CHARACTERIZATION OF PLANT GROWTH PROMOTING RHIZOSPHERIC BACTERIA FROM MANGROVES OF GOA

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MINAL MANOHAR BHOMKAR

Seat number: 22P0390007

ABC ID: 562-539-988-352

PR number: 201910472

Under the Supervision of

DR. CHANDA BERDE

Marine Microbiology

School of Earth, Ocean and Atmospheric Sciences

GOA UNIVERSITY

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Examined by:

Seal of the school

DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, "Characterization of plant growth promoting rhizospheric bacteria from mangroves of Goa" is based on the results of investigations carried out by me in the Marine Microbiology at the School of Earth, Ocean and Atmospheric Sciences, Goa University under the supervision of Dr. Chanda Berde and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understood that Goa University or its authorities will not be responsible for the correctness of observations/ experimental or other findings given the dissertation.

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Bhomkar

Minal Manohar Bhomkar

Seat no.: 22P0390007

M.Sc. Marine Microbiology

School of Earth Ocean and Atmospheric Sciences

Date: 02/05/24

Place: Goa University

COMPLETION CERTIFICATE

This is to certify that the dissertation report "**Characterization of plant growth promoting rhizospheric bacteria from mangroves of Goa**" is bonafide work carried out by **Ms. Minal Manohar Bhomkar** under my supervision in partial fulfilment of the requirement for the award of degree of **Master of Science** in the Discipline Marine Microbiology at the School of Earth, Ocean and Atmospheric Sciences, Goa University.

Date: 29/04/24

chanda

Dr. Chanda Berde

Marine Microbiology

School of Earth, Ocean and Atmospheric Sciences

Ghadi 7/11/24

Sr. Prof. Sanjeev C. Ghadi

School of Earth, Ocean and Atmospheric Sciences

Date:

Place: Goa University

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PREFACE

The research carried out for the dissertation titled **"Characterization of plant growth promoting rhizospheric bacteria from mangroves of Goa"**, was basically to understand mangrove bacteria that help plants grow better. These bacteria live in the soil around the roots of plants and provide them with essential nutrients for growth. Bacteria produce special compounds called hormones that stimulate plant growth, helping them to grow taller, develop stronger roots and produce more leaves and flowers. The study was based on characterization and screening of bacteria for various attributes including nitrogen fixation, phosphate solubilization, ammonia production, IAA production, gibberellin, exopolysaccharide and siderophores. The work started with collecting samples from Mandovi estuary of Goa. Through the documentation plant growth promoting rhizospheric bacteria across sampling sites were examined. The documentation of associated rhizospheric bacteria from sampling sites establishes a focus on various attributes of plants. Consequently, plant growth promoting properties of the bacteria including nitrogen fixation, phosphate solubilization, ammonia production, IAA production, gibberellin, exopolysaccharide and siderophores were studied. The methodologies are given in chapter where the observations and results are discussed in chapter 4 with conclusion of plant growth promoting rhizospheric bacteria. Through researching them, we can find out how they promote plant growth and pinpoint the particular strains that work best. These bacteria have ability to protect plants from pathogens, instead of relying on chemicals. By incorporating these plant growth promotion rhizospheric bacteria into their farming practice, organic farmers can enhance soil health, improve crop yields and promote overall sustainability.

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In the successful accomplishment of this dissertation project, I would like to extend my earnest appreciation to all those who have helped me in the completion of my dissertation titled **"Characterization of plant growth promoting rhizospheric bacteria from mangroves of Goa".**

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Bhomkar

Minal Manohar Bhomkar

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ABBRIVATIONS USED

Entity	Abbreviation
Chrome azurol S agar	CAS
Degree Celsius	°C
Exopolysaccharides	EPS
Gibberellin	GA_3
Grams	g
Indole acetic acid	IAA
Kilo gram	kg
Microgram	µg
Milli litre	ml
Milli meter	Mm
Minutes	Min
Nanometer	nm
Optical density	OD
Potential of Hydrogen	pH
Revolutions per minute	rpm
Room temperature	RT

ABSTRACT

The present study focus on the characterization of plant growth promoting rhizospheric bacteria from mangroves of Goa. The aim of this study to find interactions between these bacteria and different types of plants to gain deeper understanding of their relationship and how it can be benefits to the agriculture. The two sampling sites were estimated to find the various attributes including nitrogen fixation, phosphate solubilization, ammonia production, IAA production, siderophore, exopolysaccharides, Gibberellin and screening of enzymes such as amylase, lipases, and cellulase. Divar island and chorao island were selected for collecting samples. The water and sediment samples were collected for bacterial estimation wherein bacterial colonies were isolated, quantified, characterized morphologically by Gram staining and bacterial identification were done. The lab experiments were performed to estimate various attributes and selected strains were checked for plant promotion using mung seeds. The outcomes of this study aims to prove that these rhizospheric bacteria can promote the plant growth by supplying various special compounds such as nitrogen, iron etc.

KEYWORDS

Mangroves, marine ecosystem, nitrogen fixation, enzymes, siderophore, gibberellin.

CHAPTER 1

INTRODUCTION

The population of the globe, which is now estimated to be around 7 billion, is expected to rise to over 8 billion by 2020. It is quite concerning that we may not be able to feed everyone, which will need sustained increase in agricultural productivity. Hence, achieving large yields is now more important than ever for agriculture. Furthermore, the quality, nutritional value, and overall health of food have become more and more important to producers and consumers in recent years (Gamalero and Glick 2011).

Global agricultural practice is shifting towards a more environmentally friendly and sustainable model, driven by rising demand as well as awareness of the harm that excessive use of pesticides and fertilizers can do to the environment and human health (Avis et al. 2008; Leach and Mumford 2008). For instance, between 1998 and 2002, the quantity of land in European Union that is organically farmed grew by about 21% year, and it has continued to grow ever since. The consumption of organic foods climbed by 11% in Italy, the EU member state with the most agricultural producers and the greatest number of hectares dedicated to organic agriculture, in just 2007 alone (https://www.ec.europa.eu/agricuture/organic/eu-policy/data-statistics).

Microorganism found in soil that have positive effects on plant development and health offer a compelling substitute for traditional farming methods (Antoun and Prevost 2005). A growing number of producers have successfully developed, produced, marketed and applied a number of microbial inoculants in recent years (Reed and Glick 2004). While microorganisms inhabit every part of the plant, the primary source of bacteria that benefit plants is the rhizosphere. Generally speaking, these bacteria are known as plant growth promoting bacteria (PGPB) (Bashan and Holguin 1998). Both direct stimulation and biocontrol that is suppressive activity

against soil borne diseases are the usual methods they use to encourage plant development (Glick 1995). PGPB has been shown to stimulate and protect many crops on several occasions in controlled settings and field tests. Numerous soil bacteria have a beneficial influence on plants through a variety of processes, such as increased mineral nutrition, increased plant tolerance to biotic and abiotic stress, altered root growth and suppression of soil borne illness (Glick 1995; Glick et al. 1999; Kloepper et al,1989). Nitrogen fixation, phosphate solubilization, iron sequestration, phytohormone synthesis, plant ethylene level modulation, and control of phytopathogenic microbes are among the bacterial characteristics involved. An overview of primary mechanisms employed by PGPB is given in the (fig 1). The process of improving mineral nutrition to promote plant growth with particular emphasis on nitrogen fixation, phosphate solubilization and iron chelation (Avis et al. 2008).

