in arid and semi-arid areas where irrigation practices contribute to soil salinization (P.Rengasamy, 2006).

Excessive application of chemical fertilizers has been identified as a significant contributor to soil salinization, leading to adverse consequences for plant health and ecosystem sustainability (L.Zeng, 2020).Chemical fertilizers, commonly used to enhance soil fertility and crop yield, can elevate salinity stress through multiple pathways. Nitrogen-based fertilizers, such as ammonium nitrate and urea, can increase soil salinity by contributing to the accumulation of nitrate ions (G.Xu, 2020). Moreover, phosphorus fertilizers, like superphosphate, can indirectly elevate soil salinity by promoting the dissolution of insoluble salts (D.K.Gupta, 2019). Furthermore, potassium fertilizers, including potassium chloride and potassium sulfate, can directly contribute to soil salinization when applied in excessive amounts (A.L.Julkifle, 2018). The interaction between chemical fertilizers and soil salinity is influenced by various factors, including soil type, climate conditions, irrigation practices, and crop management techniques (A.Haque, 2020). For instance, sandy soils with poor water retention capacity are more susceptible to salt accumulation from fertilizer applications compared to clayey soils (Gao et al., 2017). Similarly, arid and semi-arid regions with limited rainfall and high evaporation rates are prone to salinity problems exacerbated by inappropriate fertilizer usage (M.A.Khan, 2019).

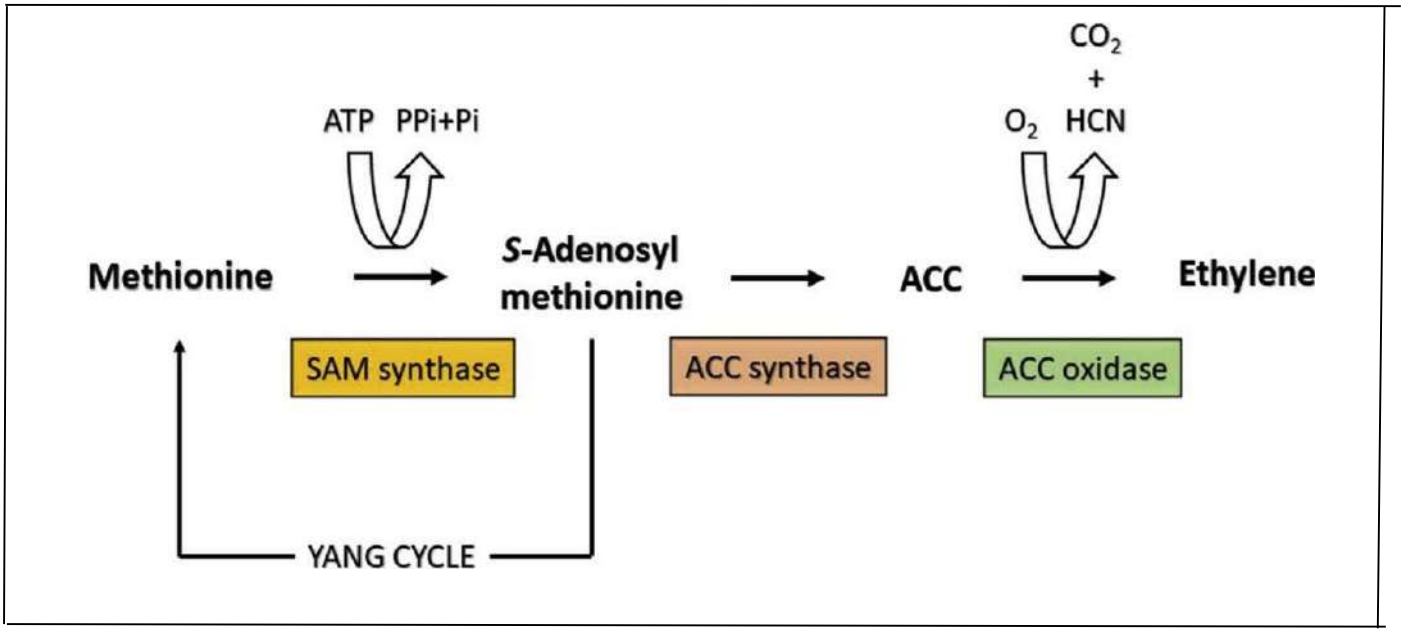
One of the most phytohormone involved in flowers, fruits & leaves development is ethylene (N. Iqbal, 2017). Ethylene is a gaseous hormone produced by all higher plants, although it is also synthesized by some bacteria, fungi and other organisms (Jordán and Casaretto, 2006).Generally as the level of stress increases due to high salinity level in plants,increase in production of ethylene occurs as stress hormone" (Glick B. , 2012). The increased level of ethylene triggers the processes leading to the senescence of various organs; leaf yellowing; the abscission of flowers, petals, leaves and even premature death (Zahir, 2009). Along with this, ethylene inhibits the nodules formation in the roots of legumes, and hence

reduces nitrogen (Nascimento F. R., 2016a).

Ethylene moves rapidly through plant tissues by passive diffusion due to its gaseous state, without any specific transporters. It is produced in a wide range of concentrations depending on the environmental conditions. At low concentrations, ethylene can induce seed germination, the elongation of plant roots, the formation of leaf and root primordia in stems and roots, and initiation of flowering. In fruits and vegetables, ethylene can induce maturation and degradation of the product. In ripening fruit ethylene concentrations may reach levels as high as 200 μL/L. Also, ethylene may be involved in the production of volatile organic compounds that are important for fruit (Choudhary, 2017.).

Ethylene moves rapidly through plant tissues by passive diffusion due to its gaseous state, without any specific transporters. It is produced in a wide range of concentrations depending on the environmental conditions. At low concentrations, ethylene can induce seed germination, the elongation of plant roots, the formation of leaf and root primordia in stems and roots, and initiation of flowering. In fruits and vegetables, ethylene can induce maturation and degradation of the product. In ripening fruit ethylene concentrations may reach levels as high as 200 μL/L. Also, ethylene may be involved in the production of volatile organic compounds that are important for fruit (D.Choudhary, 2017.).

Figure 2 ACC deaminase



Microbial inoculants, such as plant growth-promoting rhizobacteria (PGPR) and mycorrhizal fungi have been explored as bio-ameliorants to mitigate salinity stress and enhance nutrient uptake in plants (A.Kumar, 2022).

According to environmental conditions (temperature, chemical composition, moisture content, amount of soil, plants nearby), the number of microorganism changes (B.R.Glick, 1999). The microorganisms found around root of the plants having potentiality to provide growth of the plants are known as Plant Growth Promoting Bacteria (G. Santoyo, 2016). PGPB are present in the soil zone surrounding the plant root known as rhizosphere (Walker TS, 2003). Plant provides nutrition to these bacterial species; bacteria, in turns enhance the plant growth with production of different plant growth promoting bacteria (Patel RR, 2015).

PGPB eliminates the inhibitory effects of stress ethylene and facilitates long root formation in plant growing in presence of salinity (Penrose and Glick 2003). Drought , salinity and contamination of heavy metals in soil are the major environmental stresses , causing alterations in physiological, biochemical and molecular processes of plants by exerting osmotic,

oxidative as well as ionic stresses (M.Kaushal, 2016) .

The prevention of water loss is necessary to overcome the osmotic stress and maintaining osmotic balance in the plant cell, under high salinity (Wani, 2013.). As per osmotic stress, electrically neutral, low molecular weight, and highly soluble solutes termed as osmolytes are accumulated inside the plant cell (Ahn, 2011.). Plant have ability to produce several types of osmoprotectants, and most common are sugars, , sugar alcohols, ammonium compound sand few specific amino acids which help in survival under high salt stress (Singh M. K., 2015.). PGPB have ability to produce osmoprotectants or modify the biosynthesis in plant and increase salt tolerance. PGPB produces osmolytes which improves hydraulic conductivity and water potential that gives positive impact on stomatal opening and transpiration rate in the plant (Ilangumaran, 2017.)

ACC deaminase activity is most common in the plant microbiome which emphasizes importance of this activity to the interacte and communicate between plants and PGPB (Timmusk, 2011). The rhizosphere, are the preferred location for the isolation and characterization of rhizobacteria with beneficial activities, not only to find ACC deaminase, but to search for multiple mechanisms that promote plant (Wu, 2019).According to recent studies, the presence ACC deaminase has been reported not only in Rhizobium, but in several Rhizobiaceae (*Rhizobium*, *Sinorhizobium,* and *Agrobacterium*), Phyllobacteriaceae (*Phyllobacterium* and *Mesorhizobium)* and *Azospirillum* (Nascimento F. R., 2014). The specific role of a gene may be analysed by generating mutant strains, as well as isolating and expressing the target gene in heterologous hosts.

Due to salt stress, their expression leads to lower uptake of phosphate & malfunctioning in root region (Lv, 2021). PGPB can modulate expression of some key genes related to phosphate transport and improve PO42- in plants under salinity stress (Liu, 2019.).

Genes for phosphatase activity and organic acid production present in salt tolerant PGPB are over expressed which help in increased solubilization of insoluble mineral phosphate. For instance, inoculation of salt-tolerant PGPB was found to enhance acidic phosphatase activity under salinity and phosphate starved conditions (Srivastava, 2020.).Research also indicates that co-inoculation of salt-tolerant PGPB with arbuscular mycorrhizal fungi (AMF) increased expression of PHTs in many crops both in saline and non-saline conditions (Saia, 2015.).

The bacterial polysaccharides produced and secreted extracellularly during biofilm formation through cell wall anchored enzymes are termed EPS. Based on their chemical composition EPS are categorized into homo and heteropolysaccharides (Nwodo, 2012). The neutral sugars, monosaccharides, amino sugars, uronic acids, and pyruvate ketals are basic constituents of EPS (Kaushal, 2016). Several PGPB are known to produce EPS which help plants to survive under saline conditions. Under salt stress conditions, EPS produced by PGPB bind Na+ cations which reduce its (Na+) content around the root zone thereby helping in maintenance of ionic balance. EPS produced by PGPB also help in soil aggregation, water retention, and chelation of metal ions in the plant under salinity (Upadhyay, 2011). Recently, it has been revealed that the biosynthesis of lipopolysaccharide (LPS) could have a distinct role in the synthesis of EPS that can affect the salt tolerance ability of PGPB. A functioning during LPS biosynthesis reduced EPS production that also affected the salt tolerance pattern and competitive fitness of mutant strain in the rhizosphere in comparison to wild strain (Costa-Gutierrez, 2020.). EPS helped in Na+ ion sequestration while ACCD caused degradation of ACC into α-ketobutyrate and ammonia which enhanced the germination rate of wheat plant seedlings and growth under salt stressed conditions (Amna U. D., 2019.). Researchers have even suggested the amendment of bioinoculants designed for saline soils with metabolites such as EPS for better protection of the inoculated PGPB and benefits to the plant (Arora, 2021.)

In this study, the plant salinity stress was reduced with the help of plant growth promoting bacteria. As the potent organism can tolerate the salt stress, it has ability to survive in saline soil. Due to its ability to formation of ACC deaminase, it reduces the production of ACC which consequently reduces ethylene production in excessive amount. Along with this study, shows that, the isolated species have ability of nitrogen fixation, phosphate solubilization, ammonia production & exopolysachharide production .Along with this, it produces enzymes like cellulase & protease which are able to degrade fungal cell wall. After inoculating bioinoculum to the plant, it has shown rapid growth in aspect of height & weight.

REVIEW OF LITERATURE

2.0 REVIEW OF LITERATURE

2.1 Stress in plants:

Salt stress is an ecological constraint that influences plant growth and development. It is a ruinous danger to worldwide agriculture (R.Munns & M.Tester, 2008). Outdated irrigational practices and inappropriate utilization of manures has mainly contributed to excess salt in agricultural lands (Ouhibi, 2014.). Excess salt in the soil causes the accumulation of Na^+ and Cl^- ions in the soil, causing hyper osmotic and hyper ionic conditions, which obstruct plant retention of water and supplements from the soil (Ismail et al., 2014). Worldwide about 400 million hectares of land (over 6%) are affected by some sort of salinization. Of the watered farmland regions, at present, 19.5% are salt-influenced, and this percentage is increasing day by day (Zhan et al., 2019). The indiscriminate application of chemical fertilizers alters the soil's chemical composition, resulting in elevated levels of salts such as sodium, chloride, and sulfate (Huang, 2019). Prolonged exposure to high levels of soluble salts alters soil pH, leading to soil acidification or alkalization, which further exacerbates salinity stress in plants (Li, 2023). Additionally, the leaching of excess salts from fertilized soils into groundwater and surface water bodies poses a threat to aquatic ecosystems and human health (Xie, 2022).

These stressors have ability to disrupt normal plant growth and development, leading to reduced productivity and increased susceptibility to diseases (Chinnusamy, 2004) . Stress in plants can show ill effects at various levels, including molecular, cellular, physiological, and morphological levels (M. Chaves, 2009) .One of the primary responses of plants to stress is the activation of various signaling pathways that regulate gene expression and metabolic processes

to cope with the adverse conditions (Shinozaki, 2006).At the molecular level, stress responses in plants often involve the accumulation of stress-related proteins, such as chaperones and proteases, which help in protein folding, stabilization, and degradation to maintain cellular homeostasis under stress conditions (R.Mittler S. N., 2012). Additionally, plants may accumulate osmolytes such as proline and soluble sugars to maintain cellular turgor and osmotic balance under conditions of water deficit or salinity (P. M.Hasegawa, 2000).

This phenomenon negatively impacts plant growth, development, and ultimately, crop yield (J.Bose, 2014). Salinity stress occurs when soluble salts accumulate in the soil, leading to osmotic stress and ion toxicity in plants. High soil salinity disrupts water and ion uptake by plant roots, leading to nutritional imbalances. Salinity stress in plants occurs when the concentration of soluble salts in the soil surpasses the tolerance threshold of the plant species (R.Munns & M.Tester, 2008). Additionally, salt-induced oxidative stress causes cellular damage by generating reactive oxygen species (ROS), which in turn trigger lipid peroxidation, protein denaturation, and DNA damage (N.Tuteja, 2010).). Along with osmotic stress, ion toxicity, nutrient imbalance, and oxidative damage in plants, salinity stress can ultimately result in reduced growth, yield losses, and even plant death (Zhu, 2016).

The impacts of salt mainly include an imbalance in osmotic pressure, specific ion toxicity, and overproduction of reactive oxygen species (ROS) (Abbasi, 2016.). Initially, when there is an increase in salt concentration around the roots, the plant experiences osmotic stress (Abbasi, 2016.). If the salt stress persists, there is Na+ aggregation, which causes nutrient disproportion, prompting ion toxicity. Most plants belong to the category of glycophytes, which cannot tolerate high salty conditions. Hence the issue of salt stress needs to be addressed. Under salt stress, plants modify their physiological and biochemical procedures, associated with

managing ions and osmotic homeostasis and develop mechanisms for ROS detoxification up to some extent. However, there is a need to develop ways and mechanisms to mitigate salt stress.

The study on alleviation of salt stress is of prime importance to enhance overall crop efficiency, productivity, and sustainability. Rising global hunger and salinity stress make lucrative curiosity in sustainable environmental practices. Intensive research is being carried on to enhance stress tolerance, efficiency, and protection of plants. Various substances have been used to mitigate the negative effect of salt on plants (Ahmad, 2017)

2.2 Ethylene & ACC:

As one of the determining factors, abiotic conditions affect the growth and development of plants as well as define the cumulative production and productivity of food and horticultural crops (Ganie, 2020). It is evident that plant growth, development, and senescence are mediated by hormones (Wang F. A., 2019). Of these three events, plant growth and senescence are widely controlled by ethylene (ET) (Iqbal, 2017); nonetheless, ET is also considered as a multifunctional phytohormone because of its diverse role in many other plants biological processes (Wang F. A., 2019). ET directs the emergence of leaves, inflorescence, and fruits in addition to induction or inhibition of senescence based on its optimal or sub-optimal concentrations (Pierik, 2006). Even though ET emission cannot be well regulated (Tucker, 2015), the gaseous nature of ET is the key reason that restricts its applications in investigational and applied purposes. However, ethephon [(2-chloroethyl)-phosphonic acid], an ET-emitting compound having an array of practical applications, is widely used in agriculture as a substitute for ET (Hu Y. V., 2017).

In addition to the role of ET in normal developmental processes, its role in the regulation of growth and development under abiotic stress has recently emerged (Dubois, 2018.)

Furthermore, exogenous application of ACC (an ET precursor) as well as enhanced endogenous production of ethylene alleviates the salt-induced inhibition of seed germination in Arabidopsis (Divi, 2010).

Accumulating evidence indicates that the role of ET production and signaling in plant tolerance to salt stress can be either positive or negative depending on the plant species and the specific condition examined (Dubois, 2018.).

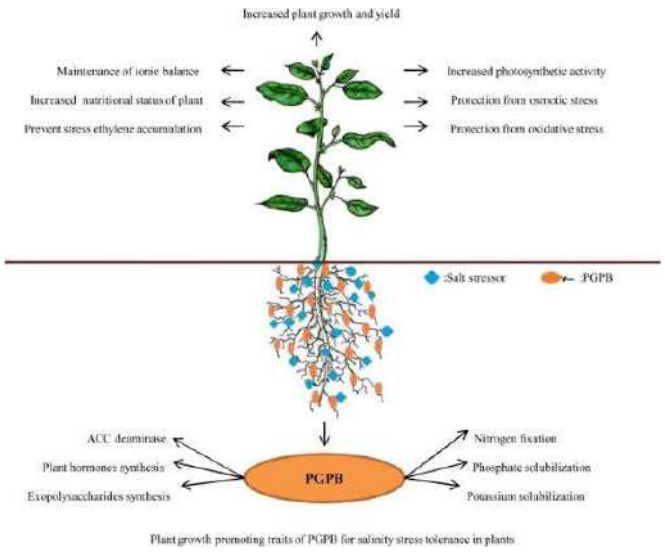
2.3 Role of Plant Growth Promoting Bacteria

The mechanisms employed by soil bacteria to facilitate plant growth are reasonably well known and understood (Glick B. , 2012). Conceptually, PGPB may affect plant growth either directly or indirectly. Direct promotion of plant growth occurs when either (i) the PGPB facilitates the acquisition of resources from the environment including nitrogen, phosphorous and iron; or (ii) modulates plant growth by providing or regulating various plant hormones including auxin, cytokine or ethylene. Indirect promotion of plant growth by PGPB occurs when a bacterium limits or prevents the damage to plants that might otherwise be caused by various pathogenic agents including bacteria, fungi and nematodes.

There are a large number of common mechanisms that PGPB use to indirectly promote plant growth including the production of antibiotics, cell wall-degrading enzymes, lowering plant ethylene levels, induced systemic resistance, decreasing the amount of iron available to pathogens, and the synthesis of pathogen-inhibiting volatile compounds (Glick B. , 2015.).Two general types of soil bacteria have been shown to have the capacity to act as PGPB; rhizospheric bacteria, that are typically found around the roots of plants; and endophytic bacteria(Lacava and Azevedo, 2013), that are found within the tissues of the plant itself (notwithstanding the fact that endophytic bacteria may also be found free-living in the soil). For the most part, rhizospheric and endophytic PGPB utilize similar, if not identical, mechanisms to promote plant growth. The main difference being that endophytic PGPB, once they are established within the tissues of the host plant, is no longer subject to the vagaries of changing soil conditions. These changing conditions, which may inhibit the functioning and proliferation of rhizospheric PGPB, include variations in temperature, soil pH and water content, and the presence of soil bacteria that may compete for binding sites on host plant root surfaces (Glick B. , 2012).