Mechanism used by plant growth promoting bacteria

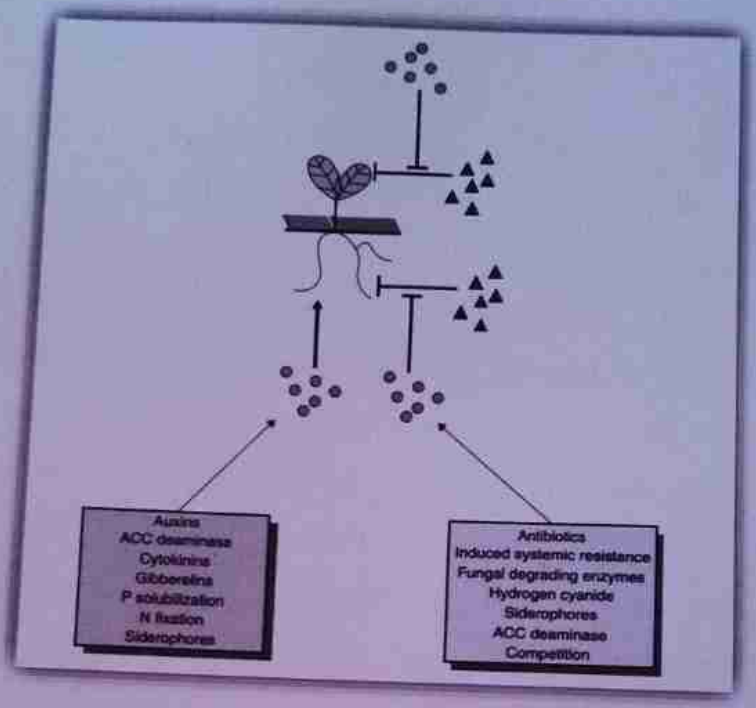


Fig 1.1 : Facilitation of plant growth by plant growth promoting bacteria (PGPB). The PGPB (circles) may either promote plant growth directly (arrow), generally by first interacting with plant roots or indirectly by preventing pathogens (triangles) from damaging the plant. Some of the bacterial traits/mechanisms that contribute to direct and indirect plant growth promotion are highlighted. Similar PGPB traits contribute to the bicontrol of root and leaf pathogens.

Provison of Nutrients

The availability of nutrients such as phosphate, nitrogen, and iron that are insufficiently present in the soil can also result in the stimulation of plant

development by bacteria. The three primary processes are phosphate solubilization, nitrogen fixation, and iron chelation via siderophores .

Phosphate solubilization

The amount of phosphorus (P) usually in soil is between 400 and 1,200 mg kg^{-1} of soil, the concentration of soluble P in soil is typically ~1 mg kg^{-1} or less (Goldstein 1994). P can be found in soil in two main insoluble forms: as orgnic forms such as inositol phosphate (soil phytate), phosphomonoesters, phosphodiesters, and phosphotriesters, and as mineral forms such as apatite, hydroxyapatite, and oxyapatite (Khan et al. 2007). P is one of the elements that is thought to restrict plant growth because it is a necessary macronutrient for plant growth and has a limited bioavailabilty. P is typically given to soils as fertilizers that are produced through very energy intensive procedures in order to meet the nutritional needs of plants (Feng el al. 2004). Plants may only utilize a limited portion of this additional P, though as metal-cation complexes precipitate out 75-90% of it, which quickly settles in the soil (Jeffries et al. 2003). Phosphate solubilizing bacteria (PSB) are responsible for solubilzation and mineralization of P, which is crucial bacterial physiological feature in soil biogeochemicsl cycles an the encoragement of plant growth (Bnayahu 1991; Rodriguez et al. 2004).

The production of low molecular weight organic acids like citric and gluconic acid serves as PSB's primary method for solibilizing inorganic P. These organic acids use their hydroxyl and carboxyl groups to bind phosphate, which chelates cations and causes soil acidification, both of which lead to release of soluble phosphate. The synthesis of chelating agents, inorganic acids , and the release of H^+ have all been linked to the solubilization of inorganic phosphate. Furthermore, by binding free P in the medium and

altering the homeostatis of P solubilization, exopolysaccharides produced by PSB indirectly contribute to the solubilization of tricalcium phosphate.

The production of phosphatases, such as phosphomonesterase, phosphodiesterase, and phosphotriesterase, which catalyze the hydrolysis of phosphoric esters, results in the mineralization of organic P. Futhermore, in the same bacterial strain, P solubilization and mineralization can occur simultaneously (Rodriguez and Fraga 1999).

Iron chelation and Siderophores

Though iron is the fourth most abundant element in the universe, it is primarily precipitated as hydroxides, oxyhydroxides and oxides in a aerobic soils, making very little of it available for assimilation by living things (Ma 2005). The quantity of iron available for assimilation ranges from 10^{-7} to 10^{-23} M at pH 3.5 and 8.5, respectively. Microbes and plants have a high requirement for iron, which is further hughlighted in the rhizosphere where fungus, bacteria, and plants compete with one another for iron (Guerinot and Ying 1994; Loper and Buyer 1991). Cellular iron deficiency in bacteria causes the synthesis of low molecular weight siderophores, which are molecules with an exceptionally high affinity for Fe^{+3}, as well as membrane receptors that can bind the Fe siderophore complex, enabling microorganisms to take up iron in order to survive (Neilands 1981).

Active iron uptake in plants mostly happens via two mechanisms (Curie and Briat 2003). Dicotyledonous and non graminaceous monocotyledonous plants utilize strategy I, which is predicated on the reduction of Fe^{+3} to Fe^{+2} and its movement into root cells as a result of the rhizosphere becoming acidified by H^{+} excretion. Utilizing specific transporter moleules (Robinson et al. 1999;

Marschner 1995; Eide et al. 1996; Vert et al. 2002), Strategy II is applied to grasses and graminaceous plants such as wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), rice (*Oryza sation*), and maize (*Zea mays*). It is based on the synthesisof Fe^{+3} chelators known as phytosiderophores and the uptake of the Fe-phytosideophore complex in root cells (Curie et al. 2001; Von Wiren et al 1994). The interplay between plant and microbially generated chemicals, as well as soil characteristics, governs the movement of iron in the rhizosphere (Robin et al. 2008; Lemanceau et al. 2009).