Interestingly, the vast majority of studies of the mechanisms used by PGPB have involved rhizospheric bacteria. Conversely, most of the studies directed toward utilizing PGPB as part of a protocol, together with plants, to remove specific contaminants from the environment (i.e., phytoremediation) have employed endophytic PGPB. There does not seem to be any scientific basis for this divide, i.e., the use of rhizospheric PGPB by one group of scientists and the use of endophytic PGPB by another group. Rather, each group emphasized studies with organisms that they were most familiar with. Given that it now quite clear that these organisms utilize essentially the same mechanisms to promote plant growth, it would appear to be advantageous to purposefully utilize endophytic PGPB to facilitate plant growth in agriculture, horticulture and silviculture as well as environmental cleanup, since they are much more likely to persist in the environment. One review article suggested that with the increasing interesting bacterial endophytic that there is a real possibility that in the future they will be used in a wide range of real-world applications (Ryan et al., 2008). These include industrial and medical applications (such as antibiotic production), use as plant yield and growth stimulators, use in the protection of plants from various pathogenic agents and as adjuncts in the phytoremediation of a wide range of organic environmental contaminants.

Figure 3 Plant growth promoting bacteria (Source: (K.K.Bhise, 2019))



Plant growth promoting traits of PGPB for salinity stress tolerance in plants

2.3.1 ACC deaminase

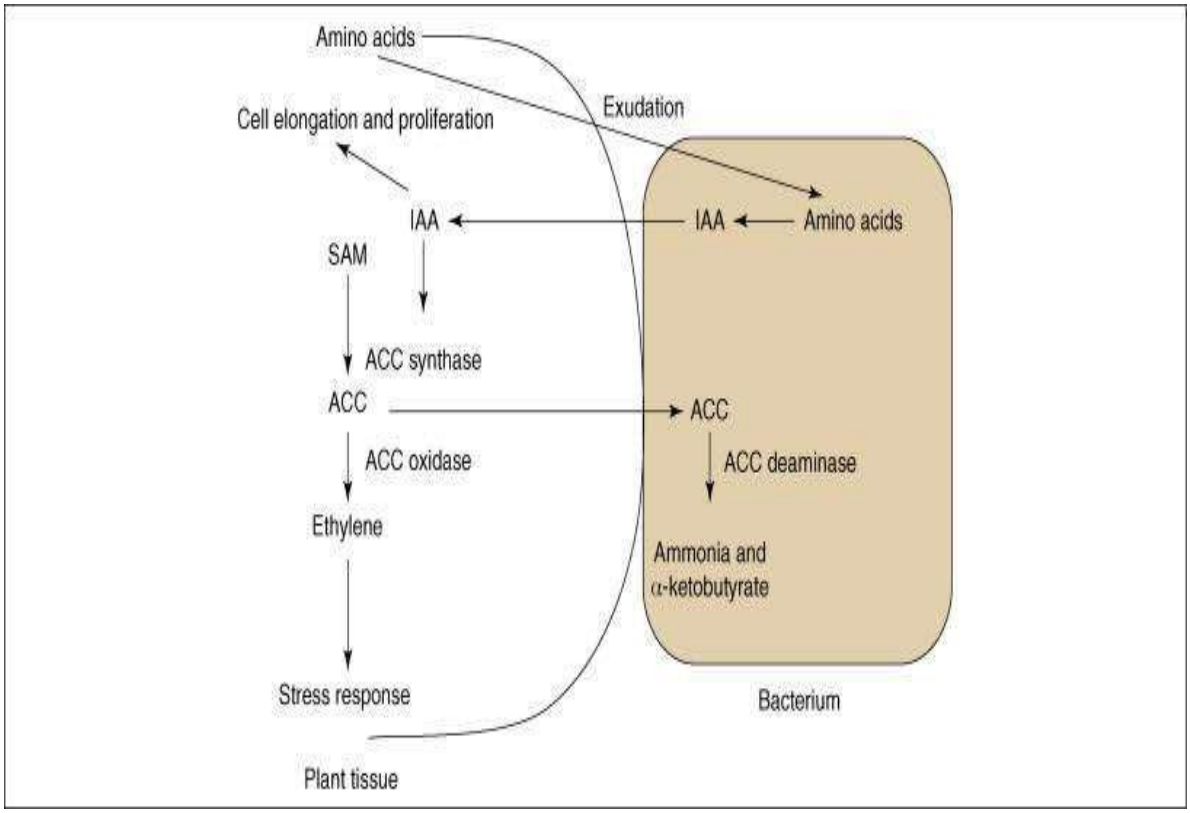
The enzyme ACC deaminase, first characterized by Honma and Shimomura (1978) has been shown to be intimately involved in the promotion of plant growth by PGPB (B.R.Glick, 1999). Following the uptake of ACC, exuded by plant roots, into a PGPB, the ACC is cleaved into α-ketobutyrate and ammonia. In this way, the PGPB is acting as a sink for ACC thereby lowering plant ACC levels, decreasing the amount of ACC within the plant that can be converted into ethylene. As a consequence of the presence of an ACC deaminase-containing PGPB, a plant that has been exposed to either biotic or abiotic stress conditions that normally induce plant growth-inhibiting levels of ethylene may be partially or even completely protected from the ethylene inhibition of plant growth. Thus, researchers have shown that (mostly rhizospheric) ACC deaminase-containing PGPB can effectively protect against growth inhibition by flooding, high salt, drought, the presence of fungal and bacterial pathogens, nematode damage, the presence of high levels of metals and organic contaminants, as well as low temperature stress (Glick B. , 2015.).On the basis of limited evidence (as far as endophytes are concerned), one group of researchers postulated that for endophytic. Examples of bacterial genes involved in colonization and plant growth-promotion.

PGPB, ACC deaminase activity is a key factor in their ability to pro-mote the growth of plant (Hardoim, 2008). They have posited that, “the decrease of plant ethylene levels relies on the ability of the ACC deaminase-positive bacteria to take up ACC before its oxidation by the plant ACC oxidase. In this context, bacterial endophytes with high locally induced ACC deaminase activities might be excel-lent plant-growth promoters, because they ameliorate plant stress by efficiently blocking ethylene production.”

More recently, the isolation and characterization of other ACC deaminase-producing endophytic PGPB has been reported (Rashid, 2012). All of these strains, from several different

bacteria, isolated from soils from five different countries, significantly promoted canola seedling root elongation. In addition, two of these ACC deaminase-producing endophytic PGPB (compared to ACC deaminase minus mutants of the same strains) were tested for the ability to promote tomato plant growth in the presence of very high levels of salt (Ali S. C., 2014)and to delay the senescence of mini carnation cut flowers (Ali S. C., 2012). Consistent with the earlier proposed model (originally developed to explain the effect of rhizospheric ACC deaminase producing plant growth-promoting bacteria on plant growth) (B.R.Glick, 1999) and the hypothesis on the environmental role of ACC deaminase-producing PGPB (Hardoim, 2008) the tested strains readily overcame the severe inhibitory stress of very high salt levels (Ali S. C., 2014).In addition, while, ACC deaminase-producing endophytic PGPB were readily taken up through the stems of cut flowers, subsequently delaying flower senescence by several days (Ali S. C., 2012),ACC deaminase-producing rhizospheric PGPB were not taken up by cut flowers and had no effect on prolonging their shelf life. Besides the above-mentioned reports, other groups have reported the ability of ACC deaminase-producing endophytic PGPB to ameliorate salt stress in Catharanthus roseus, better known as Madagascar periwinkle (Karthikeyan, 2012.), osmotic stress in pepper plants (Sziderics et al., 2007) and copper stress in canola (Zhang et al.,2011) .

Figure 5 ACC deaminase (Source: (Reddy, 2014))



2.3.2 Nitrogen fixation

Nitrogen is most important limiting nutrient for crop production and plant productivity in many part of the world. (Mitchell. et.al, 2018). Crop productivity relies heavily on the essential parameter of nitrogen (N). (Soleymani.et.al, 2011). Nitrogen fixation is divided into two parts abiotic methods (lightning), and biotic (nitrogen fixers) to fix nitrogen to the ground. In the abiotic fixation, N2 would have been oxidized with CO2 by lightning, and then NO gets converted to soluble nitrosyl hydride (Navarro.G et.al., 2011)

The inclusion of external organic matter offers a valuable supply of energy and nutrients to facilitate growth, as numerous microorganisms involved in N2 fixation are either heterotrophic or mixotrophic. (Tanga. et al, 2017). The conversion of atmospheric nitrogen (N2) into ammonia (NH3) is what biological nitrogen fixation does, making it available for plants. Biological nitrogen fixation is seen as a sustainable resource for sustainable agriculture as it aids in decreasing fertilizer nitrogen requirements. (Walley F, 2007)

Legumes such as peas, beans, lentils, soybeans, alfalfa, and clover play a crucial role in providing sustenance to both meat-producing animals and humans around the globe. In nodulated plants, crop yields are greatly enhanced, and legumes thrive in poor soils with insufficient fixed nitrogen. Nitrogen to support other types of plants. After harvest legume roots left in the soil decay, releasing organic nitrogen compounds for uptake by the next generation of plants. Farmers take advantage of this natural fertilization by rotating a leguminous one. The use of artificial fertilizers is decreased when nitrogen is fixed naturally. This not only saves money but helps to prevent the many problems brought about by excessive use of commercial nitrogen and ammonia fertilizers such as eutrophication of rivers and lakes, generation of acid rain, and overgrowth of agricultural land by non-food crops. Symbiotic nitrogen fixation is of great importance not only in production of leguminous crops but also in global N2 cycle

(Arujo A, 2008). Ammonia oxidation is believed to be the step that slows down nitrogen oxidation. (Xu G, 2012). N is the principal component of the proteins that build cell and plant tissue. Cereals and other plant species can utilize N as NO_3^- and NH_4^+, which are the available inorganic forms of N absorbed by the roots from the soil solution (Mokhele B, 2012). Despite the fact that nitrogen content in the atmosphere is highest in all atmospheric gases, there is no nitrogen in soil parent material. (Hedin L.O., 2009). Soil nitrogen input for plant nutrition and crop productivity largely depends on organic matter degradation, synthetic fertilizer applications, and biological nitrogen fixation (BNF) via nitrogenase enzyme activity. (Galloway J.N., 2008) (Vitousek P.M., 2013). Nitrogenous fertilizer production currently represents a significant expense for the efficient growth of various crops in the developed world. Synthetic N fertilizers are currently used in grain, grass, and fruit productions (about 60% for cereals and 10% with irrigated rice production). (Santi C., 2013). In contrast, the presence of salts in soil leads to a decrease in the availability of crucial macronutrients like nitrogen (N). (Gondek, 2020).

2.3.3 Phosphate solubilization

Phosphate fertilization can shift microbial communities' composition and affect biodiversity, activity and functional traits diversity (Zheng, 2017). In this regard, the functions and abundance of genes involved in microbial P-transformation under various P levels is paramount in the understanding of the full impact of P application on soil microbiota. The microbial P-transformation process is governed by genes ensuring organic P-mineralization and inorganic P-solubilization. Each one of these steps is affected by the environmental P levels as reflected by the shift in abundance and activity of microbial genes under different P levels. (Hommes, 1984.).

The abundance of P turnover genes reflects the genetic potential of soil biota to metabolize P, but the actual potential can be inferred from enzymatic activity. Multiple lines of evidence indicate that the production of phosphatase by the soil microbiome is tightly controlled by inorganic P availability and N; while N addition increases phosphatase activity (Heuck, 2018.), inorganic P supply suppresses the production and activity of phosphatase, owing to a negative feedback mechanism triggered by high P additions (Marklein, 2012.). This negative feedback creates a tight correlation between P mineralization and P input to increase the phosphatase activity to meet increased P demand (Olander, 2000).

Similarly, it has been reported that PSB (*Pantoea cypripedii* and *Pseudomonas plecoglossicida*) exhibited higher acid phosphatase, alkaline phosphatase and phytase activities when they are applied along with P rock fertilization (Chen, 2019). The same study demonstrated that the combined application of PSB and P rock increased root biomass, grain yield and P uptake in both maize and wheat. Interestingly, the source of P is also an important factor in alkaline phosphatase activity; alkaline phosphatase activity abundance increased in response to manure P but not mineral P treatment, highlighting the importance of nutrient source

on its use efficiency (Fraser, 2015a.).

In addition to the indirect effect on plants, soil P levels directly influence plant roots and trigger responsive mechanisms to control P diffusion from the soil to root cells mainly through modifications in root exudation and structure. Acquisition of P falls under the control of the roots, PSB and the P source and availability. These factors mutualize to define the crop's P acquisition traits. For instance, both P and PSB can influence the expression levels of genes responsible for P acquisition in roots (Murgese, 2020).

2.3.4 Exopolysaccharide production:

The bacterial polysaccharides produced and secreted extracellularly during biofilm formation through cell wall anchored enzymes are termed EPS. Based on their chemical composition EPS are categorized into homo and heteropolysaccharides (U.U.Nwodo, 2012). The basic constituents of EPS are monosaccharides, neutral sugars, uronic acids, amino sugars, substituent of organic esters and pyruvate ketals (S.H.Wani, 2013.). Several PGPB are known to produce EPS which help plants to survive under saline conditions. Under salt stress conditions, EPS produced by PGPB bind Na+ cations which reduce its (Na+) content around the root zone thereby helping in maintenance of ionic balance. EPS produced by PGPB also help in soil aggregation, water retention, and chelation of metal ions in the plant under salinity (Kasotia, 2016.)

Recently, it has been revealed that the biosynthesis of lipopolysaccharide (LPS) could have a distinct role in the synthesis of EPS that can affect the salt tolerance ability of PGPB Moreover, in a study, Bacillus siamensis and Bacillus methylotrophicus strains were reported to produce EPS along with IAA and ACCD. EPS helped in Na+ ion sequestration while ACCD caused degradation of ACC into α-ketobutyrate and ammonia which enhanced the germination rate of wheat plant seedlings and growth under salt stressed conditions (Amna U. D., 2019.).
There are studies that divulge the role of EPS in alleviating oxidative stress in plants.

EPS producing bacteria Gluconacetobacter diazotrophicus was reported with the potential to protect PGPB from oxidative damage and increased bacterial colonization efficiency during rice plant colonization under salt stress (Meneses, 2017). As a whole, these observations prove that how EPS production by PGPB help in the reduction of salt stress effects on plants by promoting their growth and yield. Researchers have even suggested the amendment of bioinoculants designed for saline soils with metabolites such as EPS for better protection of the inoculated PGPB and benefits to the plant (N.K.Arora, 2021). 3.7. Nutrient acquisition Salinity interferes with mineral-nutrient acquisition and influences many metabolic processes in the plant. Usually, the deposition of Na+ and Cl□ ions cause deficiency and competition for minerals such as K+, Ca+, and NO3- in plants (Hu Y. S., 2005.). PGPB may perform key roles in nitrogen (N) assimilation, and phosphate, potassium (K), zinc (Zn), and iron (Fe) uptake under saline conditions.

2.3.5 Plant growth promoting hormones:

The damaging effects of chemical fertilizers and pesticides, there is an increasing interest in improving our understanding of molecular mechanisms of interaction between plants and their rhizosphere microbial community.

It is well established that PGPR colonization is associated with profound changes in the host plant's development and hormone homeostasis. Phytohormones act as messengers to coordinate cellular activities and to regulate various cellular processes in plants, including abiotic stress responses and plant - pathogen interaction. PGPR colonization brings about many changes in plant development. These changes include, but are not limited to growth stimulation, modification of root and shoot architecture, and synthesis of secondary metabolites. As hormones regulate plant growth and development, the effects of colonization by PGPR are directly associated with changes in concentration, localizations and signaling of hormones (S. Spaepen, 2014) .

Hormones regulate or influence a range of cellular and physiological process, such as cell division, cell enlargement, bud dormancy, flowering, fruit ripening, seed dormancy, seed germination and leaf abscission. Auxin, one of the plant hormones, stimulates differentiation of phloem and xylem, root initiation on stem cutting, and also the development of branch roots. Auxin mediates the tropism (bending), response to gravity and light. (M. Kelen, 2004)

Indole -3-acetic acid (IAA) is the common natural auxin that shows all auxin doing actions and extensively affects plant’s physiology. Colorimetric method is the simplest method and has long been employed for the detection of indole-3-acetic acid (IAA) produced by plants and microorganisms (C. L. Patten and B. R. Glick, 2005). Indole-3-acetic acid is a naturally occurring and main auxin in plants as it controls many important physiological processes like cell enlargement and division, tissue differentiation, and responses to light and gravity (Lambrecht M, 2004).

Bacterial auxins have the potential to change any of these processes by altering the plant auxin pool. It depends on the amount of IAA produced and the sensitivity of plant tissue to changing levels of IAA. The roots are the most sensitive organs and respond to the changing levels of IAA by elongation of primary roots, formation of adventitious and lateral roots, or cessation of growth (PJ, 2010). Indole-3-acetic acid does not function as a hormone in bacterial cells but their ability to produce the same may have evolved as it is important in plant–bacteria relationship (Spaepen S, 2007)

Plants respond to the stress conditions by abscisic acid (ABA), an important phytoharmone. ABA is recognized as hormone, since very long time which can be up-regulated by the deficiency of water in root zone. ABA production increases in roots and shoots of plants due to water deficiency and osmotic stress caused by soil salinity. (C. Cabot, 2009)

The relationship between salt tolerance and ABA accumulation is due to the accumulation of compatible solutes like sugars and proline in root vacuoles, $Ca2+$ and $K+$, which neutralize the effect of $Na+$ and Cl (A. Gurmani, 2011). A higher level of ABA was found in the Arabidopsis plants mediated by PGPB compared to the control under drought (Bresson J., 2013)

The PGPB-inoculated plant exhibits higher water retains due to higher root biomass. The plant experienced significantly lower water loss through transpiration, which may reflect a better drought resistance strategy. This enhanced tolerance was correlated with a PGPB-induced delay in the transition from plant vegetative to reproductive developmental stage, which causes higher biomass accumulation before the flowering time of the plant. Higher concentrations of ABA in the leaves might lead to lower transpiration rate and resulting in a reduced plant growth rate. (Zhou C., 2016)

2.3.6 Fungal cell wall degrading enzymes

Plant cells have thick cell walls comprised mainly of polysaccharides and lignin, an aromatic polymer. These polymers have adapted to create intricate composite materials that make plant cells very strong against attacks from pathogens (Somerville, et al., 2004). Plant polysaccharides are mainly composed of cellulose, which is a homopolymer of glucose linked by β-1,4 bonds; hemicellulose, which is a diverse, branched polysaccharide made up of various polymers linked by β-1,4 bonds including xylan, glucuronoxylan, xyloglucan, glucomannan, and arabinoxylan backbones with diverse side chains. (Scheller & Ulvskov, 2010).

OBJECTIVE

- Collection of soil samples
- Isolation of salt tolerant microorganisms
- Screening of PGPB on the basis of Plant Growth Promoting abilities:
    - ACC deaminase production
    - Nitrogen fixation
    - Phosphate solubilization
    - Ammonia production
    - Exopolysaccharide production
    - Fungal cell wall degradation enzymes: Protease enzyme , Cellulase enzyme
- Preparation of effective bioinoculum.
- Seed germination with bioinoculum.
- Pot trials on *Vigna radiata* by using bioinoculum of potent organisms.

MATERIAL & METHODOLOGY

3.0 MATERIAL & METHODOLOGY

3.1 Collection of soil sample

- The study focused on soil samples collected from various locations within Maharashtra, India. The selected regions, including Miraj, Kasba Bawda, Shivare & Morouchi villages, are known for grappling with salinity issues, posing significant challenges to soil quality and agricultural productivity.
- Soil sampling was meticulously conducted to ensure representative samples from both saline-affected regions and control areas.
- A systematic approach was employed, with samples collected from multiple depths to capture the variability in soil physicochemical properties.
- The collected soil samples underwent comprehensive physicochemical analysis to assess key parameters influencing soil fertility and health. The following parameters were evaluated:

- Electrical Conductivity (EC):
  - EC measurements were performed using a conductivity meter following the standard protocols outlined by the American Public Health Association (APHA, 1998).
  - This parameter provides insights into soil salinity levels, crucial for understanding soil suitability for various crops.
- pH:
  - Soil pH, a fundamental determinant of soil health, was measured using a pH meter calibrated with standard buffer solutions.
  - pH levels influence nutrient availability and microbial activity in the soil.