Siderophore have a dual role in protecting human health and promoting plant development (Robin et al. 2008). When a plant is exposed to environmental stressors such as heavy metal pollution, the role of soil bacteria in imroving plant iron nutrition becomes even more crucial. Microbial metabolites, in particular siderophores, which can bind to iron, magnesium, manganese, chromium (III), gallium (III), cadmium, copper, nickel, arsenic, lead and zinc as well as radionuclides like plutonium (IV) (Malik 2004; Nair et al. 2007), can have impact on the mobility of metals in soil. Additionally, siderophores may lessen the pressures that hugh soil concentrations of heavy metals impose on plants by providing iron to the plants (Diels et al. 2002; Belimov et al. 2005; Braud et al. 2006).

Nitrogen Fixation

The use of chemical sources of nitrogen which are obtained at the expense of petroleum, has increased in agricultur. In addition to being expensive, the manufacture of chemical nitrogen fertilizers pollutes the environment and depletes nonrenewable resources. An economically and environmentally sound option would be to use biological nitrogen fixation in addition to, and eventually in instead of, mineral fertilizers. Nitrogen is abundant in the

atmosphere, but before it can be digested by plants and used as a building block of proteins, nucleic acids and othe biomolecules, it must first be converted to ammonia (Bockman 1997). The most significant microorganisms currently employed in agriculture to increase plant nitrogen content are variety of Rhizobia, each of which is unique to a certain group of plants. As bacterial inoculants, other nitrogen fixing bacteria, most notably *Azospirillum* spp., are also used; however, it is generally believed that fixed nitrogen supply represents just a very minor portion of what free living bacteria accomplish for the plant (James and Olivares 1997).

The nitrogenase enzyme, which is a two componet metalloenzyme made up of (a) dinitrogenase reductase, a dimer of two identical subunits that contains the sites for mgATP binding and hydrolysis and provides the reducing power to the dintrogenase and (b) the dinitrogenase component that contains a metal cofactor, is used by nitrogen-fixing (diazotrophic) bacteria to fix atmospheric nitrogen. It would be advantages if rhizobial carbon resources were directed toward oxidative phosphorylation, which results in the synthesis of ATP, rather than glycogen synthesis, which leads in the storage of energy as glycogen, as nitrogen fixation takes a significant quantity of ATP (Dean and Jacobson 1992).

Modulation of Phytochrome Levels

Auxins, cytokinins, gibberellins, ethylene, and abscistic acid (ABA) (Salisbury and Ross 1992) are phytohormones that are important in cotrolling the growth and development of plants. Plants often lack sufficient quantities of endogenous phytohormones to grow to their full potential when exposed to unfavorable environmental conditions (De Salamone et al. 2005). In vitro , a variety of rhizosphere bacteria generate or modify phytohormones. As a

result, the hormonal balance of the plant may be impacted by numerous PGPB that have the capacity to change phytohormone levels. The effects of PGPB's synthesis of gibberllins, cytokinins, and IAA on plant growth. The regulation of ethylene production by bacteria that produce ACC deaminase and their function in promoting plant growth in both natural and stressful environments.

IAA

Auxins affect plant cell and tissue division, extension and differentiation in addition to promoting the germination of seeds and tubers, speeding up the development of xylem and roots, regulating vegetative growth processes, intiating lateral and accidental roots, mediating responses to light and gravity, florescence and fructification of plants, as well as photosynthesis, pigment formation, biosynthesis of various metabolites, and resistance to stressful environments (Tsakelova et al. 2006). While a number of auxins found in nature have been identified the most researched auxins is indole-3-acetic acid (IAA), and the terms auxin and IAA are often used interchangeably. Nonetheless, the majourity of IAA in plants is typically found in conjugated forms, which are primarily involved in IAA catabolism transportation, storage and preservation (Seidel et al. 2006).

Plant responses vary depending on the kind of plant, the specific tissue involved, and the stage of development of the plant. Various auxin concentrations have different impacts on plant physiology. Plant species and the sensitivity of the plant tissue to auxin determine the real range of effective auxin concentrations; levels below this range have no impact, while higher concentration impede growth. It is estimated that approximately 80% of soil bacteria are capable of producing auxin. Numerous types of soil bacteria, including streptomycetes, methylobacteria, cynobacteria, and archaea, have

been found to possess the ability. While some of these microbes are free-living or symbiotic PGPB, others are engaged in plant pathology. The improvement of lateral and accidental rooting, which results in better mineral and nutrients uptake and root exudatation and in the promotes bacterial proliferation on the roots is one of the primary consequences of bacterial IAA.

Gibberellins and cytokins

Numerous research studies have demostrated that a wide range of soil bacteria, with PGPB in particular are capable of producing both gibberellins and cytokinins. As a result, certain strains of *Bacillus subtilis, Pantoea polymyxa, Rhizobium* spp., *Rhoospirillum rubrum, Azotobacter* spp. and *Pseudomonas fluorescens* have been found to produce cytokinins in their cell-free medium. Furthermore, it has been documented that certain PGPB that produce gibberellin or cytokinin can promote plant growth. Neverthless, a thorough knowledge of the function of hormones produced by bacteria as well as the regulation of bacterial production of these plant harmones is presently lacking. Therefore, a large portion of our understanding of the function of cytokinins and gibberellins produced by bacteria is derived from investigations on plant physiological responses to exogenous administration of pure hormones to developing plants. Lastly, certain phytopathogen strains have the ability to produce cytokinins. On the other hand, it seems that PGPB create fewe cytokinins than phytopathogens, which explains why PGPB has a stimulatory effect on plant growth whereas pathogen-produced cytokines have an inhibitory effect (Glick 2012).

Antibiotics and Lytic Enzymes

The manufacture of various antibiotics is a characteristic of PGPBs that is commonly linked to the bacterium's capacity to inhibit the growth of plant diseases, primarily fungus. A number of these antibiotics, together with their mechanisms of action and degree of specificity, have been throughly investigated; some of these biocontrol strains have even gone on sale. An issue with overly relying on bacteria that produce antibiotics as bicontrol agents is that some phytopathogens may become resistant to particular antibiotics as a result of the increased use of these strains. Some biocontrol bacteria produce enzymes including chitinase, cellulases, β-1,3 glucanases, proteases, and lipases that can lyse a portion of cell walls of many pathogenic fungi (Glick 2012).

1.1 BACKGROUND

Mangroves are incredible ecosystem that support a wide range of bacterial species, including those that promote plant growth. These plant growth promoting bacteria play a vital role in enhancing soil fertility, nutrient uptake, and disease resistance in plants. By harnessing the agricultural practices and increase crop productivity. These bacteria form a beneficial symbiotic relationship with plants, providing them with various benefits. One of the key ways these bacteria help plants is by fixing atmospheric nitrogen into a form that plants can use. Nitrogen is an essential nutrient for plant growth and these bacteria help in converting atmospheric nitrogen into a usable form, promoting healthier and greener plants. Additionally, plant growth promoting bacteria can also enhance plant tolerance to abiotic stresses such as drought, salinity, and extreme temperatures. They can help to improve water and nutrient uptake, increase root growth and stimulate the production of stress-respective compounds in plants. This can be particularly beneficial in region facing challenging environmental conditions. Overall, the use of plant growth promoting bacteria in agriculture holds great promise for sustainable and environmentally friendly farming practices.

1.2 AIM AND OBJECTIVE

AIM: To find interactions between these bacteria and different types of plants to gain deeper understanding of their relationship and how it can be benefits to the agriculture.

OBJECTIVE:

- To isolate plant growth promoting bacteria from mangroves ecosystem.
- Morphological and Gram characteristics of bacterial isolates.
- Screening of PGPR for various attributes: nitrogen fixation, phosphate solubilization, Ammonia production, production of IAA, gibberellin, exopolysaccharide (EPS), siderophores.
- Screening of enzymes: lipase, cellulase, amylase.
- Characterization of IAA and EPS.
- To promote growth of mung bean plants using selected strains of PGPR.
- Biochemical identification of selected strains.

1.2 HYPOTHESIS/ RESEARCH QUESTION

HYPOTHESIS:

- In order to characterize the rhizospheric bacteria from Goan mangroves that promote plant growth, my theory is that these bacteria are essential to the growth of plants.
- Through researching them, we may find out how they can promote plant growth and pinpoint the particular strains that work best.

RESEARCH QUESTION:

- The research question in the characterization of plant growth promoting rhizospheric bacteria from mangroves in Goa is to identify and understand the specific bacterial strains that have the potential to enhance plant growth in this unique ecosystem.
- By focusing on the mangroves of Goa, we aim to address the gap in knowledge regarding the diversity and functional capabilities of these bacteria and their impact on plant growth.
- This research question will help us uncover the potential applications of these bacteria in sustainable agriculture practices.

1.4 SCOPE

- Investigating the diversity of these bacteria across different plant species and environments.
- Studying the impact of environmental factors on the bacterial-plant interactions.
- Exploring the potential for biofertilizers and biocontrol agents derived from these bacteria.
- Assessing the long term effects of these interactions on soil health and ecosystem sustainability.
- Investigating the role of these bacteria in enhancing plant resilience to abiotic stresses such as drought or salinity.
- Collaborating with farmers and agricultural organizations to implement and validate the practical applications of this research.

CHAPTER 2

LITERATURE REVIEW

Mangrove habitats, which are found in the area where land and sea meet, have the potential to provide biotechnological resources. Brazil has one of the world's largest mangrove ecosystem along its vast coastline. According to Castro, samples of mangrove forests were previously taken from the Brazilian state of Sao Paulo. Three locations were evaluated:(A) the oil contaminated Bertioga location; (B) the uncontaminated Bertioga location with anthropogenic affects; and (C) the uncontaminated Cananela location with minimal anthropogenic consequences. In this study three different plant species—*Rhizophora melega*, *Laguncularia racemosa*, and *Avicennia nitida*—were shown to harbor endophytic bacteria. These isolates, totaling 115, were assessed for their capacity to solubilize phosphorous and fix nitrogen. The amount of indole acetic acid synthesis in bacteria that passed both of these tests was investigated further. After choosing two strains with high levels of indole acetic acid synthesis to utilize as reforestation tree inoculants, the plants' development was assessed in a field setting. Phosphorus solubilization index was low in P*seudomonas fluorescens* (strain MCR1.10) and high in *Enterobacter* sp. (strain MCR1.48), the other strain of bacteria utilized. *Acacia polyphylla*, a reforestation tree, was employed. The results show that the MCR148 endophyte successfully support the plant's growth and fitness, which can be used in the production of *Acacia polyphylla* seedlings. The inoculation of this strain resulted in an increase in *Acacia polyphylla* shoot dry mass. Consequently, by carefully examining the biotechnological potential of mangrove endophyte isolates with an emphasis on promoting plant growth, we were able to identify a strain that might supply a restricted amount of nutrients and hormones to aid in plant growth (Castro, Dourado et al., 2017).

Enzymes have been extracted and refined form plants, animals, and microorganisms; of these, microorganisms are the most widely used source of enzymes due to their wide range of biochemical variety, ease of mass growth, and simplicity in genetic modification. Because microbial enzymes are more stable and active than similar enzymes generated from plants or animals, marine microorganisms are becoming increasingly popular as a source for novel enzymes. Enzymes with unique features are becoming more and more in demand as biotechnology has advanced. The significant differences between the enzymes produced by marine microorganisms and homologous enzymes from terrestrial microorganisms may be attributed to the complexity of the marine environment, which includes high salinity, high pressure, low temperature, and unique lightning conditions. These enzymes find application in fine chemicals, food additives, and medications. Researchers have recently extracted a wide range of unique-activities enzymes from fungus, actinomycetes, marine bacteria, and other marine microorganisms; some of these compounds have already found industrial usage. Specifically, a sizable number of potential drugs have been discovered. Since they are still in the early stages of discovering their vast genetic and biochemical diversity, marine microorganisms are currently gaining a lot of attention as a novel and potentially useful source of enzymes (Zhang and Kim, 2010).

Every plant tissue contains phytomicrobiome microbes, which together with the plant make up the halobiont. Plants carefully control the make-up and activity of the bacterial population they are linked with. In exchange for the plant's many functions and advantages, these microorganisms give the microbial community additional metabolites and reduced carbon. In general, soils are moist environment with high reduced carbon content that sustain

large soil microbial populations. This paper provides an update on the use of PGPR in agriculture, covering their acquisition, processing, and sale as inexpensive commercial inputs. We begin by outlining the notion and function of the phytomicrobiome as well as the agricultural setting that supports food security in the twenty-first century. Subsequently, the process by which PGPR promotes plant growth are examined. These mechanisms involve the exchange of signals between plant roots and PGPR, and they also influence plant responses to antibiotic stress by inducing systemic resistance. On the application front, methods for enhancing PGPR inoculant colonization of the rhizosphere are covered. The uses of PGPR in 21st – century agriculture and the path towards the commercialization of PGPR-based technologies are covered in the paper's concluding sections (Backer, Rokem et al., 2018).