- The study aimed to isolate and characterize salt-tolerant bacteria from the collected soil samples, offering potential insights into agricultural sustainability and soil remediation strategies.
- The isolation process involved the following steps:

➢ Preparation of Soil Suspension:

- Ten grams of soil from each location were mixed with 100 ml of sterile saline solution (0.85%) in 250 ml Erlenmeyer flasks.
- This ensured uniform extraction of bacteria from the soil matrix.

➢ Serial dilution:

- Serial dilutions of the soil suspension were prepared to obtain dilutions suitable for bacterial colony counting.
- For this, initially the dilution blanks were made up to 10^{-6}.
- The dilution blank tubes consist of distilled water.
- The set of dilution blank was sterilized.
- After sterilization, the sample was transferred serially from 10^{-1} to 10^{-6}.

➢ Inoculation, incubation

- To isolate bacterial culture sterile nutrient agar plates were prepared.
- In Erlenmeyer flask, dehydrated nutrient agar powder (2.8 gm) was suspended along with agar (1.0 gm) in 100 ml distilled water were transferred. The contents were sterilized.
- After sterilization, the nutrient agar was transferred to sterile petriplates.
- Aliquots of the diluted soil suspensions were spread onto sterile nutrient agar plates using the spread plate method.

- Nutrient agar provided a favorable growth medium for the isolation of diverse bacterial strains present in the soil samples. The plates were incubated at 30°C for 24 hours.
- After incubation, individual bacterial colonies were observed on the plates.
- To ensure purity, colonies with distinct morphological characteristics were selected and streaked onto fresh nutrient agar plates.
- This process of sub culturing helps eliminate contaminants and ensures the isolation of pure bacterial cultures.

The isolated bacteria were then maintained on nutrient agar slants at suitable storage conditions. Regular sub culturing and maintenance of pure cultures are essential to preserve the viability and characteristics of the isolates for further studies.

3.2 Salt tolerance test:

Salt stress is a major environmental factor that adversely affects plant growth and productivity.

- To evaluate the salt stress tolerance of the isolates, nutrient agar plates supplemented with varying concentrations of NaCl were prepared.
- NaCl concentrations ranging from 2 to 8 % were chosen to simulate different levels of salinity commonly encountered in agricultural soils.
- A control plate containing a minimal concentration of 0.5 % NaCl was also included for comparison.
- In Erlenmeyer flask, dehydrated nutrient agar powder (2.8 gm) was suspended along with agar (1.0 gm) & respected NaCl concentrations in 100 ml distilled water were transferred.

- The contents were sterilized. After sterilization, the nutrient agar was transferred to sterile petriplates.
- Each isolated bacterial strain was streaked onto the NaCl-supplemented plates and the control plate, followed by incubation at 30°C for 48 hours.
- The incubation period allowed the bacteria to grow and express their tolerance to salt stress. After incubation, the plates were examined for bacterial growth.

The extent of growth on the NaCl-supplemented medium was compared with that on the control plate to determine the effect of salt stress on bacterial growth. This initial assessment provided valuable insights into the salt stress tolerance of the isolated PGPB and identified potential candidates for further investigation.

3.3 ACC deaminase

The qualitative assessment of ACC deaminase activity in PGPB involved a systematic approach to determine the ability of these bacteria to utilize 1-aminocyclopropane-1-carboxylate (ACC) as a nitrogen source under varying salinity conditions. The experimental setup employed rigorous controls and precise conditions to ensure the reliability and repeatability of the results.

Table 1 DF minimal medium

Glucose	0.2 gm
Gluconic acid	0.2 gm
Citric acid	0.2 gm
KH2PO4	0.4gm
Na2HPO4	0.6 gm
MgSO4.7H2O	Trace

CaCl2	Trace
Na2MoO4	Trace
KI	Trace
NaBr	Trace
MnCl2	Trace
CoCl2	Trace
CuCl2	Trace
AlCl3	Trace
NiSO4	Trace
Agar powder	1gm
Distilled water	100 ml

- Preparation of DF medium:
    - DF medium i.e. Dworkin & Foster media was prepared by addition of following components in 250 ml Erlenmeyer flask containing 100 ml distilled water.
    - All the components were mixed & then after sterilization of the medium, the medium was poured into sterile petriplates. Each plate was supplemented with 3 mmol-1 ACC, serving as the sole nitrogen source. ACC is a precursor of ethylene in plants and is converted into ammonia and α-ketobutyrate by ACC deaminase-producing bacteria. The utilization of ACC as a nitrogen source is indicative of ACC deaminase activity in the bacteria.
- To initiate the assessment, fresh bacterial cultures were obtained & inoculated onto DF minimal medium agar plates.
- The inoculated plates were then carefully incubated at a constant temperature of 30°C.

- This temperature was chosen based on previous literature indicating the optimal growth conditions for the bacteria under study.
- Incubation at a consistent temperature allowed for uniform colony development and facilitated accurate assessment of bacterial activity. These plates served as the growth substrate for the bacteria and provided a controlled environment for observing colony development.

3.4 Nitrogen fixation ability

Nitrogen fixation ability of isolates was verified on Ashby's agar medium.

Table 2 Ashby's agar

Manitol	0.2 gm
K_2HPO_4	0.2 gm
$MgSO_4.7H_20$	0.2 gm
NaCl	0.2 gm
K_2SO_4	0.2 gm
Agar powder	1 gm
$CaCO_3$	0.5 gm
Distilled water	100 ml

- Preparation of Ashby's agar medium:
  - The Erlenmeyer flask with 100 ml of distilled water was provided with components given in the table above.
  - The media components were sterilized after mixing in water.
  - CaCO3 was separately sterilized.

- After sterilization, the other media components and $CaCO_3$ were mixed together.
- The sterilized media was poured in sterile petriplates.

3.5 Phosphate solubilization

Phosphate solubilization activity of isolates was checked on Katzelson & Bose agar medium.

Table 3 KB agar

Glucose	1 gm
KCl	0.02 gm
$MgSO_4.7H_20$	0.02 gm
$(NH_4)_2SO_4$	0.05 gm
$FeSO_4$	trace
$MnSO_4$	trace
Yeast extract	0.05 gm
Agar powder	2 gm
Tricalcium phosphate	0.5 gm
Distilled water	100 ml

- Preparation of Katzelson & Bose agar medium.:
  - The Erlenmeyer flask with 70 ml of distilled water was provided with all the components (except Tricalcium phosphate).
  - The media components were sterilized after mixing in water.
  - Along with this Tricalcium phosphate (0.5 gm) was separately sterilized in 30 ml distilled water.

- After sterilization, the other media components and Tricalcium phosphate were mixed together.
- The sterilized media was poured in sterile petriplates.
- The isolates were spot inoculated on the media and observed for clear zone.

- The isolates were spot inoculated on the media and observed for clear zone.

3.6 Ammonia production

- Separate inoculation procedures were carried out for each experimental condition.
- Each isolate culture was aseptically inoculated into 5 ml of peptone water. This step ensured that each tube represented a specific salt concentration for subsequent analysis.
- Preparation of peptone water:
    - 1.5 gm of peptone powder supplemented with 2 gm NaCl was added to 100 ml distilled water.
    - The components were mixed well & distributed in quantity of 5 ml in each test tube. Later the filled test tubes were sterilized
- Preparation of Nesseler's reagent:
    - Nessler's reagent, a crucial component of the ammonia production assay, was prepared beforehand according to established protocols.
    - This reagent is essential for detecting the presence of ammonia in the experimental samples.
- Following inoculation, each tube was tightly sealed to prevent contamination and placed in an incubator set at 30°C.
- The incubation period extended for 48 hours to allow sufficient time for bacterial growth and potential ammonia production.
- Upon completion of the incubation period, 0.3 ml of Nessler's reagent was meticulously added to each inoculated tube. This step was executed with precision to ensure uniformity across all samples.
- Following the addition of Nessler's reagent, the tubes were carefully observed for any color changes.
- The development of a brown to yellow coloration indicated a positive test for ammonia

production. This visual observation was conducted systematically to accurately record the results.

- To mitigate the influence of non-bacterial factors on color changes, uninoculated medium served as a blank control. This control was essential for distinguishing color variations solely attributable to bacterial activity.

3.7 Exopolysaccharide production

The bacterial strains under investigation were cultivated in a nutrient-rich medium to facilitate their growth and the production of extracellular polymeric substances (EPS).

- Preparation of medium:

Table 4 Exopolysaccharide production medium

Yeast extract	1 gm
Casamino acids	0.75 gm
Trisodium citrate	0.3 gm
KCl	0.2 gm
MgSO4·7H2O	2 gm
MnCl2·4H2O	0.036gm
FeSO4·7H2O	5 gm
Distilled water	100 ml
Agar powder	1.5 gm

- The medium composition consisted of above components.These components were carefully selected to provide essential nutrients and promote bacterial metabolism.
- The components were added in 100 ml distilled water & transferred in Erlenmeyer flasks.
- The flasks were sterilized.

- The cultures were prepared in Erlenmeyer flasks Incubation of the cultures was carried out at a controlled temperature of 30°C for duration of 5 days. This incubation period was chosen based on preliminary experiments and literature review, aiming to optimize bacterial growth and EPS production while considering practical constraints.
- Following the incubation period, the cultures were subjected to centrifugation at 8000 rpm for 15 minutes at 4°C. This step facilitated the separation of bacterial cells and other solid components from the liquid phase, resulting in the formation of a supernatant.
- The supernatant, containing the secreted EPS, was carefully collected and transferred to a separate vessel.
- Cold absolute ethanol was then added drop wise to the supernatant under continuous stirring. The addition of ethanol led to the precipitation of EPS molecules, forming a visible precipitate in the solution. The formation of EPS was assessed based on the visual observation of precipitate formation, confirming the ability of the bacterial strains to synthesize extracellular polymeric substances under the specified culturing conditions.

The cultivation and inoculation procedures were meticulously designed to provide optimal conditions for bacterial growth and EPS production. Each component of the growth medium was selected based on its ability to support bacterial metabolism and facilitate EPS synthesis

3.8 Cellulase production

- In this research project, the cellulase activity of the isolate was assessed using a standard procedure involving inoculation on 1% carboxyl methyl cellulose (CMC) agar plates followed by an incubation period at 30°C for 48 hours. The method used was adapted from Kasana et al. (2008)
- Preparation of CMC agar:

Table 5 1% CMC agar

CMC powder	0.2gm
Peptone	0.5gm
NaCl	0.5 gm
Beef extract	0.5 gm
Agar powder	1 gm
Distilled water	100 ml

  - To prepare the agar plates, a solution of 1% carboxyl methyl cellulose the above m-components were dissolved in distilled water.
  - Agar powder was also added.
  - This solution was then autoclaved to ensure sterility.
  - Once cooled to a suitable temperature, the CMC solution was mixed with nutrient agar to achieve a final concentration of 1% CMC in the agar medium.
  - The mixture was then poured into Petri dishes and allowed to solidify.
- The isolates, previously obtained and maintained under suitable conditions, was

inoculated onto the prepared CMC agar plates using a sterile inoculation loop.

- Care was taken to spread the inoculums evenly across the surface of the agar.
- Following inoculation, the plates were incubated at a constant temperature of 30°C for a period of 48 hours. This temperature was chosen based on previous studies indicating optimal conditions for cellulase activity.
- After the designated incubation period, the plates were carefully examined for the presence of a clear zone around the inoculation site.
- The formation of a clear zone indicated the degradation of cellulose by the enzyme cellulase, produced by the isolate.
- To confirm the presence of cellulase activity, iodine solution (0.666% KI; 0.333% iodine) was added to the plates, covering the entire surface.
- The plates were then allowed to stand for 5 minutes, allowing the iodine solution to react with any remaining cellulose.
- The development of a clear zone around the inoculation site, followed by the addition of iodine solution, served as a positive indicator of cellulase activity. The clear zone indicated the hydrolysis of cellulose by the enzyme, resulting in the formation of soluble products. The addition of iodine solution confirmed the absence of intact cellulose in the cleared area, as iodine reacts with starch, producing a distinct blue-black color. Thus, the absence of color change in the clear zone further supported the presence of cellulase activity.
- To ensure the validity of the results, appropriate controls were included in the experiment. Negative controls, consisting of uninoculated agar plates, were incubated and subjected to the same procedures as the experimental plates.

3.9 Protease production

The cultures were utilized for assessing its protease production ability. The cultures were maintained on agar slants and stored at 4°C for further use.

- Preparation of skimmed milk agar:

Table 6 Skimmed milk agar

Nutrient agar	2.8 gm
Agar powder	1.0 gm
Distilled water	100 ml
Skimmed milk	3 ml

- Skim milk agar plates were prepared by adding 3% (w/v) skim milk to nutrient agar medium which are separately autoclaved.
- The mixture was then poured into sterile petri plate and allowed to solidify under aseptic conditions.

- A single colony of each isolate were carefully picked from the agar slant using a sterile inoculating loop and streaked onto the surface of the skim milk agar plates.
- The plates were then placed in an incubator set at 30°C for 72 hours.
- After the incubation period, the plates were examined for the presence of clear zones around the colonies. The formation of clear zones indicated the hydrolysis of casein in the skim milk agar by the protease enzyme secreted by isolates. The diameter of the clear zones was measured using a ruler to quantify the protease activity.

Standard microbiological safety practices were followed throughout the experiment to prevent contamination and ensure the safety of the researchers involved. This included wearing appropriate personal protective equipment, proper handling and disposal of microbial cultures and biohazardous

materials, and disinfection of work surfaces.

3.10 Preparation of bioinoculum

- To prepare the bacterial inoculums, we followed a modified version of the method described by Penrose and Glick (2003).
- The potent isolates were cultured in nutrient broth until it reached the logarithmic growth phase.
- The bacterial cells were then harvested via centrifugation, washed with sterile saline solution to remove any residual media, and finally resuspended in sterilized distilled water to achieve the desired cell densit

3.11 Seed germination & pot trial

- Surface sterilization of *V. radiata*. seeds were essential to ensure the efficacy of the bacterial inoculation.
- This was achieved by immersing the seeds in 70% ethanol for one minute, followed by four rinses with sterile distilled water.
- Subsequently, the sterilized seeds were treated with the prepared potent isolates' inoculum by soaking them in broth inoculated with the bacterial culture. This step ensured thorough seed colonization by the beneficial bacteria.
- After treatment, the seeds were sown into pots filled with soil.
- The pot study was conducted, taking into account the seasonal variations in temperature and humidity. Ambient temperatures ranged from 30 to 35°C, while humidity levels exceeded 30%.
- To induce salt stress, the plants were subjected to varying concentrations of NaCl solutions at 48-hour intervals. This allowed us to assess the response of *V. radiata L*. to salt stress in the presence of potent isolate inoculation.

RESULTS AND DISCUSSION

4.0 RESULTS & DISCUSSION

4.1 Results

4.1.1 Collection of soil

After collection of soil, the soil was initially checked for parameters like colour, pH & electrochemical conductivity. The observation for this was as follows:

Table 7 Soil properties

Location	Soil colour	Soil pH	Soil EC
Miraj	Black	8.0	4.3
Kasba Bawda	Black	8.5	4.0
Shivare	Red	8.0	4.4
Morouchi	Brown	8.0	4.4

4.1.2 Isolation of bacteria:

After incubation of nutrient agar plates of serial dilution at 30°C for 24 hours the organisms were found. Around 50+ isolates were isolated from soil samples of four locations.

4.3 Salt tolerance of bacteria:

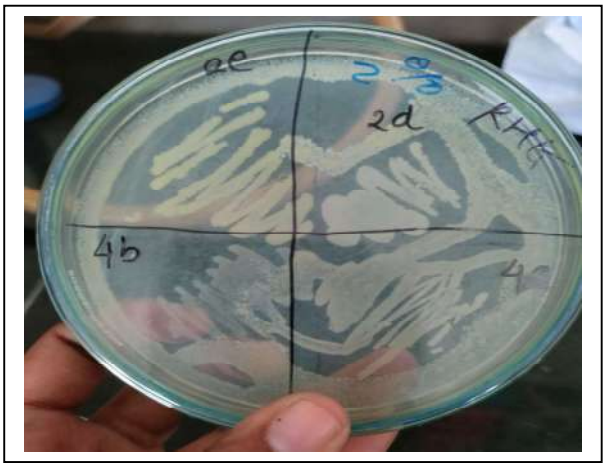
The salt stress tolerance of the isolated PGPB was assessed by streaking them onto nutrient agar plates supplemented with varying concentrations of NaCl. Concentrations ranging from 20 to 80 g L^{-1} were selected to simulate different salinity levels encountered in agricultural soils, with a control plate containing minimal NaCl (0.5 g L^-1) for comparison. Among 50+ isolates, 20 isolates were found to be salt tolerant.

Table 8 Salt tolerance of bacteria

	2%	3%	4%	5%	6%	7%	8%	9%	10%
1a	+	+	+	+	+	+	+	-	-
1b	+	+	+	+	+	+	+	-	-
1c	+	+	+	+	+	+	+	-	-
1d	+	+	+	+	+	+	+	-	-
1e	+	+	+	+	+	+	+	+	+
2a	+	+	+	+	+	+	+	+	-
2b	+	+	+	+	+	+	+	+	-
2c	+	+	+	+	+	+	+	-	-
2d	+	+	+	+	+	+	+	-	-
2e	+	+	+	+	+	+	+	-	-
3a	+	+	+	+	+	+	+	-	-
3b	+	+	+	+	+	+	+	+	-
3c	+	+	+	+	+	+	+	+	-
3d	+	+	+	+	+	+	+	+	-
4a	+	+	+	+	+	+	+	-	-
4b	+	+	+	+	+	+	+	+	+
4c	+	+	+	+	+	+	+	+	+
4d	+	+	+	+	+	+	+	+	+
4e	+	+	+	+	+	+	+	+	+
4f	+	+	+	+	+	+	+	+	+