Rhizobacteria that support plant growth are soil bacteria that live on or on the surface of roots. They have a directed or in directed role in plant growth and development by producing and secreting a variety of regulating compounds in the rhizosphere. Rhizobacteria that promote plant growth generally work two ways: either directly by aiding in the uptake of resources (nitrogen, phosphorus, and essential minerals) or indirectly by regulating plant hormone levels or by lessening the negative effects of different pathogens on plant growth and development in the form of biocontrol agents. Plant Growth Promoting Rhizobacteria have been shown in numerous studies to improve the health and production of various plant species in both stressed and normal environments. Plant-beneficial rhizobacteria have the potential to reduce the world's reliance on dangerous agricultural chemicals that upset agro-ecosystems. The current ideas on the rhizosphere and plant growth-promoting rhizobacteria are highlighted in this review. Furthermore, with current

advancement and research, precise outlooks on the many mechanisms of rhizobacteria-mediated plant growth promotion have been detailed in depth. In order to highlight current trends and facilitate the development of future insights, the most recent paradigms about the applicability of these beneficial rhizobacteria in various agro-ecosystems have been thoroughly presented under both normal and stressful settings (Ahemad, Kibret, 2013).

Plant growth may be positively impacted by the microbe-plant interaction in the rhizosphere, or it may be neutral, variable, or harmful. Plant growth-promoting rhizobacteria are the rhizobacteria that positively impact plant development. Bacteria that actively invade the rhizosphere are referred to as rhizobacteria. Fourteen soil bacterium isolates, including two identified plant Azotobacter and growth-promoting rhizobacteria (PGPR) strains *Bacillus cereus UW85* and *vinelandii Mac259* were investigated in vitro. The parameters that were evaluated included the synthesis of indole acetic acid (IAA), phosphate solubilization, dinitrogen fixation, and manufacture of siderophores (Fe-III chelating agents). Ferric chloride was used in a colorimetric test to measure IAA formation. catalyst for perchloric acid. Solubilization of phosphate and siderophore plating the bacteria in order to qualitatively test the production Chrome azurol S agar and Pikovskaya agar, respectively. The capacity the nitrogenase activity of was used to calculate the fixation of dinitrogen. using gas chromatography, the bacterium. The outcomes demonstrated that IAA was generated by twelve isolates, ranging from 2.09 to 33.28 µmol ml1. Four isolates showed a favorable ability to solubilize precipitated phosphate (BS 58, BTS, TCaR 61, and BTCaRe 65). Mac 259 was one of the seven isolates that produced a positive siderophore. The isolates were all devoid of nitrogenase activity. Only one isolate (TS 3)

showed no signs of any of the characteristics examined. It was discovered that the reference strain Mac 259 and isolate TCeRe 60 both have characteristics that produced siderophores and IAA. In addition to generating siderophores and IAA, four P-solubilizing bacteria (BS 58, BTS, TCaR 61, and BTCaRe 65) were also shown to be. Additional research is required to determine whether using these PGPR isolates can improve plant development. The purpose of this study was to evaluate the ability of twelve soil bacterial isolates-IAA, phosphate solubilizers, nitrogenase, and siderophore (Fe-III chelating agent) to provide plant growth promotion (Husen, 2003).

Many bacteria that live in environments where iron availability is limited release ferric iron-specific ligands, also known as siderophores, which help sequester and move iron. Here, they describe how certain plant growth-promoting rhizobacteria produce siderophores. The production of siderophores by rhizobacteria that promote plant development was identified by the use of the chrome azurol S assay, a universal siderophore test that is not dependent on siderophore structure. Recent studies have demonstrated these chemicals' role in promoting plant development. Plant growth promoting rhizobacteria (PGPR) are root colonization bacteria that either directly or indirectly promote plant growth. Through enhanced uptake of nitrogen and phosphorus, the synthesis of chemicals that stimulate plant growth, and the uptake of iron through siderophores, they aid in the growth of many annual crops. Siderophores with higher binding potentials are produced by PGPR. Ten PGPR isolates have been examined for siderophore synthesis in the current investigation (Gupta and Gopal, 2008).

Beneficial microorganisms called halotolerant plant growth promoting rhizobacteria (PGPR) are used to lessen biotic and abiotic pressures on plants.

In this study samples of bacteria were extracted from the Sundarban mangroves' saline soil. Twenty of the 156 bacterial samples that were identified has their potential for salt tolerance examined. Using morphological, biochemical, and molecular methods, these isolates were described. They were divided into four taxa based on 16s rRNA sequencing, including *Arthrobacter* sp. (01 isolate), *Pseudomonas, Bacillus* (17 isolates), *Kocuria rosea* (01 isolation), and *plecoglossicida* (01 isolate). The halotolerant isolates with characteristics that promoted plant growth were chosen, such as the creation of ammonia, phosphate and zinc solubilization, indole acetic acid synthesis, and siderophore. Additionally, in high salinity conditions, the impact of two halotolerant isolates, GN-5 and JR-12, which displayed the most notable PGP activities, was assessed in pea plants. When compared to non-inoculated control plants, the isolates increased survivability by encouraging germination (36 to 43%), root-shoot growth, and pea plant weight. Both of these halo-tolerant isolates shown antagonistic activity against *Macrophomina phaseolina* (Tassi) Goid NAIMCC-F-02902, the pathogen that causes aggressive root rot disease, in a later dual culture confrontation assay. The isolates that were discovered may have an antagonistic effect on the disease known as root rot and be employed as prospective bioagents for saline soils. Many bacterial isolates that are salt-tolerant have been obtained from the saline Sundarban mangrove ecosystem. The characterization of these isolates provides information about the activity of particular isolates that promote plant growth and impart salinity resistance during the germination of pea seeds and the growth of seedlings. The generation of siderophores, zinc solubilization, phosphate solubilization, and IAA production are among the salinity-tolerant PGPR's key traits that these investigations have revealed. Due to the existence of enzymatic activities,

these isolates additionally demonstrated biocontrol capability and resistance against the aggressive soil pathogenic fungus and phaseolina that cause the illness known as root rot in legumes. This can also be utilized to create bio-formulations that target other crops that are stressed by salt by using these isolates (GN-5 and JR-12). (Mishra, Sahu et al., 2023).

In recent times, there has been a surge in agricultural output to meet the worldwide need for food, which is being driven by population growth. Artificial fertilizers, which have multiple negative consequences on human and environmental health, are a major component of conventional farming techniques. Because of this, environmentalists and sustainability academics have focused more on alternative crop fertilization methods. Biofertilizers are microbial mixtures made up of naturally occurring rhizobacteria that promote plant growth (PGPR). PGPRs can either directly or indirectly stimulate plant growth by solubilizing nutrients in the soil, producing hormones that stimulate plant growth, and producing siderophores, which are metabolites that sequester iron. This paper critically advocates for the continued use of PGPR biofertilizers in sustainable agricultural production while also evaluating their history and current use. As a result, it refreshes our knowledge of how PGPR biofertilizers have evolved in crop productivity. This kind of information can help assess their potential and eventually open the door to more widespread usage. It is helpful to establish sustainable agriculture systems to have this conversation. Even though these bioresources have been used sparingly in various parts of the world, the findings are encouraging, and more research and development could increase their effectiveness (Aloo, Tripathi et.al, 2022).

Plant health, production, and soil fertility are all determined by interactions between microbes and plants in the rhizosphere. Plant growth-promoting bacteria (PGPBs) are bacteria that, through a variety of mechanisms, can protect plants from abiotic stresses and disease. Those bacteria that form close associations with plants, like endophytes, may be more effective in promoting plant growth. Plant growth promotion (PGP) features can be used to evaluate a number of significant bacterial traits, including biological nitrogen fixation, phosphate solubilization, ACC deaminase activity, and siderophore and phytohormone synthesis. This paper provides a summary of the significance of soil-plant-microbe interactions for the creation of effective inoculants, given that PGPB are well-researched microorganisms that comprise a wide range of readily available beneficial bacteria. As part of the search for new technologies for agricultural crops, a variety of methodologies are combined with these bacteria, including the identification of traits that promote plant growth, the identification of bacterial strains, as well as assays of seed inoculation in laboratory conditions and cultivation experiments in the field. Consequently, several genera, including *Azospirillum, Bacillus,* and *Rhizobium*, may be the main contenders when this search reveals a prospective bacterial inoculant that is suitable for reintroduction in the environment. Lastly, the development of novel and effective inoculants for agriculture depends on the hunt for beneficial bacteria. Investments in technology that can help boost the inoculum's efficiency and the bacterial adhesion rate to the seeds, two other critical elements for a successful inoculation are also significant. Therefore, adding helpful bacteria to the soil is a sustainable agronomic strategy that can lower production costs because it tends to be less aggressive and environmentally harmful than chemical fertilization (de Souza, Ambrosini et al., 2015).

Naturally existing soil bacteria known as plant growth-promoting rhizobacteria (PGPR) actively invade plant roots and aid in the growth of plants. Early agricultural plant inoculation with specific PGPR strains increases biomass production by directly influencing the growth of the roots and shoots. A variety of effects on early-season plant growth may arise from inoculating ornamentals, forest trees, vegetables, and agricultural crops with PGPR. These effects include the improvement of seedling germination, stand health, plant, plant height, shoot weight, nutrient content of shoot tissues, early bloom, chlorophyll content, and increased nodulation in legumes. Numerous pathways have been described by PGPR to affect growth, yield, and nutrient uptake. They generate plant hormones, support other beneficial bacteria or fungi, boost nitrogen fixation in legumes, encourage free-living nitrogen-fixing bacteria, increase the supply of other nutrients, such as phosphorus, sulfur, iron, and copper, control bacterial and fungal diseases, and aid in the management of insect pests. PGPR has attracted a lot of research interest, and more and more PGPR are now being sold commercially for a variety of crops. A number of reviews have covered particular facets of PGPR's growth promotion. They have covered a variety of microorganisms that function as PGPR in this review, along with their processes and advantageous traits. Capacity to control the rhizosphere to improve the survival and competitiveness of these advantageous microbes will determine the success of these products. In addition to inoculant formulation and delivery, rhizosphere management will necessitate taking crop cultural practices and soil conditions into account (Saharan, Nehra, 2011).

Low molecular weight substances known as siderophores are secreted by bacteria in low-iron environments. In comparison, the marine environment

has very low quantities of soluble iron. As a result, in the diluted environment, marine bacteria secrete a variety of siderophores that aid in their competition for iron. The current investigation details the isolation of bacteria that produce siderophores in the marine environment. Three isolates (S31, S34, and W16) out of the 48 collected were determined to be strong siderophore producers. Using the CAS assay, the siderophore production was verified. These isolates are identified by molecular analysis, biochemical analysis, and morphological analysis as belonging to the genera *Bacillus* and *Pseudomonas*. Thus, the current work offers a helpful starting point for future research on siderophore synthesis in marine environments. Our research offers Solid proof that marine bacteria make siderophores in order to meet their iron requirements. Marine siderophores contribute significantly to the maintenance of the iron cycle in aquatic habitats because of their capacity to chelate iron. Along with helping to create biosensors and biofilms, these siderophores can also aid in the development of medicinal medications. The goal of this study was to isolate and characterize strong marine bacteria that produce siderophores, which may be used to stimulate plant growth in saline-sodic coastal environments (Uchgaonkar, Padmadas et al.,2018).

It is well known that soil bacteria found in root zones promote plant development. Either direct or indirect mechanisms mediate this positive effect. The generation of plant hormones like auxins and the availability of biologically fixed nitrogen are frequently cited as the causes of the direct impacts. The suppression of bacterial, fungal, and nematode pathogens as well as the generation of volatile metabolites, ammonia, siderophores, and antibiotics are the indirect consequences. The potential for certain bacteria found on mangrove roots to aid in the growth of mangrove seedlings is

discussed in this research. From the rhizosphere soil of a mangrove species (*Rhizophora mucronata*), 48 bacterial strains were identified. The strains' capacities to (a) fix nitrogen, (b) solubilize phosphate, (c) create ammonia, and (d) manufacture the plant growth hormone (IAA) were examined. They also examined the impact of the strains on *R. mucronata* shoot growth. It was determined that *Azotobacter vinelandii* and *Bacillus megaterium* were the two strains that caused the development of mangrove seedlings to double. This makes it more likely that the bacterial species can be used to promote the growth of mangrove seedlings (Kathiresan and Selvam, 2006).

Endophytes are microorganisms that live inside the living tissues of plants and do not immediately cause harm to the plants. It is interesting to note that every one of the roughly 300,000 plant species that call the earth home is a host to endophytes. Only a small number of endophyte species up to 1 million, according to estimates have been fully characterized. The endophytic microorganism's ability to fix nitrogen, produce phytohormones, biocontrol phytopathogens in the root zone, or improve nutrient and mineral availability can all contribute to the host plants growth stimulation. The current study Investigated the bioprospecting properties of endophytic bacteria that were isolated from the leaves of two types of salt-marsh plants and five mangroves. 104 distinct bacterial strains were obtained from leaf samples of mangrove plants in the Tamil Nadu district of Pichanvaram. A total of thirty-six isolates with rapid growth were chosen and their biological activities were examined. 36 isolates in total 28 isolates (77%) had antibacterial activity, 94.4% had pectinase activity, 58.3% had protease activity, and 52.7% had inulinase and invertase activity. 22 (61.1%) and 25 (69.4%) endophyte isolates respectively, shown plant growth-promoting activity, such as the synthesis of ammonia and

acetoin; 26 isolates (72.2%) demonstrated nitrogen-fixing activity. Seven isolates (19.4%) generated indole acetic acid (IAA), while six isolates (16.6%) demonstrated phosphate solubilization activity. Additionally, it was shown that 12 (33.3%) and 20 (55.5%) endophytic bacterial isolates had malachite green and phenol degrading capabilities, respectively. While ~31 isolates (86.1%) were resistant to *vancomycin* and *bacitracin*, more than 20 endophytic bacteria were responsive to drugs like streptomycin and trimethoprim. Thirty-four (94.4%) and thirty-six (86.6%) endophytic isolates showed growth on media having concentrations of up to 7.5 and 10% NaCl, respectively. Further research is warranted as the current work has demonstrated that endophytic bacteria with the potential for bioprospecting can be found in mangroves. In the current investigation, 104 bacterial colonies were found in the leaves of mangrove and salt marsh plants. On the other hand, there are no noteworthy reports of endophytic bacteria found in mangroves. Compared to rhizospheric bacteria, endophytic bacteria typically exist at lower population densities 25. Thirteen plant samples containing 36 bacterial isolates were chosen for additional analysis based on discernible morphological variations (Gayathri, Saravanan et al., 2010).