Fig 6 Salt Tolerance Result

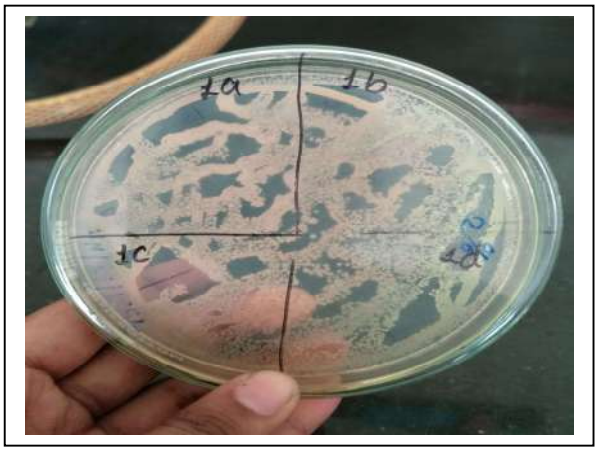
3c 3d



2e 2d

4b 4c

1a 1b

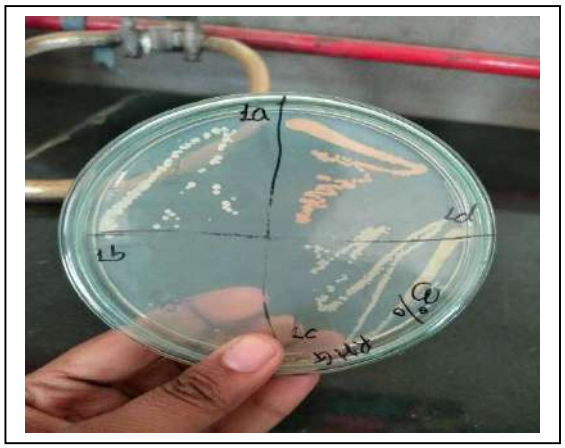


1c 1d

2d 1e

2c 2b

1a **1d**

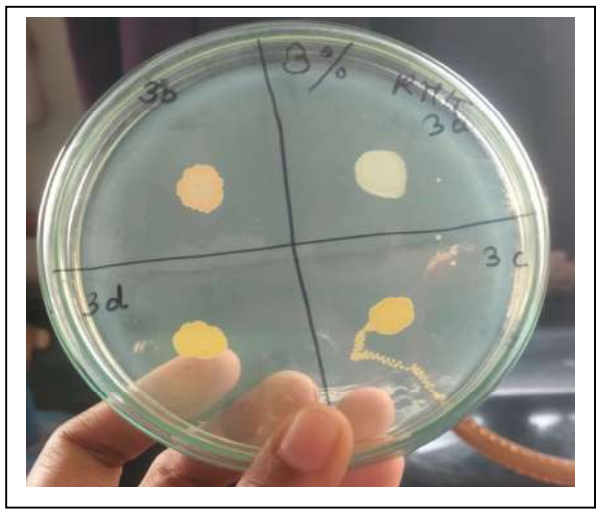


1b **1c**

2a **1e**

2b **2e**

3b **3a**



3d **3c**

2e **2d**

4a **4b**

1d 1c

1a 1b

2a 1e

2c 2b

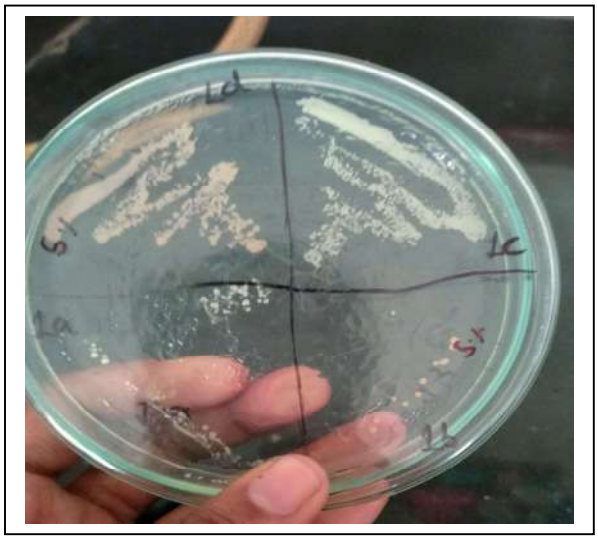
2e **2d**

4a **4b**

3d **3e**

3a **3d**

1d **1c**



2a 2b

1e **2b**

2a 2c

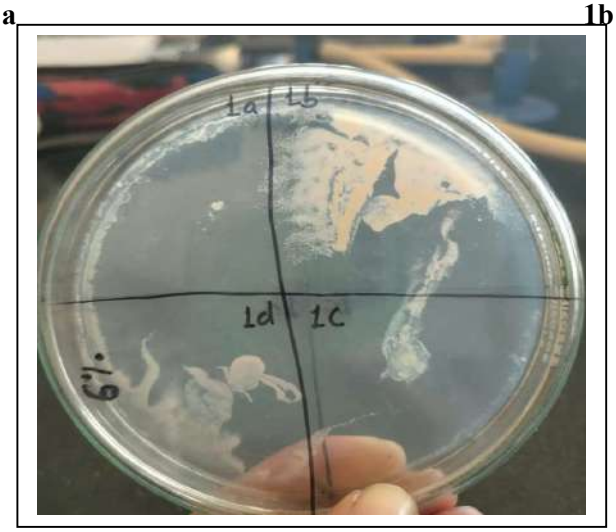
3b 3a

3d 3c

2e 2d

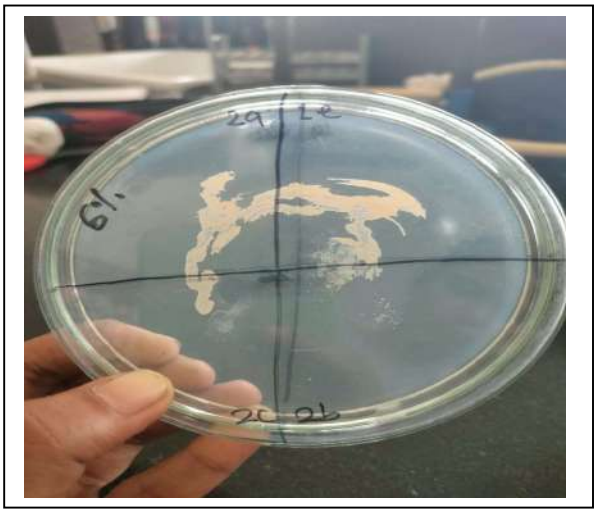
4b 4a

1a 1b



1d 1c

2a 1e



2c 2b

2e 2d

3b 3a

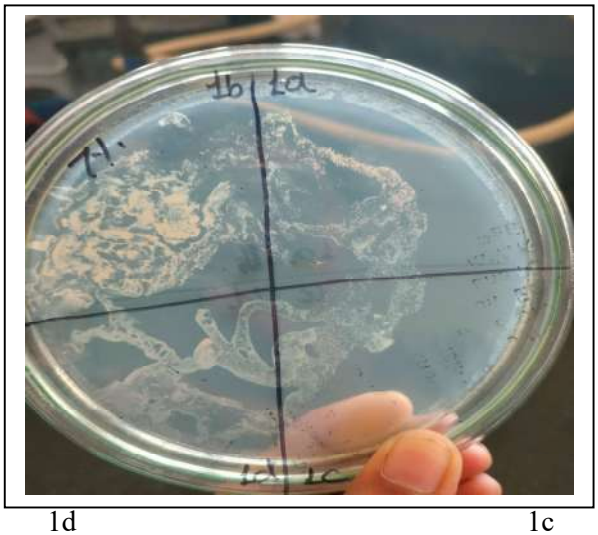
3d 3c

4b 4a

4c 4d

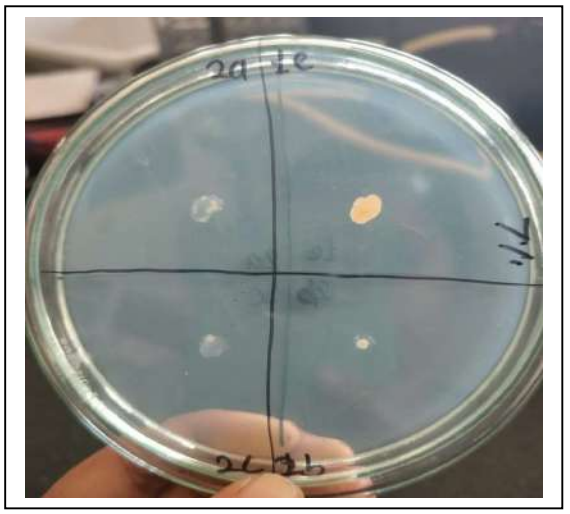
4f 4e

1b 1a



1d 1c

2a 1e

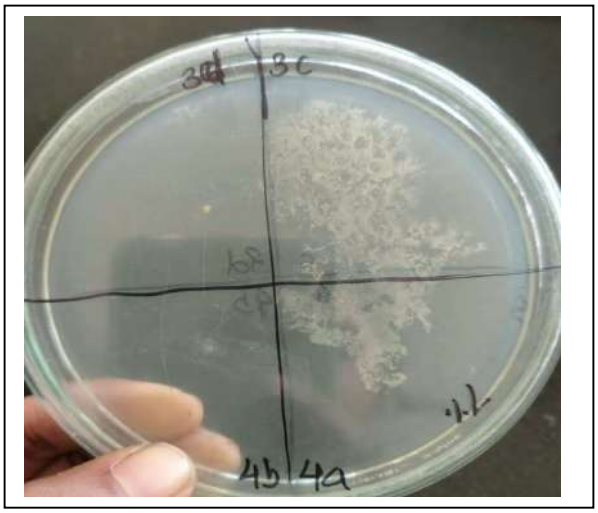


2c 2b

2e 2d

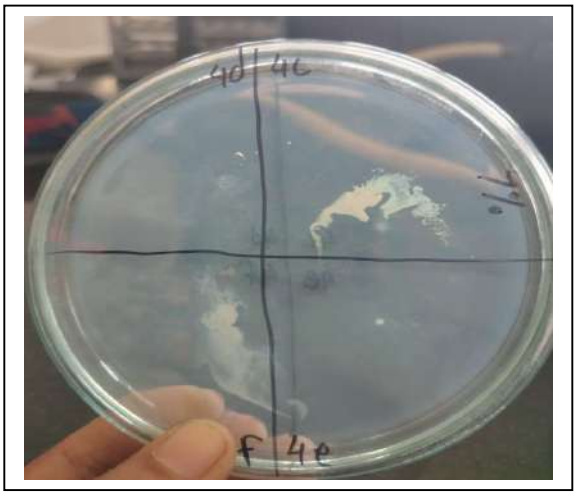
3b 3a

3d 3c



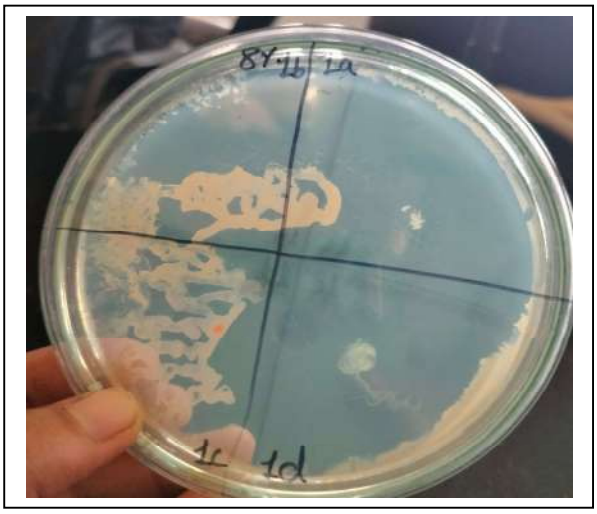
4b 4a

4d 4c



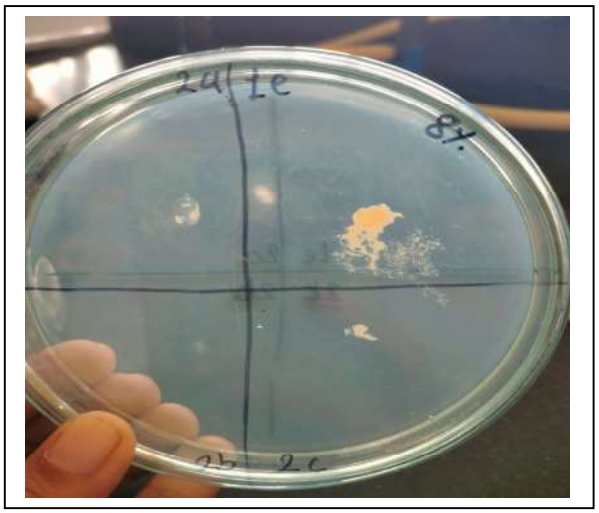
4f 4e

1b 1a



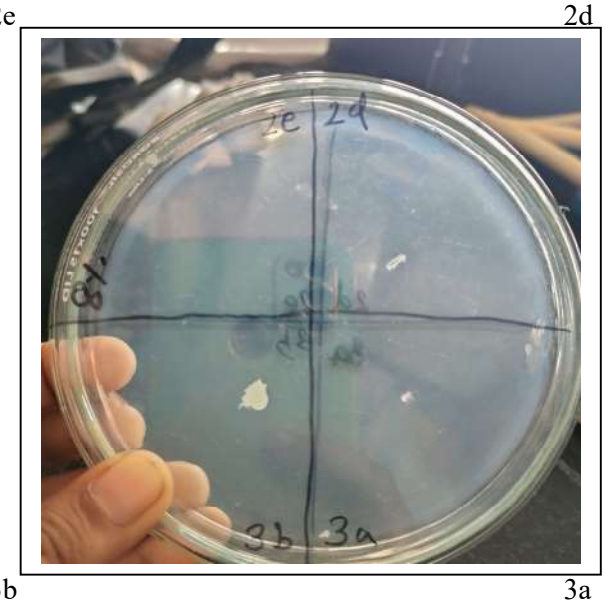
1c 1d

2a 1e



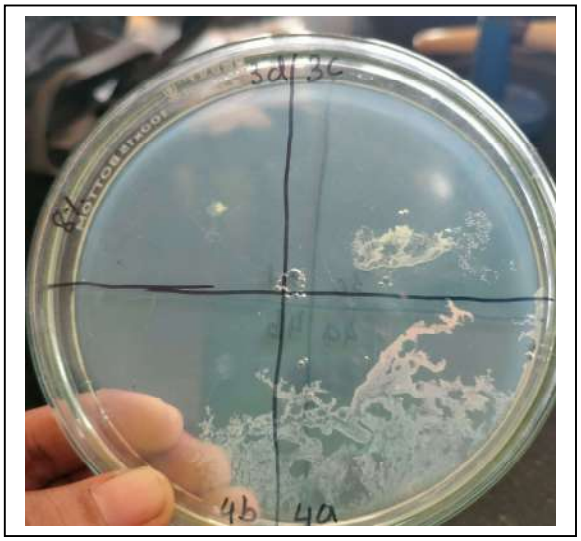
2b 2c

2e 2d



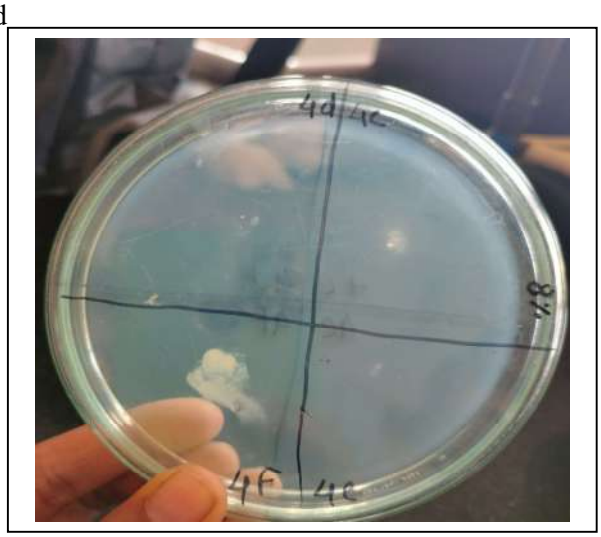
3b 3a

3d 3c



4b 4a

4d 4e



4f 4e

The colony characters of these organisms were noted down. The colony characters are as Table 4.3

Table 9 Colony characteristics of salt tolerant bacteria

	Size	Shape	Colour	Margin	Opacity	Elevation	Surface	Consistency
1a	3 mm	Circular	White	Entire	Opaque	Low convex	Smooth	Moist
1b	1 mm	Circular	Orange	Entire	Opaque	Convex	Smooth	Sticky
1c	1 mm	Circular	White	Entire	Opaque	Convex	Smooth	Sticky
1d	1 mm	Circular	Light pink	Entire	Opaque	Convex	Smooth	Sticky
1e	1 mm	Circular	Orange	Entire	Opaque	Convex	Smooth	Sticky
2a	2 mm	Circular	Light pink	Entire	Opaque	Convex	Smooth	Sticky
2b	2 mm	Circular	White	Entire	Opaque	Convex	Smooth	Sticky
2c	1 mm	Circular	White	Entire	Opaque	Convex	Smooth	Sticky
2d	3 mm	Circular	Yellow	Entire	Opaque	Convex	Smooth	Sticky
2e	0.5mm	Circular	Yellow	Entire	Opaque	Convex	Smooth	Sticky
3a	0.5mm	Circular	White	Entire	Translucent	Convex	Smooth	Moist
3b	3 mm	Circular	Orange	Entire	Opaque	Flat	Smooth	Moist
3c	2 mm	Circular	Yellow	Entire	Opaque	Flat	Smooth	Moist
3d	0.5mm	Circular	White	Entire	Opaque	Convex	Smooth	Moist
4a	1 mm	Circular	Light orange	Entire	Opaque	Flat	Smooth	Moist
4b	1 mm	Irregular	Bluish tinge	Undulated	Translucent	Convex	Smooth	Sticky
4c	1 mm	Circular	Pink	Entire	Translucent	Flat	Smooth	Moist
4d	1 mm	Irregular	White	Entire	Translucent	Convex	Smooth	Sticky
4e	1 mm	Irregular	White	Entire	Translucent	Low convex	Smooth	Sticky
4f	1 mm	Circular	Light orange	Undulated	Translucent	Low convex	Smooth	Sticky

4.4 ACC deaminase test:

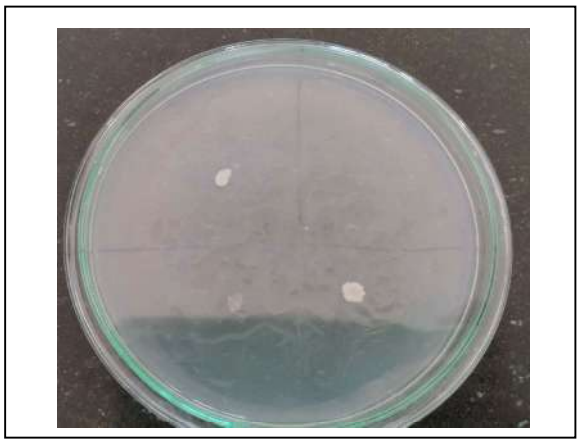
All the isolates were observed for ACC deaminase activity. According to observations, the growth of potent organisms was visible on DF agar medium supplemented with ACC. **15** isolates shown growth on DF medium which indicates the ability of the isolates to produce ACC deaminase.