Thousands of microbial species depend on the vital habitat that mangrove sediments provide. Biofilms, or surface attached aggregates encased in an extracellular polymeric matrix, are home to microorganism. Ten distinct bacterial isolates were employed in this investigation, and soil samples were procured from mangrove regions located in Nijampatnam, Andhra Pradesh. Nutrient agar medium was used for the isolation processes (NAM). The bacterium that formed biofilms was isolated using marine water. PVC pipes were used to create a biofilm-forming apparatus together with sterile glass

slides. After a month, ten morphologically distinct bacteria were isolated from the glass slides scraped from the apparatus. Each of these isolates completed morphological characterization. The ten isolates that are currently available have distinct morphologies and include *Pseudomonas, Staphylococcus, Micrococci, Klebsiella* sp.1, *Klebsiella* sp.2, *E. coli*, *Enterobacter, Proteus* sp.1, *Proteus* sp.2, and *Citrobacter*. It was found that *Proteus* sp.1 and Enterobacter had maximum colony sizes of 8 mm. EPS and biofilm formation efficiency were monitored over a 24- to 96-hour incubation period. Maximum generation of biofilm (98 mg/100 ml) and exopolysaccharide (96 mg/100 ml) in *Enterobacter* was seen after 72 hours of incubation. Antimicrobial compounds can be present in high concentrations in biofilms. The possibility that bacterial biofilm contributes to the pathophysiology of disease has prompted a greater emphasis on identifying illnesses that may be biofilm-related. It is clear that after 48 hours of incubation, the marine isolates, including *Pseudomonas, Klebsiella* sp.1, *Klebsiella* sp.2, *Enterobacter*, and *Proteus* sp., produced the highest amounts of EPS and biofilm. The detection of EPS and biofilms in this study was made possible by the use of the tube technique and CRA. My findings indicate that more research is required to determine whether biofilm exopolysaccharides have industrial potential (Venkatalakshmi, 2022).

Understanding heterotrophic bacteria and their activities in the mangrove environment is crucial. This includes knowledge of their production, abundance, dispersion, and role in the cycling of nutrients as well as their position at the base of the microbial food web. Screening was done for total heterotrophic bacteria (THB) from sediment from Pitchavaram mangroves. Eight isolates, including *Bacillus subtilis, Streptococcus* sp., *Staphylococcus*

sp., *Pseudomonas* sp., *Photobacterium* sp., *Enterobacteriaceae* sp., *Escherichia coli*, and *Azotobacter* sp., were chosen based on the morphology of the colonies and their identification by phenotypic and biochemical characteristics. Exopolysaccharide (EPS) production was assessed in all isolates, and it was shown that *Pseudomonas* sp. And *Azotobacter* sp. Produced a significant amount of EPS. The current study came to the conclusion that the Total Heterotrophic Bacterial (THB) community in the Pitchavaram mangrove sediment may be characterized using phenotypic and biochemical analysis, which can also help us understand how these ecosystems function. In addition to their pattern of distribution and role in the cycling of nutrients, they also function as a tool for the biodegradation of nutrients in the food web. It was discovered that *Pseudomonas* sp. (Isolate 1) and *Azotobacter* sp. (Isolate 6) were more effective in making EPS, and they underwent additional analysis to produce alginate (Lakshmipriya and Sivakumar, 2012).

The rhizosphere zone of plants is home to a vast array of microorganisms, including bacteria, which can both positively and negatively affect plant development. The presence of rhizobacteria that stimulate plant growth (PGPR) is responsible for the positive result. This work involved the genetic identification of a bacterial strain that was isolated from the lupin rhizosphere as *Serratia marcescens* (OK482790). A number of genetic and biochemical traits were validated both in vivo and in vitro to establish the OK482790 strain's potential for PGPR. The in vitro findings showed that several lytic enzymes (protease, Lipase, cellulase, and catalase), antimicrobial substances (siderophores, hydrogen cyanide), ammonia, nitrite, and nitrate, as well as the latter's capacity to convert nitrate into nitrite. For the strain OK482790, an in

vitro and in silico screening suggested a potential denitrification-DNRA-nitrification pathway. The nitrite and nitrate genes that encode the Nar membrane-bound sensor proteins (NarK, NarQ, and NarX) were found by the genome screening. Together with nitroreductases (NTR) and numerous oxidoreductases, nitrate and nitrite reductase expressing genes (NarI, NarJ, NarH, NarG, and NapC/NirT) and (NirB, NirC, and NirD) are also discovered. The in vivo findings on wheat seedlings verified that the soil inoculation of OK482790 strain greatly enhanced the growth of the seedlings. According to this study, *S. marcescens* OK482790 participates in the nitrogen cycling process through the denitrification-DNRA-nitrification pathway. It also has the capacity to produce a number of compounds and enzymes that contribute to the advantageous role of plant-microbe interactions in maintaining plant growth and development for a safer environment (Hamada and Soliman, 2023).

Inoculants known as Plant Growth Promoting Rhizobacteria (PGPR) are frequently employed to increase the growth and productivity of agricultural crops. Thus, it is crucial to screen for the selection of efficient PGPR strains. In this investigation, 180 bacteria were isolated from the rhizosphere of various wild plants that were gathered in the Turkey's Erzurum and Kırsehir regions. Tests were conducted to determine the nitrogen fixing (NF) and the capacity of phosphate solubilizing (PS). Sixteen isolates (named AS1, AS2, AS3–AS16) were discovered to contain varying degrees of both NF and PS potential. Additionally, the auxin (IAA) and ACC deaminase production potentials of these 16 isolates were examined. A pot and a field experiment were carried out to look at how PGPR isolates affected the growth of spring wheat (*Triticum aestivum L.*). Only the six most successful isolates were

evaluated in field trials based on the outcomes of the pot tests, and certain growth and yield metrics were examined after harvest. Were examined. The findings indicated that the six isolates (AS1, AS3, AS4, AS6, AS8, and AS15) significantly ($P<0.05$) improved plant height, dry matter content, and protein content. These successful isolates were identified as *Cellulomonas turbata* (AS1), *Pseudomonas putida* (AS3), *Bacillus cereus* (AS4), *Enterobacter cloacae* (AS6), *Bacillus megate-rium* (AS8), and Bacillus megaterium (AS15) based on a few culture, cytological, and fatty acid profiles. Thus, it may be concluded that introducing these strains into wheat may result in higher yields as well as a possible replacement for expensive nitrogen-phosphate fertilizer in wheat production (Sezen, Ozda, 2016).