Table 10 ACC deaminase activity

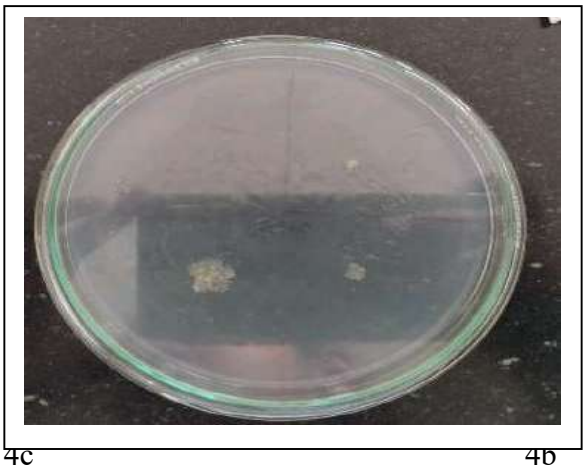
1a	**1b**	**1c**	**1d**	**1e**	**2a**	**2b**	**2c**	**2d**	**2e**
+	+	+	-	+	-	+	+	-	+
3a	**3b**	**3c**	**3d**	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**
-	+	+	+	+	+	-	+	+	+

Figure 7 ACC deaminase

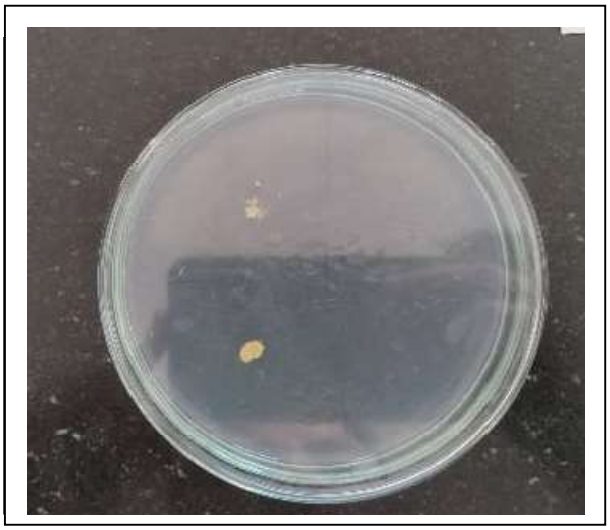
4c 4d 3d 3c

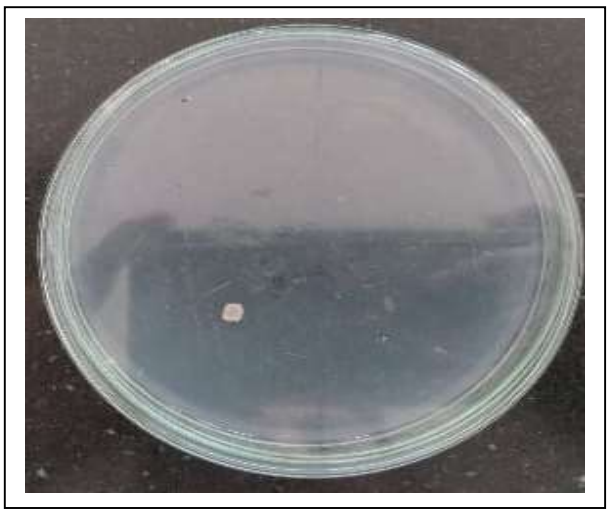


4f 4e 4c 4b



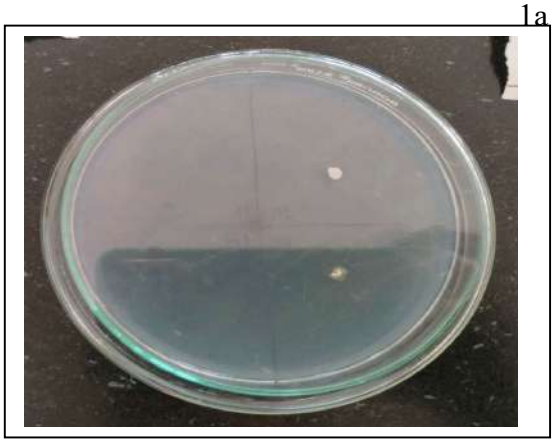
2d 2e 2a 1e





3b 3a 2c 2b

1b 1a



1d 1c

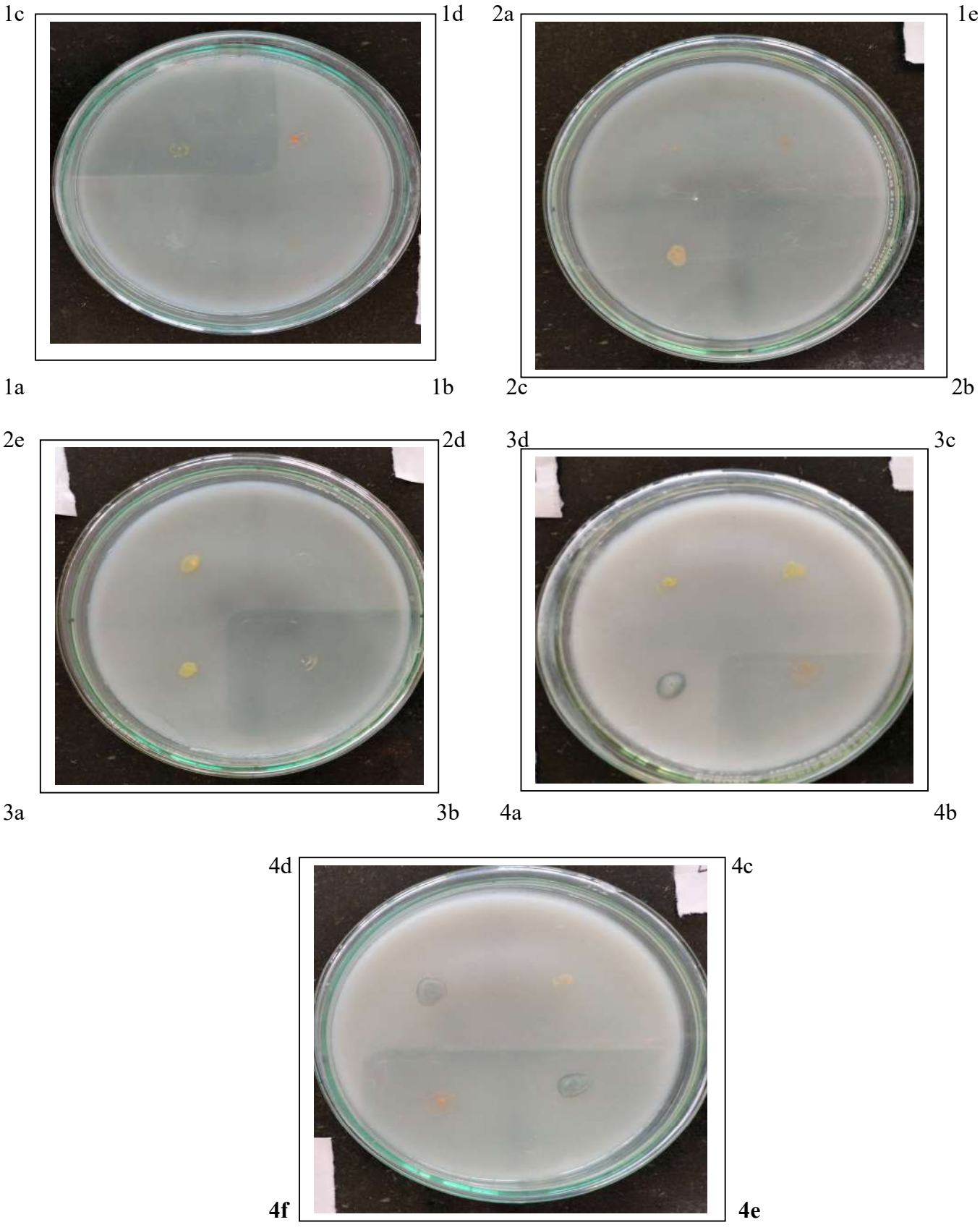
4.5 Nitrogen fixation ability:

The isolates were spot inoculated on Ashby's agar medium. After 48 hours, the clear zone around colonies of **3** isolates were observed. The spot inoculated organisms shown the positive results were:

Table 11 Nitrogen fixation ability

1a	**1b**	**1c**	**1d**	**1e**	**2a**	**2b**	**2c**	**2d**	**2e**
-	-	-	-	-	-	-	-	-	-
3a	**3b**	**3c**	**3d**	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**
-	-	-	-	-	+	-	+	+	-

Fig No.8 Nitrogen Fixation Result



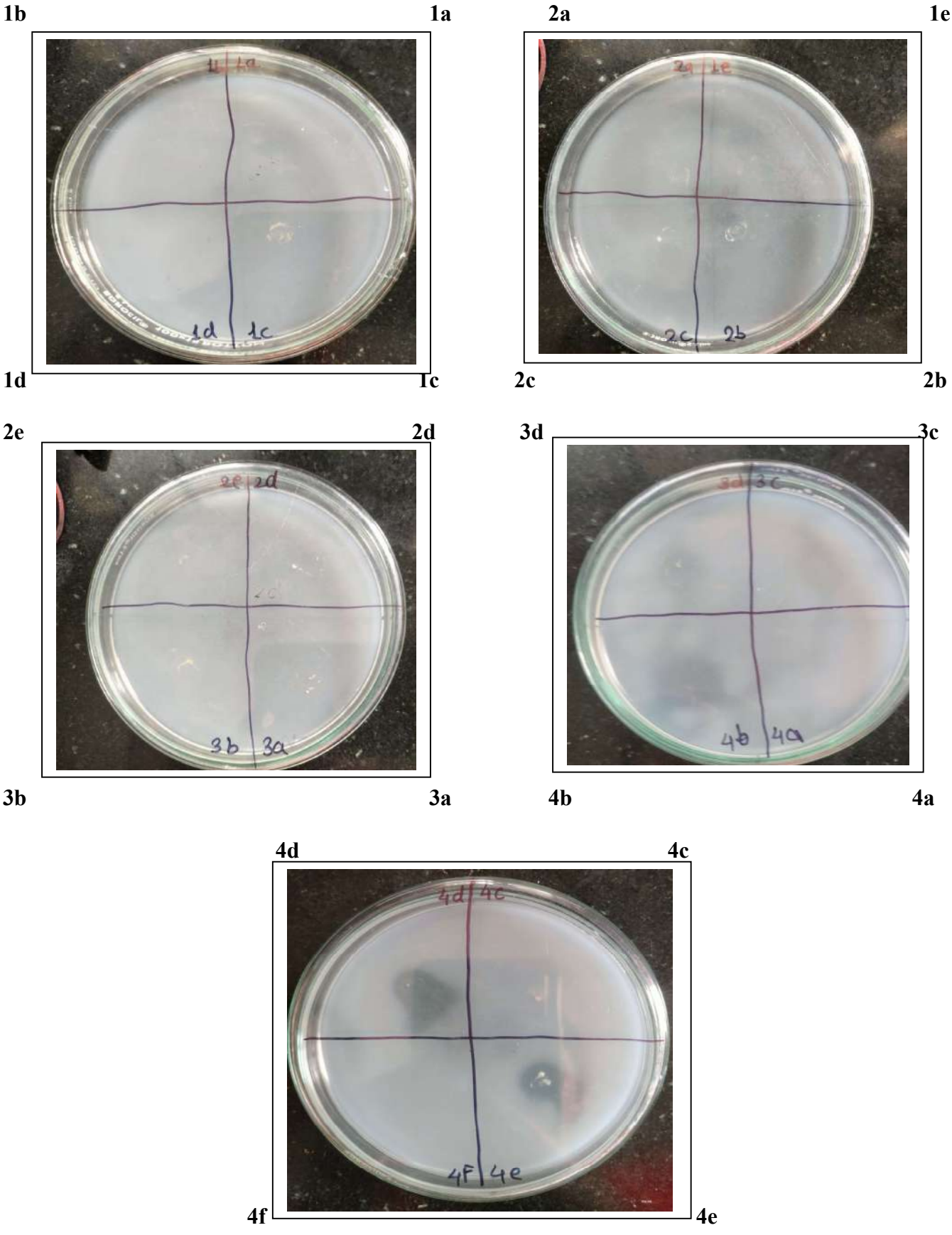
4.6 Phosphate solubilization

On Katzelson & Bose agar medium the spot inoculated **4** isolates showed the clear zone around colony after 48 hours. The spot inoculated organisms shown the positive results:

Table 12 Phosphate solubilization

1a	**1b**	**1c**	**1d**	**1e**	**2a**	**2b**	**2c**	**2d**	**2e**
-	-	-	-	-	-	-	-	-	-
3a	**3b**	**3c**	**3d**	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**
-	-	-	+	-	+	-	+	+	-

Fig No.9 Phosphate Solubilization Result



4.7 Ammonia production

After 24 hours, turbidity was observed in the peptone water. After addition of Nesslers' reagent, brown to yellow colour was observed. Among all the isolates, **15** isolates showed ammonia producing ability.

Table 13 Ammonia production

1a	**1b**	**1c**	**1d**	**1e**	**2a**	**2b**	**2c**	**2d**	**2e**
+	-	-	+	+	-	-	+	+	+
3a	**3b**	**3c**	**3d**	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**
+	+	+	-	+	+	+	+	+	+

Fig No.10 Ammonia production Result

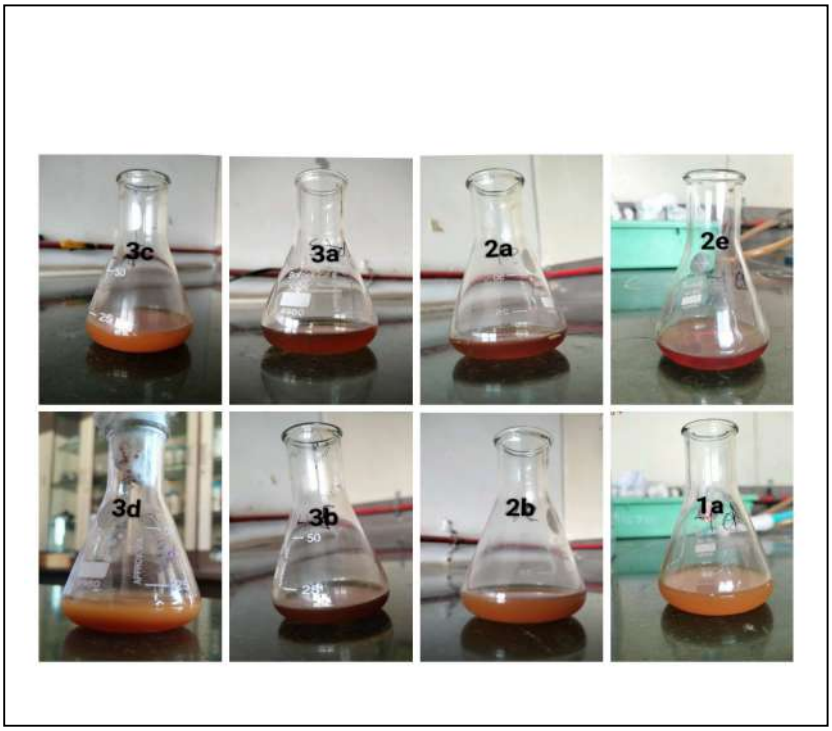
4.8 Exopolysaccharide production:

From all the isolates only 6 isolates shown ability to produce Exopolysaccharide.

Table 14 Exopolysaccharide production

1a	1b	1c	1d	1e	2a	2b	2c	2d	2e
-	-	-	-	-	-	-	+	-	-
3a	**3b**	**3c**	**3d**	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**
-	-	+	-	-	+	-	+	+	+

Fig No.11 Exopolysaccharide production Result



Production of fungal cell wall degradation

4.9 Protease production:

On skimmed milk agar medium the spot inoculated 7 isolates showed the clear zone around colony after 48 hours. The spot inoculated organisms shown the positive results:

Table 15 Protease production

1a	**1b**	**1c**	**1d**	**1e**	**2a**	**2b**	**2c**	**2d**	**2e**
+	+	-	+	+	+	+	+	-	-
3a	**3b**	**3c**	**3d**	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**
-	-	+	-	+	+	+	-	-	+

Figure 4 Protease production

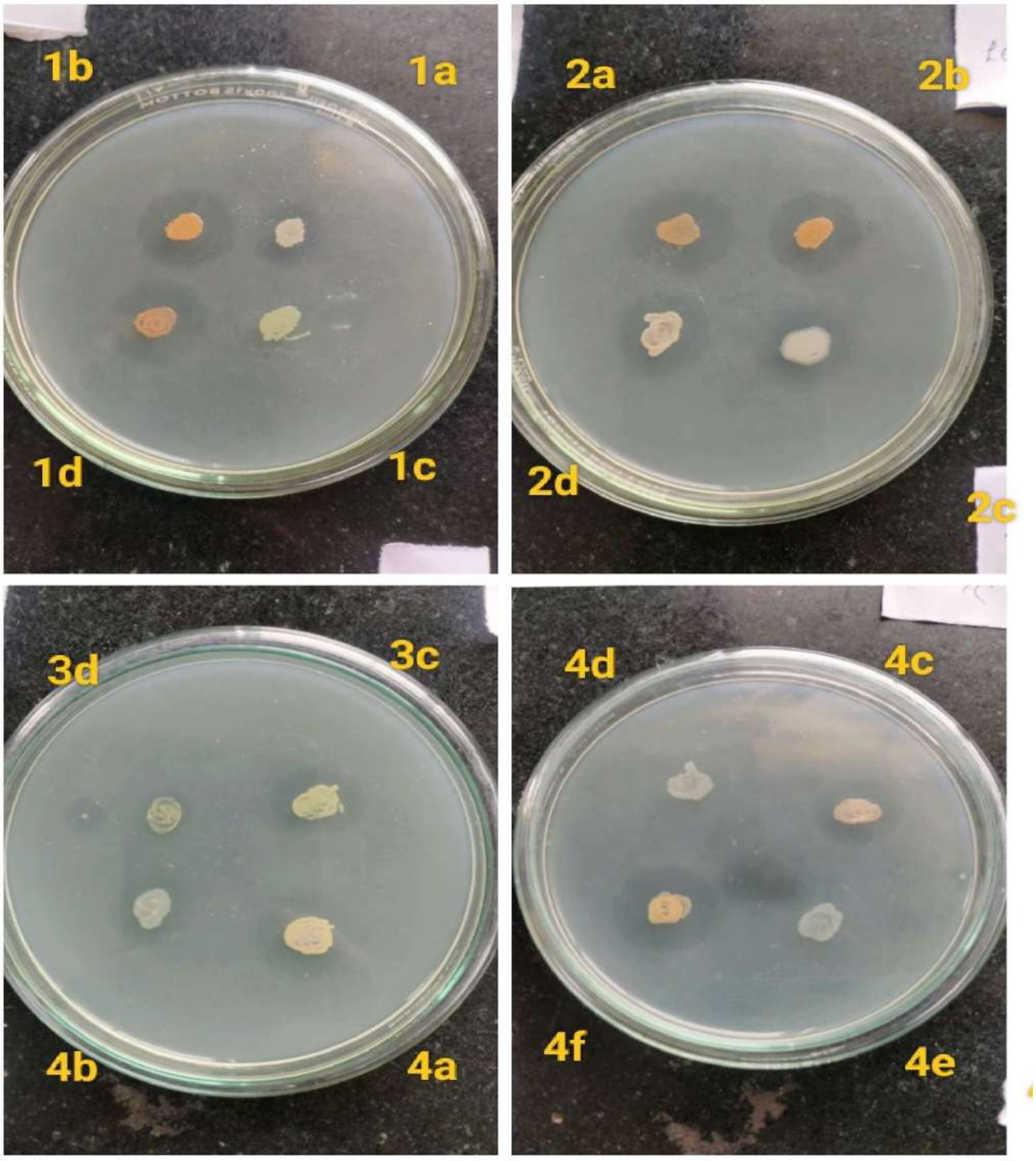


Figure 12 Protease production

4.10 Cellulase production:

The isolates were spot inoculated on CMC agar medium. After 48 hours, iodine was added to the plate. The clear zone around colonies of 8 isolates was observed. The spot inoculated organisms shown the positive results were:

Table 16 Cellulase production

1a	1b	1c	1d	1e	2a	2b	2c	2d	2e
-	-	-	-	-	+	-	+	+	+
3a	**3b**	**3c**	**3d**	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**
-	+	+	+	-	-	+	-	-	+

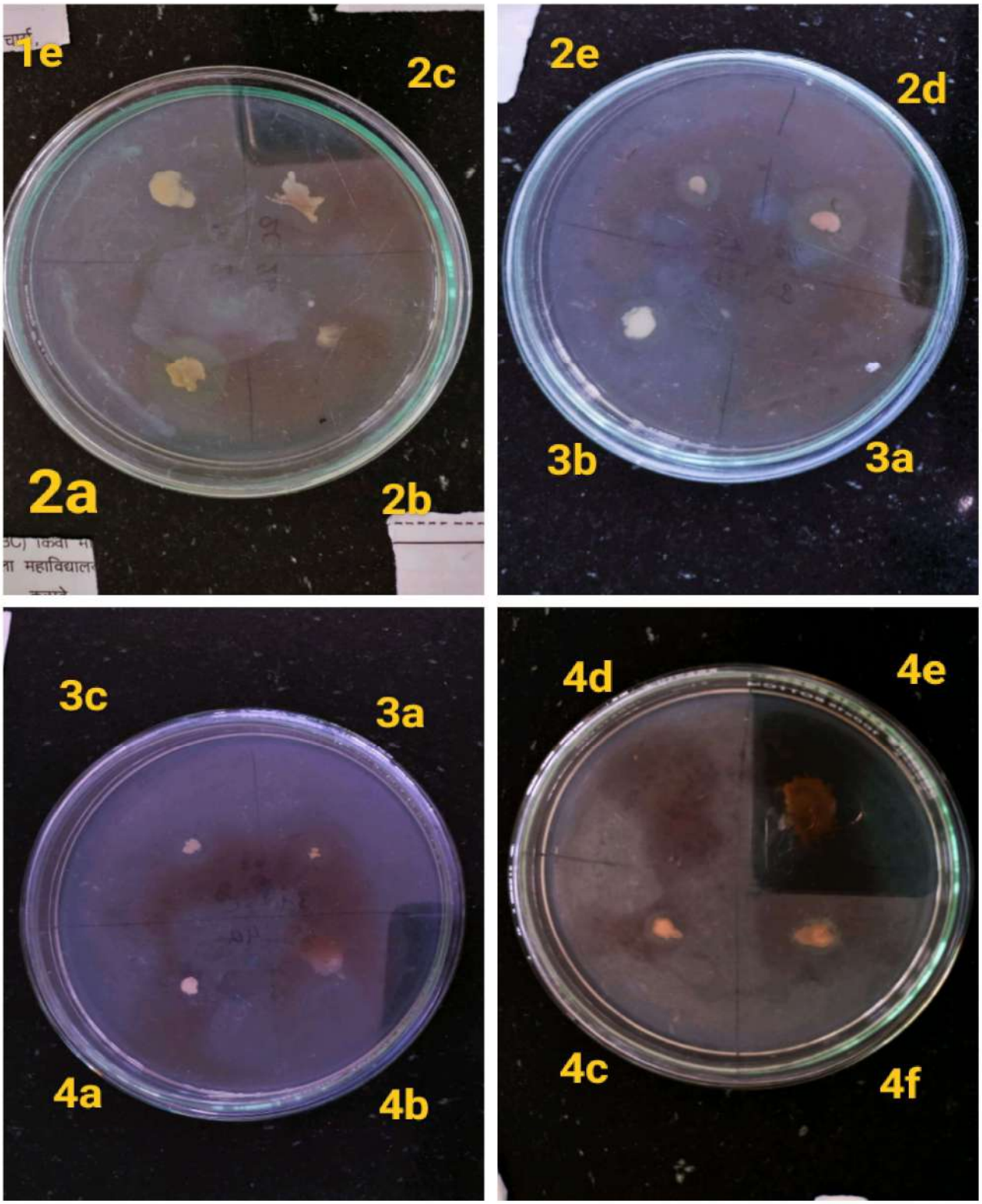


Figure 13 Cellulase production

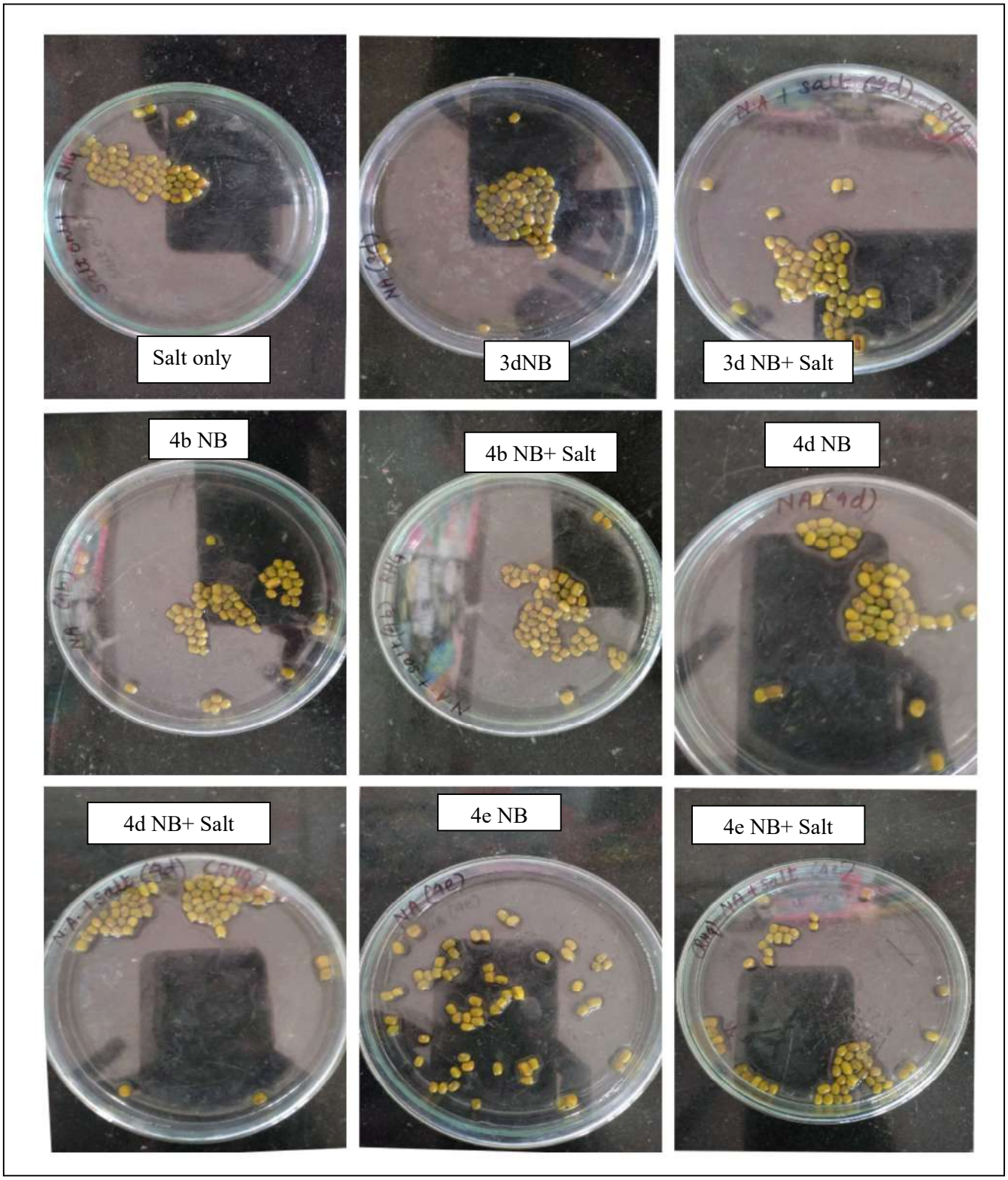
Table 17 Results

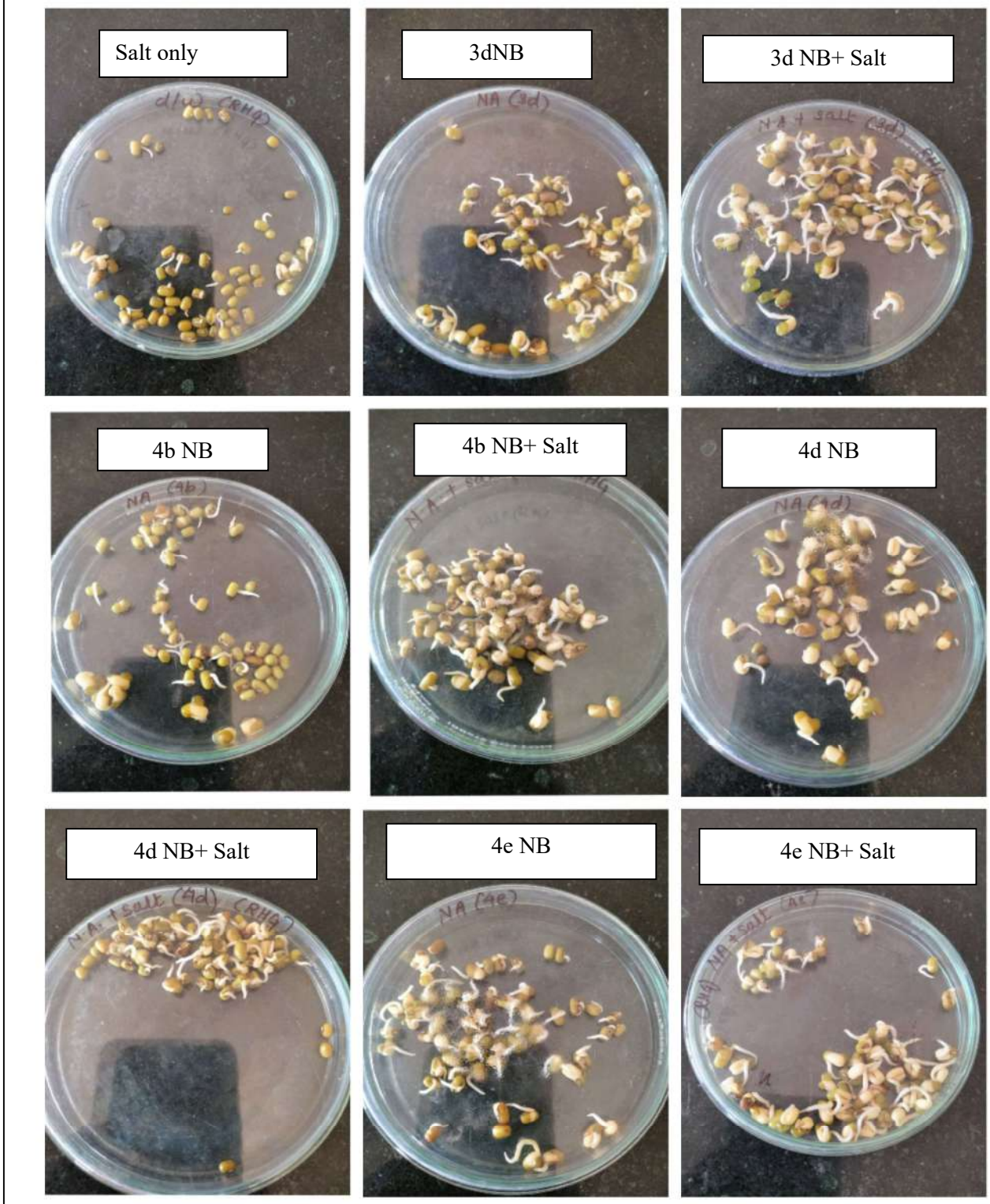
	ACC deaminase	N_2 fixation	Phosphate solubilization	Ammonia	Exopolysaccharide	Protease	Cellulase
1a	+	-	-	+	+	+	-
1b	+	-	-	-	+	+	-
1c	+	-	-	-	+	-	-
1d	-	-	-	+	+	+	-
1e	-	-	-	+	+	+	-
2a	-	-	-	-	+	+	+
2b	+	-	-	-	+	+	-
2c	+	-	-	+	+	+	+
2d	-	-	-	+	+	+	+
2e	+	-	-	+	+	+	+
3a	+	-	-	+	+	+	-
3b	+	-	-	+	+	+	+
3c	+	-	-	+	+	+	+
3d	+	-	+	-	+	-	+
4a	+	-	-	+	+	+	-
4b	+	+	+	+	+	+	-
4c	-	-	-	+	+	+	+
4d	+	+	+	+	+	-	-
4e	+	+	+	+	+	-	-
4f	+	-	-	+	+	+	+

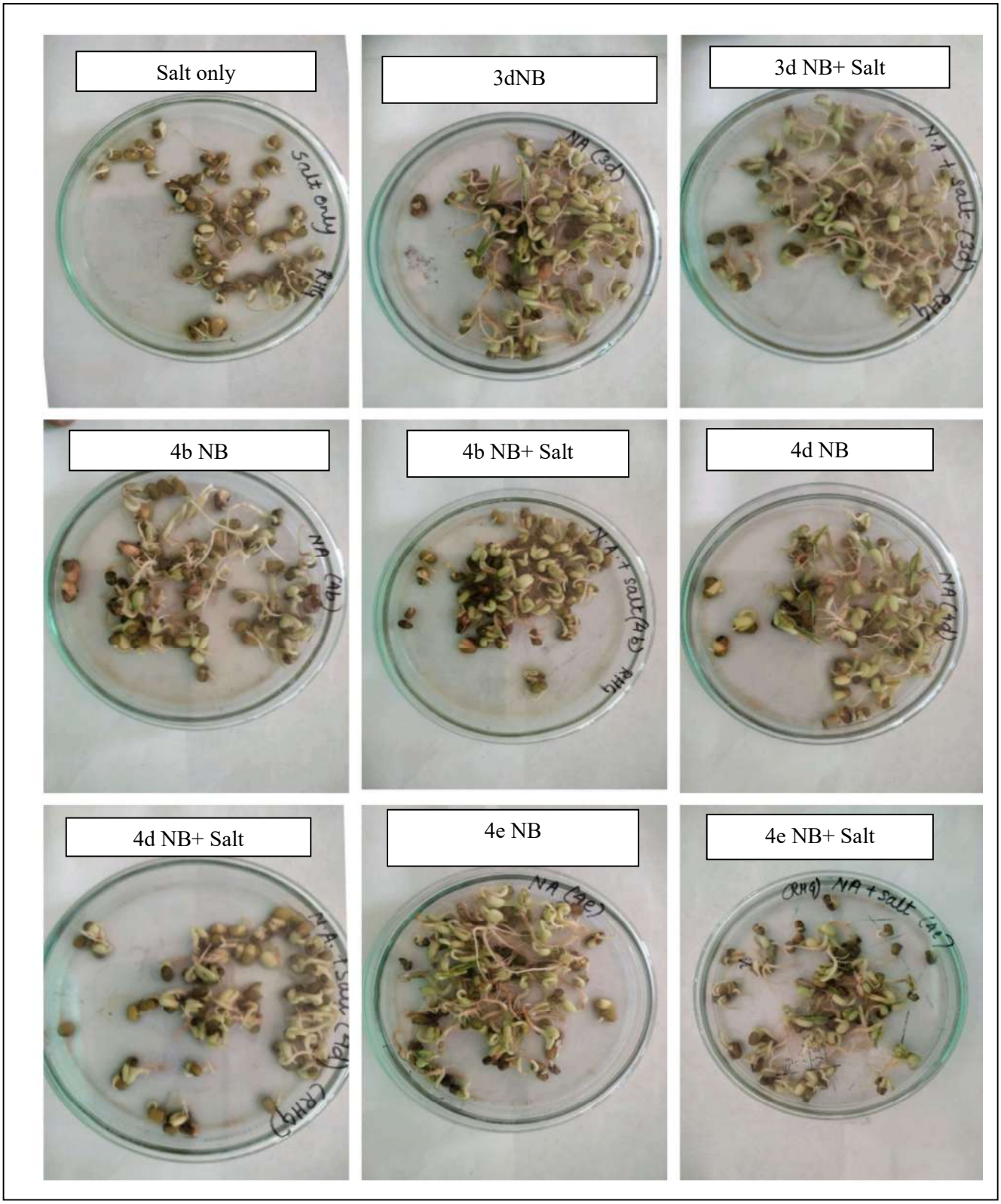
According to the above observations, the isolated organisms **3d, 4b, 4d** & **4e** were found to be potent

Seed germination & pot trials:

Seed germination of *Vigna radiata* in petriplates was carried out for 7 days. The growth pattern was observed in plates. The inoculum of **4b** showed the highest growth.




Salt only
3dNB
3d NB+ Salt
4b NB
4b NB+ Salt
4d NB
4d NB+ Salt
4e NB
4e NB+ Salt


Salt only
3dNB
3d NB+ Salt
4b NB
4b NB+ Salt
4d NB
4d NB+ Salt
4e NB
4e NB+ Salt

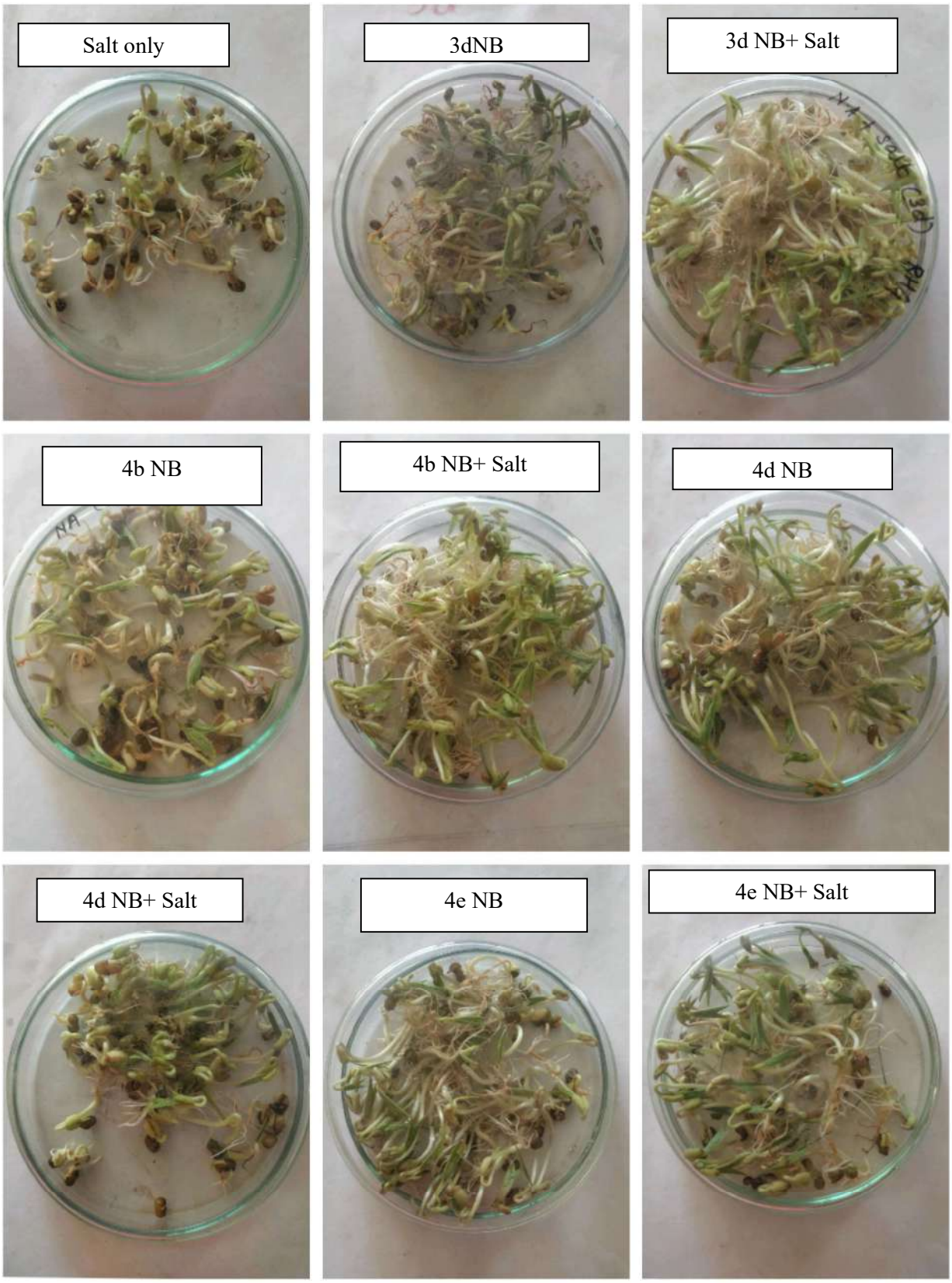

Salt only
3dNB
3d NB+ Salt
4b NB
4b NB+ Salt
4d NB
4d NB+ Salt
4e NB
4e NB+ Salt

Table 18 Seed germination & pot trials

	Without salt stress	With salt stress
3d	0.14 gm	0.16 gm
4b	0.21 gm	0.23 gm
4d	0.13 gm	0.12 gm
4e	0.17gm	0.14 gm
Control	Only water: 0.11 gm	Only 0.5% NaCl: 0.0 gm

The observations *Vigna radiata* in pot trials were been checked & the observations are as follows:

Table 19 Pot trials

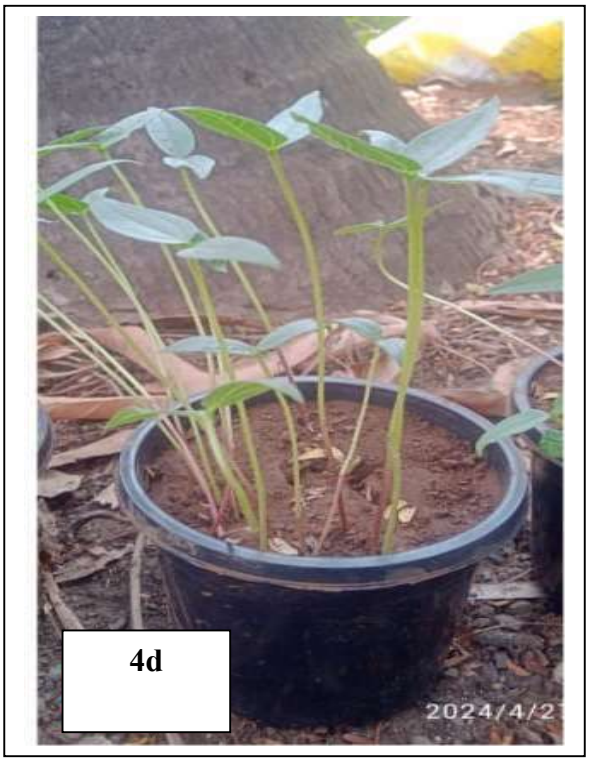
	Height of plant on day 10	Weight of plant on day 10
3d	13.9 cm	0.20 gm
4b	15 cm	0.31 gm
4d	13.5 cm	0.18 gm
4e	14.5 cm	0.26 gm
Control	12 cm	0.14 gm

According to all the observations, **4b** organism was found to be most potent isolate. Hence, further biochemical testing was carried out.


3d


4e


4b

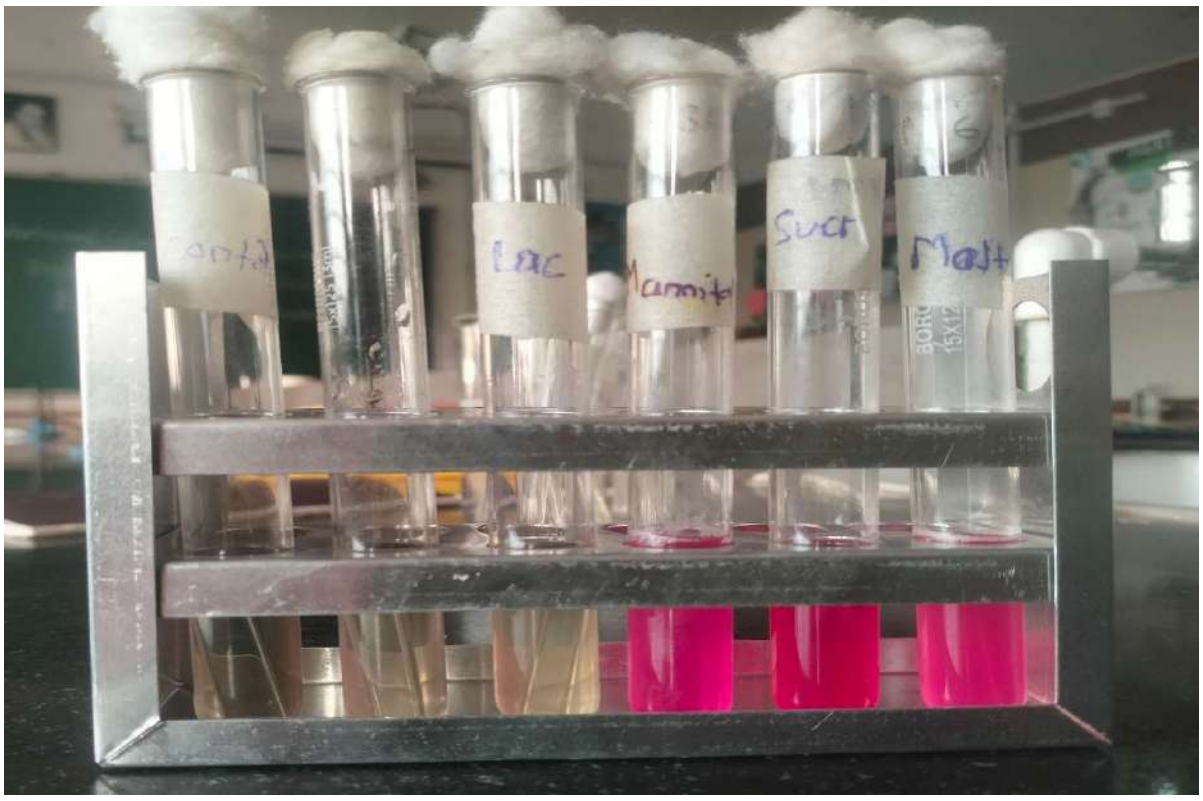

4d

Biochemical testing:

a. **Sugar fermentation:**

Table 20 Sugar fermentation

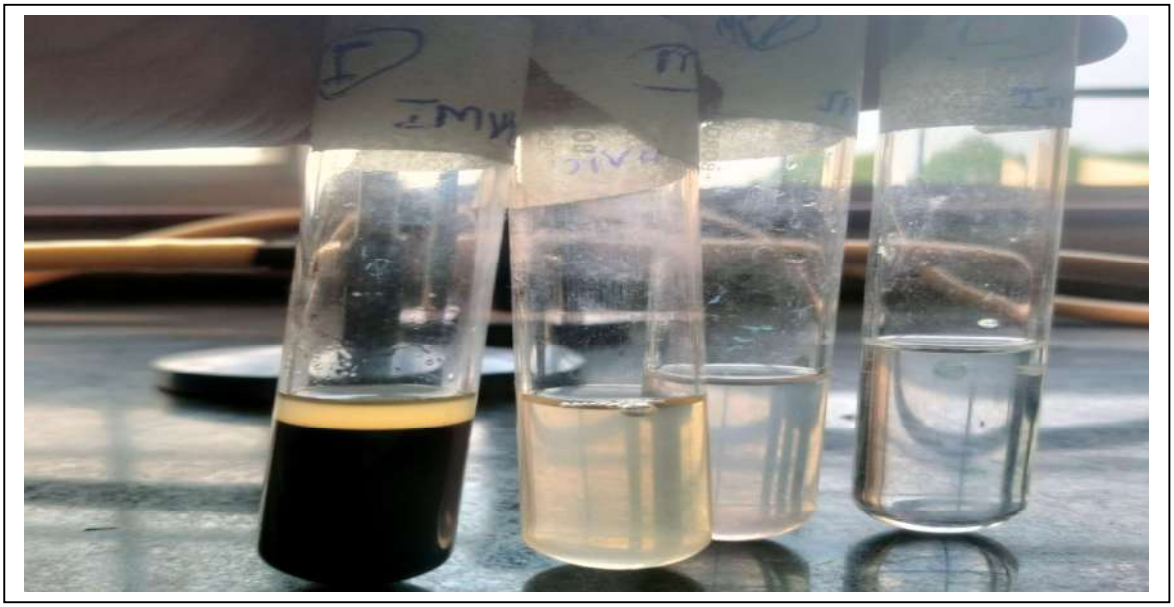
Sugar	Result
Glucose	+
Sucrose	+
Manitol	+
Maltose	+
Lactose	-



b. IMViC test

Table 21 IMViC test

	Result
Indole	-
Methyl red	-
Vogus Prosakar	+
Citrate	-



c. Other biochemical tests

Table 22 Other biochemical tests

	Result
Amylase	+
H2S production	-
Oxidase	-
Catalase	-

4.2 Discussion:

The processes by which plant growth-promoting microorganisms support plant growth and development in the face of both biotic and abiotic stresses are both direct and indirect. Agro ecosystems are affected by salt stress in terms of crop food/feed yield output and quality because of a variety of primary (osmotic stress, decreased nutrient uptake and growth) and more complicated secondary physiological disbalances caused by salt. (Song, et al., 2017) . This capacity for salt tolerance contributes to the presence of PGPB in salinity-affected soil. These results are in accordance with those obtained on *Enterobacter* spp. which could tolerate 7 % levels of NaCl stress (Deepa and others 2010) .In a present study the salt stress tolerance of the isolated PGPB was assessed by streaking them onto nutrient agar plates supplemented with varying concentrations of NaCl. Concentrations ranging from 20 to 80 g L^{-1} were selected to simulate different salinity levels encountered in agricultural soils, with a control plate containing minimal NaCl (0.5 g L^-1) for comparison. Among 50+ isolates, 20 isolates were found to be salt tolerant.

In previous study, ACC is then finally converted to ethylene by ACC oxidase. The Yang cycle consists in recycling methylthio and ribose moieties from S-adenosylmethionine into methionine, thereby maintaining high levels of ethylene in plants and fruits. In present study The qualitative assessment of ACC deaminase activity in PGPB involved a systematic approach to determine the ability of these bacteria to utilize 1-aminocyclopropane-1- carboxylate (ACC) as a nitrogen source under varying salinity conditions. The experimental setup employed rigorous controls and precise conditions to ensure the reliability and repeatability of the results. (Wang et al., 2018) ACC deaminase activity in PGPB is one of the main mechanisms that is involved in the bacterial promotion of plant growth (Kang et al, 2019). In previous the result was isolated 13 rhizospheric strains with ACC deaminase activity from saline soils from

Xinjiang, China. Three isolates of the genus *Bacillus* stood out among the others, as they significantly increased the plant fresh and dry weight, the length of the roots and the aerial portions of pepper plants (*Capsicum annuum* L.) under salt stress (Wang et al., 2018). In present study all the isolates were observed for ACC deaminase activity. According to observations, the growth of potent organisms was visible on DF agar medium supplemented with ACC. **15** isolates shown growth on DF medium which indicates the ability of the isolates to produce ACC deaminase.

Phosphorus (P) is one of the most important minerals required for plant growth occupying a strong position among soil macro nutrients. Soil P deficiency is often fulfilled by phosphate fertilizers. According to the optimum pH, the phosphatase enzyme is classified into two groups: (i) alkaline phosphatase (maximum activity at an alkaline pH > 7) and (ii) acidic phosphatase (maximum activity at pH < 6), where both are produced by PSB depending upon external conditions (Neal, 2018). According to the previous findings, these strains can be used as a potentially suitable biofertilizer for the management of phosphate in saline affected agriculture fields. In Present study Katzelson & Bose agar medium the spot inoculated 4 isolates showed the clear zone around colony after 48 hours. The spot inoculated organisms shown the positive results.

Nitrogen is a major component of chlorophyll, the most important pigment required for photosynthesis, and plays a critical role for plant growth and production (Wang D. G., 2012). Nitrogen is an essential component of urea and amino acids (proteins), nucleic acids (DNA and RNA), adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NAD) in all living cells (Bulen).In previous study Plant endophytes and bacteria inhabiting the rhizosphere have been reported to enhance nodule formation and tolerance of biotic and abiotic stress in controlled conditions. In present study the isolates were spot inoculated on Ashby's agar

medium. After 48 hours, the clear zone around colonies of **3** isolates was observed. The spot inoculated organisms shown the positive results.

Bacterial ammonia production was detected by using peptone water (J.G.Cappuccino, 2008) . Bacteria were grown in 1.5 % w/v peptone water at 30 °C for 2 days with constant shaking at 150 rpm. Cell-free supernatants of fermented broth were added with 5 % Nessler reagent. Nesslerization of sterile un-inoculated peptone water was served as reference. Colour change of supernatants from pale to deep yellow was determined at absorbance 425 nm. The amount of produced ammonia was measured through a standard curve established by determining the different concentration (0−100 mM) of authentic ammonia at absorbance 425 nm. In previous study the presence of deep yellow colour in nesslerized spent broth has shown that all selected isolates were efficient in emission of high concentration ammonia ranging from the lowest 60.3 mM in *Br. gelatini* strain JD04 up to the highest 75.3 mM in *P*.

These isolates were capable to catalyze liberation of ammonia from organic detritus that is present in natural environments. In present After 24 hours, turbidity was observed in the peptone water. After addition of Nesslers' reagent, brown to yellow colour was observed. Among all the isolates, 15 isolates showed ammonia producing ability.

Exopolysaccharides, are produced by an array of microorganisms like bacteria, cyanobacteria, microalgae, yeasts, and fungi (Boonchai R, 2014) These exopolysaccharides impart defence against a wide range of environmental stresses like drought, metals, salt and temperature (Sayyed RZ, 2015). Agricultural productivity and crop yields can be affected by various environmental stressors such as drought, salinity, high temperatures, and heavy metals, all of which adversely affect plant growth and development, and eventually lead to global food scarcity. Recent investigations have identified several species of bacteria that impart stress

tolerance properties to plants through various activities such as EPS production and biofilm formation, which help increase the nutrient uptake and water retention capacity of plants. In present study from all the isolates only 6 isolates shown ability to produce Exopolysaccharide.

Cellulose is the most abundant biomass on the earth. Cellulases are inducible enzymes which are synthesized by large number of microorganisms either cell-bound or extracellular during their growth on cellulosic materials (K.Shanmugapriya1*, 2012). In previous study the isolate appeared white colonies on CMC agar. A microscopic examination of the isolate revealed that it was a Gram positive bacterium with a centrally oval shape spore and produced CELLULASE K.Shanmugapriya, et al. 512 enzyme cellulase. Furthermore, the biochemical analysis of the isolates was performed and identified to be Bacilli species. . - Negative and + Positive reaction. Biochemical characteristics of the bacterial isolate. In present study, the isolates were spot inoculated on CMC agar medium. After 48 hours, iodine was added to the plate. The clear zone around colonies of 8 isolates was observed. The spot inoculated organisms shown the positive results.

Hauser described two species of the genus: *Proteus vulgaris* and *Proteus mirabilis* (Aboh E.A., 2015). In previous study the genus *Proteus* includes Gram-negative, facultative anaerobic, heterotrophic, and proteolytic rods being human opportunistic pathogens. The taxonomic classification of these bacteria has changed several times. Lately, the only *Proteus* species with no clinical significance, *Proteus myxofaciens*, has been postulated to be moved from the genus *Proteus* to a new genus *Cosenzaea* (Giammanco GM, 2011) . In present study On skimmed milk agar medium the spot inoculated 7 isolates showed the clear zone around colony after 48 hours. The spot inoculated organisms shown the positive results.

CONCLUSION AND SUMMARY

5.0 CONCLUSION & SUMMARY

The introduction of plant growth-promoting bacteria (PGPB) as a means to mitigate salinity stress in plants has garnered significant attention in recent years. Salinity stress poses a significant threat to agricultural productivity worldwide, affecting approximately 20% of cultivated land. High salt concentrations in soil can impair plant growth and development by disrupting cellular processes and reducing water uptake. However, certain bacteria have evolved mechanisms to tolerate high salt concentrations and can even facilitate plant growth under saline conditions.

One such mechanism involves the production of ACC deaminase, an enzyme that catalyzes the conversion of 1-aminocyclopropane-1-carboxylate (ACC) into α-ketobutyrate and ammonia. ACC is a precursor of ethylene, a plant hormone that regulates various physiological processes, including stress responses. By reducing the levels of ACC, PGPB can decrease ethylene production, thereby alleviating the inhibitory effects of salt stress on plant growth.

In addition to ACC deaminase activity, PGPB can also contribute to plant growth promotion through other mechanisms, such as nitrogen fixation, phosphate solubilization, and production of exopolysaccharides (EPS). Nitrogen fixation is the process by which certain bacteria convert atmospheric nitrogen into a form that can be readily utilized by plants. This provides an additional source of nitrogen, which is essential for plant growth and development. Phosphate solubilization involves the release of phosphorus from insoluble compounds in the soil, making it more accessible to plants. Ammonia production by PGPB can also serve as a source of nitrogen for plants, further enhancing their growth potential.

Moreover, the production of EPS by PGPB can improve soil structure and water retention, thereby enhancing plant resilience to environmental stresses, including salinity. EPS are complex polymers secreted by bacteria that can form a protective matrix around microbial cells, helping them to survive adverse conditions. This matrix can also bind soil particles together, improving soil aggregation and stability.

Furthermore, PGPB can exert biocontrol effects against plant pathogens through the production of enzymes such as cellulase and protease. Cellulase enzymes degrade cellulose, a major component of fungal cell walls, thereby inhibiting fungal growth and colonization. Similarly, protease enzymes can degrade proteins in fungal cell walls, disrupting their structural integrity and reducing their virulence.

The efficacy of PGPB in promoting plant growth and alleviating salt stress has been demonstrated in numerous studies. Inoculation of plants with PGPB has been shown to enhance root growth, increase nutrient uptake, and improve overall plant vigor under saline conditions. These beneficial effects are often accompanied by changes in the expression of stress-responsive genes and modulation of plant hormone levels.

A study conducted by in this project proves the potential of PGPB isolated from saline soil was evaluated for their ability to promote plant growth and mitigate salt stress in *Vigna radiata* . The isolated bacteria were found to possess multiple beneficial traits, including ACC deaminase activity, nitrogen fixation, phosphate solubilization, EPS production, and biocontrol potential against fungal pathogens.

The experimental setup involved inoculating *Vigna radiata* with the selected PGPB strains and subjecting them to varying levels of salinity stress. Plant growth parameters, such as height, biomass was measured periodically to assess the impact of PGPB inoculation on plant performance under salt stress.

The results of the study revealed that inoculation with PGPB significantly improved the growth and physiological status of *Vigna radiata* under saline conditions. Plants treated with PGPB exhibited increased root length, shoot height, and biomass compared to untreated controls.

In conclusion, the study provides compelling evidence for the potential of PGPB as a sustainable approach for mitigating salinity stress and enhancing plant productivity in saline environments. The multifaceted mechanisms employed by our isolate 4b, includes ACC deaminase activity, nitrogen fixation, phosphate solubilization, EPS production, and biocontrol activity, contribute to their ability to promote plant growth and alleviate salt stress. This study suggests the use of this organism as an ideal bioinoculum commercially to reduce impact of salinity stress & to promote plant growth significantly

Further research is warranted to optimize PGPB inoculation strategies and to elucidate the molecular basis of PGPB-plant interactions under saline conditions.

BIBLIOGRAPHY

6.0 BIBLIOGRAPHY

1. R.Munns & M.Tester. (2008). Mechanisms of salinity tolerance. *Annual review of plant biology*.
2. A. Gurmani, A. B. (2011). *Alleviation of salt stress by seed treatment with abscisic acid (ABA), 6-benzylaminopurine (BA) and chlormequat chloride (CCC) optimizes ion and organic matter accumulation and increases yield of rice (Oryza sativa L.).*
3. A.Haque, M. A. (2020). Effect of Climate Change on Soil Salinity and Associated Impacts on Food Security in the South-West Coastal Region of Bangladesh. *Journal of Soil Science and Environmental Management*.
4. A.Kumar, J. V. (2022). Plant Growth-Promoting Rhizobacteria (PGPR): Current Scenario and Future Prospects. In Plant Microbiome: Stress Response. *Springer, Singapore.*
5. A.L.Julkifle, J. &. (2018). Effects of different potassium fertilizers on soil salinity and crop yield under saline conditions. *Communications in Soil Science and Plant Analysis*.
6. Abbasi, H. J. (2016.). Salt stress manifestation on plants, mechanism of salt tolerance and potassium role in alleviating it: a review. . *Zemdirb. Agric. 103 (2), 229–238.*
7. Aboh E.A., G. F. (2015). Microbiological assessment of well waters in Samaru, Zaria, Kaduna, State, Nigeria.
8. ACC demainase result. (Fig No.1).
9. ACC Demainase Result. (Fig No.1).
10. Ahmad, B. K. (2017). Exogenously sourced g-irradiated chitosan-mediated regulation of growth, physiology, quality attributes and yield in Mentha piperita L. Turk. *J. Biol. 41 (2), 388–401.*
11. Ahn, C. P. (2011.). Increased salt and drought tolerance by d-ononitolproduction in transgenic Arabidopsis thaliana. *Biochem. Biophys. Res. Commun.*
12. Ali, S. C. (2012). Delay of flower senescence by bacterialendophytes expressing 1-aminocyclopropane-1-carboxylate deaminase. *J.Appl. Microbiol. 113, 1139–1144.*
13. Ali, S. C. (2014). Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase. .*Plant Physiol. Biochem. 80, 160–167*.
14. Amna, U. D. (2019.). Mechanistic elucidation of germination potential and growth of wheat inoculated with exopolysaccharide and ACCdeaminase producing Bacillus strains under induced salinity stress. *Ecotoxicol.Environ. Saf. 183, 109466*.
15. Amna, U. D. (2019.). Mechanistic elucidation of germination potential and growth of wheat inoculated with exopolysaccharide and ACC deaminase producing Bacillus strains under induced salinity stress. *Ecotoxicol.Environ.*
16. Arora, N. E.-J. (2021.). Editorial: salt tolerant rhizobacteria: for better productivity and remediation of saline soils. *Front.Microbiol.*
17. Arujo A, F. M. (2008). *Potent of Biological Nitrogen Fixation as indicator of soil pollution, Nitrogen Fixation research progress,.* New York : Nova Science Publisher.
18. B.R.Glick, C. L. (1999). Biochemical & genetic mechanism used by plant promoting bacteria. *World Scientific*.
19. Boonchai R, K. J. (2014). Effect of nutrient starvation on nutrient uptake and extracellular polymeric substance for microalgae cultivation and separation.
20. Bresson J., V. F. (2013). *The PGPR strain Phyllobacterium brassicacearum STM196 induces a reproductive delay and physiological changes that result in improved drought tolerance in Arabidopsis, New Phytologist.*
21. Bulen, W. a. (n.d.). he nitrogenase system from Azotobacter: two-enzyme requirement for N2 reduction, ATP-dependent H2 evolution, and ATP hydrolysis. *2017*.
22. C. Cabot, J. S. (2009). *Abscisic acid decreases leaf Na+ exclusion in salt-treated Phaseolus vulgaris L.* Plant Growth Regul.
23. C. L. Patten and B. R. Glick. (2005). *"Bacterial biosynthesis on indole-3-acetic acid.* Canadian Journal of Microbiology.
24. Chen, Q. L. (2019). Identification and characterization of the phosphate-solubilizing bacterium Pantoea sp. S32 in reclamation soil in Shanxi, China. *Front. Microbiol. 10,*.
25. Chinnusamy, V. K. (2004). Molecular genetic perspectives on cross-talk and specificity in abiotic stress signalling in plants. *Journal of Experimental Botany*.
26. Choudhary, D. V. (2017.). Plant-Microbe Interaction: an Approach to Sustainable Agriculture. *Springer, New Delhi, India.*
27. Costa-Gutierrez, S. L.-D.-U. (2020.). Plant growth promotion by Pseudomonas putida KT2440 under saline

stress: role of eptA. *App. Microbiol.*-.
28. D.Choudhary, A. V. (2017.). Plant-Microbe Interaction: an Approach to Sustainable Agriculture. *Springer, New Delhi, India.*
29. D.K.Gupta, ,. F. (2019). Phosphorus and Plant Response to Salinity. In Phosphorus in Agriculture: 100% Zero. *Springer, Cham.*
30. Divi, U. R. (2010). Brassinosteroid-mediated stress tolerance in Arabidopsis shows interactions with abscisic acid, ethylene and salicylic acid pathway . *BMC Plant Biol. 10.*
31. Dubois, M. V. (2018.). The pivotal role of ethylene in plant growth. . *Trends Plant Sci. 23, 311–323.*
32. *FAOSTAT.* (2015). Retrieved September 29, 2016, from www.faostat.fao.org.
33. Fraser, T. L. (2015a.). Soil bacterial phoD gene abundance and expression in response to applied phosphorus and long-term management. . *Soil Biol. Biochem. 88, 137–147.*
34. G. M. Naik, P. A. (2020). Mung Bean. In Postharvest Handling of Pulses (pp. 123–141). *Springer Nature Switzerland AG. doi: 10.1007/978-3-030-41377-4_6* .
35. G. Santoyo, G. H. (2016). Plant growth promoting bacterial endophytes. *Elsevier*.
36. G.Xu, X. &. (2020). Plant nitrogen assimilation and use efficiency. *Annual Review of Plant Biology*.
37. Galloway J.N., T. A. (2008). *Transformation of the Nitrogen Cycle.* Recent Trends, Questions, and Potential Solutions.
38. Ganie, S. A. (2020). Vascular plant one zinc-finger (VOZ) transcription factors: novel regulators of abiotic stress tolerance in rice (Oryza sativa L.). *Genet. Resour. Crop Evol. 67, 799–807.*
39. Giammanco GM, G. P. (2011). Syst Evol Microbiol.
40. Glick, B. (2012). Plant growth-promoting bacteria: mechanisms and applications. *Scientifica*.
41. Glick, B. (2015.). Stress control and ACC deaminase. *Principles of Plant-Microbe Interactions. Springer, Cham, pp. 257–264.*
42. Gondek, M. W. (2020). *Soluble salts in compost and their effects on soil and plants.* Compost Sci. Util.
43. H.Alderman. (2007). *Managing risk to increase efficiency & reduce poverty.* Washington DC: World Development Report 2008.
44. Hardoim, P. v. (2008). Properties of bacterialendophytes bacterialendophytes and their proposed role in plant growth. . *Trends Microbiol. 16,463–471*.
45. Hedin L.O., B. E. (2009). *The Nitrogen Paradox in Tropical Forest Ecosystems.* Annu. Rev. Ecol. Evol. Syst.
46. Heuck, C. S. (2018.). Effects of long-term nitrogen addition on phosphorus cycling in organic soil horizons of temperate forests. *Biogeochemistry 141, 167–181.*
47. Hommes, R. P. (1984.). Evidence of a quinoprotein glucose dehydrogenase apoenzyme in several strains of Escherichia coli. *FEMS Microbiol. Lett. 24, 329–333.*
48. Hu, Y. S. (2005.). Drought and salinity: a comparison of their effects on mineral nutrition of plants. . *J. Plant Nutr. Soil Sci. 168, 541–549.*
49. Hu, Y. V. (2017). Regulation of seedling growth by ethylene and the ethylene-auxin crosstalk. *Planta 245, 467–489.*
50. Huang, L. W. (2019). Effects of salinity stress on growth, ion homeostasis, and osmotic adjustment of sugar beet (Beta vulgaris L.) in hydroponic culture. *Journal of Soil Science and Plant Nutrition*.
51. Ilangumaran, G. S. (2017.). Plant growth promoting rhizobacteria in amelioration of salinity stress: a systems biology perspective. *Front. Plant Sci.*
52. Iqbal, N. K. (2017). Ethylene role in plant growth, development and senescence: interaction with other phytohormones. . *Front. Plant Sci. 8, 475.*
53. J.Bailey-Serres, L. H. (2019). Flooding, acclimatization, and adaptation. *Annual Review of Plant Biology*.
54. J.Bose, M. &. (2014). ROS homeostasis in halophytes in the context of salinity stress tolerance. *Journal of Experimental Botany*.
55. J.G.Cappuccino, N. S. (2008). Microbiology:A Laboratory Manual.
56. J.J. Dethier, A. E. (2011). Agriculture & development : Abrief review of the literature. *Elsevier*.
57. K.K.Bhise, P. (2019). MItigation of salinity stress in plants using plant growth promoting bacteria. *Synbiosis*.
58. K.Shanmugapriya1*. (2012).
59. Kang et al, M. e. (2019).
60. Karthikeyan, B. J. (2012.). ACC deaminase containingdiazotrophic endophytic bacteria ameliorate salt stress in Catharanthus roseusthrough reduced ethylene levels and induction of antioxidative defensesystems. *Symbiosis 56, 77–86*.
61. Kasotia, A. V. (2016.). Amelioration of soybean plant from saline-induced condition by exopolysaccharide

producing Pseudomonas mediated expression of high affinity K ⁺ -transporter (HKT1) gene. *Curr. Sci. 111, 1961–1967.*
62. Kaushal, M. W. (2016). Rhizobacterial-plant interactions: strategies ensuring plant growth promotion under drought and salinity stress. *Agricul. Ecosys. Environ.*
63. L.Zeng, M. &. (2020). Evaluation of Salt Tolerance in Rice Genotypes by Multiple Agronomic and Physiological Indices. *Agronomy Journal*.
64. Lambrecht M, O. Y. (2004). *Indole-3-acetic acid: a reciprocal signaling molecule in bacteria-plant interactions.* Trends Microbiol.
65. Li, F. C. (2023). Soil pH as a predictor of soil salinity under different land uses in the arid oasis of northwest China. *Science of the Total Environment*.
66. Liu, X. J. (2019.). Phosphate-solubilizing Pseudomonas sp. strain P34-L promotes wheat growth by colonizing the wheat rhizosphere and improving the wheat root system and soil phosphorus nutritional status. *J. Plant Growth Regul.*
67. Lv, S. W. (2021). Variation of PHT families adapts salt cress to phosphate limitation under salinity. *Plant Cell Environ.*
68. M. Chaves, J. F. (2009). Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Annals of Botany*.
69. M. Farooq, A. W. (2009). Plant drought stress: effects, mechanisms and management. *Agronomy for Sustainable Development*.
70. M. Hasanuzzaman, K. N. (2017). Plant response to drought and salinity stress and its tolerance mechanisms. *Frontiers in Plant Science*.
71. M. Kelen, E. Ç. (2004). *"Separation of abscisic acid, indole-3-acetic acid, gibberellic acid in 99R (Vitis berlandieri × Vitis rupestris) and rose oil (Rosa damascena Mill.) by reverse phase liquid chromatography,".* Turkish Journal of Chemistry.
72. M.A.Khan, S. S. (2019). Impact of soil salinity on crop productivity and mitigation strategies in Saline Soil-based Agriculture by Halophytes. *Springer, Cham.*
73. M.Kalyani. (2015). Mungbean (Vigna radiata L. Wilczek): Retrospect and Prospects. In Mungbean [Vigna radiata (L.) Wilczek]: Improvement in Production and Utilization (pp. 1–23). . *Springer International Publishing. doi: 10.1007/978-3-030-41376-7_1*.
74. M.Kaushal, S. P. (2016, September 01). Rhizobacterial-plant interactions: Strategies ensuringn plant growth promotion under drought & salinity stress. *Agriculture, Ecosystem & Environment*.
75. Marklein, A. H. (2012.). Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. *New Phytol. 193, 696–704.*
76. Meneses, C. G. (2017). Gluconacetobacter diazotrophicus exopolysaccharide protects bacterial cells against oxidative stress in vitro and during rice plant colonization. *Plant Soil 416,133–147.*
77. Mitchell. et.al. (2018).
78. Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in plant science*.
79. Mittler, R. (2006). Abiotic stress, the field environment and stress combination. *Trends in plant science*.
80. Mokhele B, Z. X. (2012). *Nitrogen assimilation in crop plants and its affecting factors.* Can J Plant Sci.
81. Murgese, P. S. (2020). Ameliorative effects of PGPB on yield, physiological parameters, and nutrient transporter genes expression in barattiere (Cucumis melo L.). . *J. Soil Sci. Plant Nutr. 20, 784–793.*
82. N. Iqbal, N. K. (2017). Ethylene role in plant growth, development & senescene: interraction with other phytohormones. *Front Plant Sci.*
83. N.K.Arora, E. D.-J. (2021). Editorial: salt tolerant rhizobacteria: for better productivity and remediation of saline soils. *Front.Microbiol. 12, 660075*.
84. N.Tuteja, S. &. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*.
85. Nascimento, F. R. (2014). New insights into 1-aminocyclopropane-1-carboxylate (ACC) deaminase phylogeny, evolution and ecological significance. *PLoS One 9*.
86. Nascimento, F. R. (2016a). Role of ACC deaminase in stresscontrol of leguminous plants. In: Subramanian, G. (Ed.),. *Plant Growth Promoting Actinobacteria*.
87. Navarro.G et.al. (2011).
88. Neal, A. B. (2018). biogeography and the effects of land management upon bacterial non-specific Acid phosphatase Gene diversity and abundance.
89. Newman, B. (2007). *A Bitter Harvest: Farmer Suicide and the Unforeseen Social, Environmental and*

Economic Impacts of the Green Revolution in Punjab, India. Punjab: Development Report No. 15.
90. Nwodo, U. G. (2012). Bacterial exopolysaccharides: functionality and prospects. *Int. J. Mol. Sci.*
91. O.P. Yadav, D. ,. (2019). India's evergreen revolution in cereals. *Curr Sci 116*.
92. Olander, L. V. (2000). Regulation of soil phosphatase and chitinase activity by N and P availability. . *Biogeochemistry 49, 175–191*.
93. Ouhibi, C. A. (2014.). Salt stress mitigation by seed priming with UV-C in lettuce plants: growth, antioxidant activity and phenolic compounds. *Plant Physiol. Biochem.*
94. P. K.Joshi, S. K. (2020). Understanding the demand for pulses in India: A meta-analysis approach. *Journal of Agribusiness Marketing and Management, 11(1), 1-17*.
95. P. M.Hasegawa, R. A. (2000). Plant cellular and molecular responses to high salinity. *Annual review of plant biology*.
96. P.Pandey, V. M. (2017). Impact of combined abiotic and biotic stresses on plant growth and avenues for crop improvement by exploiting physio-morphological traits. *Frontiers in Plant Science*.
97. P.Rengasamy . (2010.). Soil processes affecting crop production in salt-affected soils. *Funct.Plant Bio*.
98. P.Rengasamy. (2006). World salinization with emphasis on Australia. *Journal of Experimental Botany*.
99. Patel RR, P. D. (2015, March 31). Alleviation of salt stress in germination of Vigna radiata L. by two halotolerant Bacilli sp. isolated from saline habitats of Gujarat. *Plant Growth Regulation*, p. 76.
100. Pierik, R. T. (2006). The Janus face of ethylene: growth inhibition and stimulation. . *Trends Plant Sci. 11, 176–183.*
101. PJ, D. (2010). *The plant hormone concept: concentration, sensitivity and transport In: Davies PJ (ed) Plant hormones.* physiology, biochemistry, and molecular biology. Kluwer Academic Publishers.
102. R. Gilliham, M. &. (2015). Salinity tolerance of crops–what is the cost? *New Phytologist*.
103. R.Mittler, S. M. (2004). Reactive oxygen gene network of plants. *Trends in plant science*.
104. R.Mittler, S. N. (2012). ROS signaling: the new wave? *Trends in plant science*.
105. R.Munns., & M. Tester. (2008). Mechanisms of salinity tolerance. *Annual review of plant biology*.
106. Rashid, S. C. (2012). . Isolation and characterization of newplant growth-promoting bacterial endophytes. . *Appl. Soil Ecol. 61, 217–224.*
107. Reddy, P. (2014). Potential role of PGPR in agriculture. *Plant Growth Promoting Rhizobacteria for horticultural crop protection*.
108. S. Selvakumar, K. R. (2017). Groundwater quality & its suitability for drinking & irrigational use in South Tiruchirappalli district, Tamil Nadu , India. *Applied water science*.
109. S. Spaepen, S. B. (2014). *Phenotypical and molecular responses of A. thaliana roots as a result of inoculation with the auxin-producing bacterium Azospirillum brasilense.* New Phytologist, 201 .
110. S.H.Wani, N. ,. (2013.). Compatible solute engineering in plants for abiotic stress tolerance - role of glycine betaine. . *Curr. Genomics 14,157–165.*
111. Saia, S. R. (2015.). Soil inoculation with symbiotic microorganisms promotes plant growth and nutrient transporter genes expression in durum wheat. *Front. Plant Sci.*
112. Santi C., B. D. (2013). *Biological nitrogen fixation in non-legume plants.* Ann. Bot. .
113. Sault Toleranace Result. (Fig No.1).
114. Sayyed RZ, P. P. (2015). Plant growth promotion and root colonization by EPS producing Enterobacter sp. RZS5 under heavy metal contaminated soil. .
115. Shinozaki, K.-S. &. (2006). Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Annual review of plant biology*.
116. Singh, M. K. (2015.). Roles of osmoprotectants in improving salinity and drought tolerance in plants: a review.in improving salinity and drought tolerance in plants: a review. *Rev. Environ. Sci.. Rev. Environ. Sci.*
117. Singh, S. S. (2019). Green revolution in India: Success and challenges. *International Journal of Current Microbiology and Applied Sciences, 8(2), 2278-2285*.
118. Soleymani.et.al. (2011).
119. Song, T., Xu, H., Sun, N., Jiang, L., Tian, P., Yong, Y., . . . Cui, G. (2017). *Metabolomic Analysis of Alfalfa (Medicago sativa L.) Root-Symbiotic Rhizobia Responses under Alkali Stress.* Front. Plant Sci.
120. Spaepen S, V. J. (2007). *Indole-3-acetic acid in microbial and microorganism plant signaling.* FEMS Microbiol.
121. Srivastava, S. S. (2020.). Prescience of endogenous regulation in Arabidopsis thaliana by Pseudomonas putida MTCC 5279 under phosphate starved salinity stress condition. *Sci. Rep.*
122. T Mohapatra, P. R. (2022). Indian Agriculture: Achievements and Aspirations. In J. M. H Pathak,

Indian Agriculture after Independence.
123. Tanga. et al. (2017).
124. Timmusk, S. P. (2011). Bacterial distribution in the rhizosphere of wild barley under contrasting microclimates. *PLoS One*.
125. Tucker, M. W.-K. (2015). Research Tool: ethylene preparation: treatment with ethylene and its replacements. *In: Wen, C.-K. (Ed.), Ethylene in Plants. Springer*.
126. U.U.Nwodo, E. A. (2012). Bacterial exopolysaccharides: functionality and prospects. *Int. J. Mol. Sci. 13, 14002–14015.*
127. Upadhyay, S. S. (2011). Exopolysacharraride-producing plant growth-promoting rhizobacteria under salinity condition. *Pedosphere*.
128. V.Jawahar, K. K. (2021). Response of Mung Bean Varieties (Vigna radiata L.) to Application Rates and Methods of Blended NPS Fertilizer at Humbo. . *Agriculture, 11(12), 1220. [doi: 10.3390/agriculture11121220*.
129. Vitousek P.M., M. D. (2013). *Biological nitrogen fixation: rates, patterns and ecological controls in terrestrial ecosystems.* Philos. Trans. R. Soc. B: Boil. Sci.
130. W.Walelign, F. M. (2021). Response of Mung Bean Varieties (Vigna radiata L.) to Application Rates and Methods of Blended NPS Fertilizer at Humbo. *Agriculture*.
131. Walker TS, B. H. (2003). Root exudation and rhizosphere biology. *Plant Physiol 132:44–51*.
132. Walley F, C. G. (2007). *Nitrogen economy of pulse production in the northern great plains,.* Agron J.
133. Wang et al. (2018).
134. Wang et al. (2018).
135. Wang, D. G. (2012). *A nodule-specific protein secretory pathway required for nitrogen-fixing symbiosis.*
136. Wang, F. A. (2019). Ethylene is involved in red light-induced anthocyanin biosynthesis in cabbage (Brassica oleracea). . *Int. J. Agric. Biol. 21, 955–963.*
137. Wani, S. S. (2013.). Compatible solute engineeringin plants for abiotic stress tolerance - role of glycine betaine. *Curr. Genomics 14*.
138. Wu, Y. Z. (2019). Antifungal and plant growth promotion activity of volatile organic compounds produced by Bacillus amyloliquefaciens. *Microbiology Open*.
139. Xie, X. Z. (2022). Impacts of salinity stress on soil fertility and crop productivity in coastal areas of China: A review. *Land Degradation & Development*.
140. Xu G, F. X. (2012). *Plant nitrogen assimilation and use efficiency.* Annu Rev Plant Biol.
141. Zahir, Z. G. (2009). Comparative effectiveness of Pseudomonas and Serratia sp. containing ACC-deaminase for improvinggrowth and yield of wheat (Triticum aestivum L.) under salt-stressed conditions. *Arch.Microbio.*
142. Zheng, B. H. (2017). Long-term nitrogen fertilization decreased the abundance of inorganic phosphate solubilizing bacteria in an alkaline soil. . *Sci. Rep. 7, 1–10.*
143. Zhou C., M. Z. (2016). *Rhizobacterial strain Bacillus megaterium BOFC15 induces cellular polyamine changes that improve plant growth and drought resistance.* International Journal of Molecular Sciences,.
144. Zhu, J. K. (2016). Abiotic stress signaling and responses in plants. *Cell*.
145. Zoellick, R. (2011, January 23). NEWSWEEK .