Given that Plant-N remobilization is closely linked to plant senescence, plant growth-promoting rhizobacteria (PGPR) may offer a biological substitute for fixing atmospheric N2 and delaying N remobilization in maize plants to boost crop production. Thus, out of a number of bacterial strains isolated from maize roots at two locations in Malaysia, four PGPR strains were chosen. Plant growth promotion tests and biochemical plant growth-promoting (PGP) capabilities were used to screen the PGPR strains in vitro. The reference strain utilized was *Bacillus subtilis* UPMB10. These bacteria were identified as *Klebsiella* sp. Br1, *Klebsiella pneumoniae* Fr1, *Bacillus pumilus* S1r1, and *Acinetobacter* sp. S3r2. Through in vitro studies, N2 fixation, phosphate solubilization, and auxin production were found to be positive in all PGPR strains. The N2 fixation abilities of PGPR in association with maize were assessed using the 15N isotope dilution technique in a greenhouse experiment with reduced Fertilizer-N input (a third of recommended Fertilizer-N rate) at two harvests, namely, prior to anthesis (D50) and ear harvest (D65). The

findings showed that PGPR inoculation had an impact on the dry biomass of the top, root, and ear, total N content, and bacterial colonizations in the non-rhizosphere, rhizosphere, and endosphere of maize roots. Specifically, the *B. pumilus* S1r1-inoculated plants performed better overall than the other treatments. With 30.5% (262 mg N2 fixed plant−1) and 25.5% (304 mg N2 fixed plant−1), they produced the maximum N2 fixing capacity of maize top at D50 and D65, respectively, of the total N requirement. In maize, PGPR inoculation delayed N remobilization and plant senescence, a sign of increased grain yield. Significant associations between PGPR strains and harvest time for metrics related to N uptake and at. % 15Ne of tassel show this. The reduced N content in the tassels of maize treated with PGPR, specifically B. pumilus S1r1, K. *pneumoniae* Fr1, B. subtilis UPMB10, and *Acinetobacter* sp. S3r2 at D65 harvest, further supports the phenomena. This work shows that B. pumilus S1r1 PGPR inoculation can biologically fix atmospheric N2 and offer a different method (apart from plant breeding) to delay N remobilization in maize plants, resulting in better ear yield (up to 30.9%) with less Fertilizer-N input (Kuan, Othman et al., 2016).

CHAPTER 3

METHODOLOGY

Sampling

The rhizosphere soil sample and water sample collected across four corners of the mangroves of Saint Estevam Island (15.523415°N and 73.933774°E) Betqui Goa, Chorao (15.513077°N and 73.870444°E) Goa and Britona, Alto Porvorim (15.518436°N and 74.84641°E) Goa. The soil samples were collected under the root surface of the mangrove plant and transferred aseptically into laboratory using polythene bags, also the collected water samples were filled in plastic bottles.

Viable count and Isolation of cultures

A five -fold serial dilutions from 10^0 to 10^{-8} was done to reduce bacterial concentrations to a required concentration for the test method. 1ml of aliquot of the stock solution (soil sample) was added to the labelled tube 10^0 which contain 4 ml of 0.85% normal saline. 1ml of aliquot from 10^0 was transferred to 10^{-1} containing 4 ml of saline.

1 ml from 10^{-1} was transferred to 10^{-2} and the steps were repeated till it reached up to 10^{-8}. The last 3 dilution factor tubes 10^{-8}, 10^{-7} and 10^{-6} were taken into consideration as the least concentrates, which were spread with 0.1 ml of each dilution onto a Nutrient agar medium. The plates were incubated at room temperature for 24 hours. This was performed same for the water sample. After 24 hours the colonies were counted to determine the viable count, morphological character and Gram character of each isolate. Total 31 rhizobacterial isolates from the mangrove soil and water sample were isolated on the Nutrient agar and then preserved on nutrient agar slants for further processing.

Colony characteristics

Characteristics of a colony such as shape, margin, elevation, colour and texture were observed. When recording colony morphology, it is important to record colour, optical properties (translucence, sheen) and texture (moist, mucoid or dry).

Microscopic identification

The basic technique used for classifying bacteria under microscope by Gram staining method isolates stains with crystal violet for 1 min then with iodine which is mordant makes dye less soluble so it adheres to cell wall then rinse with alcohol which act as decolourizer rinse till last drop of it becomes colourless. Stain again it with safranin which is counterstain.

Screening of Phosphate solubilizing microorganisms (Kyaw et al. 2021)

All the bacterial cultures obtained were screened for phosphate solubilization. The cultures were spot inoculated on the Pikovskaya agar. The plates were incubated for 48-72 hours and clear zones around the bacterial colonies were taken as evidence for phosphate solubilization.

Screening of Nitrogen fixing microorganisms

A total of 36 rhizobacteria were screened to show the fixation of nitrogen using Ashby's Mannitol agar. The culture was streaked onto the plates and growth was observed for 1 week.

Screening for enzyme activity

All isolates were spot inoculate on NTYE media containing $MgSO_4$, kCl, CaCl, Crude salt, Yeast extract, Tryptone, NaCl and agar and adding 1% of Starch to check amylase activity, 1% of Carboxy-methyl- cellulose for

cellulase activity and 1% of casein (Skimmed milk) for protease activity. The isolates were grown at room temperature for 24 hours. After 24 hours of period the enzyme activity was checked using reagents such as Gram's iodine for amylases producers, Congo red for cellulose producers, Methyl red for lipase producers.

Screening of Indole acetic acid (IAA) producing microorganisms (Kyaw et al. 2021)

Isolates were grown in nutrient broth with 0.1% tryptophan and incubated at room temperature for 48 hours. The isolates were centrifuged at 8000 rpm for 10 min and to determine amounts of IAA production by each isolate, the supernatant was mixed with 2 ml of Salkowski reagent. Incubated it in dark for 30 min and observe for pink coloration.

Screening of Gibberellin producing microorganisms (Memon 2023)

All the isolates obtained were screened for the production of gibberellins. The culture was inoculated in the tubes containing nutrient broth. The tubes were incubated at room temperature for 48 hours. The culture was then centrifuged at 8000 rpm for 10 min. The supernatant was taken and pH was adjusted to 2.5 using 0.1 N HCl. The culture supernatants were extracted using ethyl acetate. Amount of gibberellic acid in ethyl acetate was measured by UV spectrophotometer at 254 nm against the control blank.

Screening of Exopolysaccharide producing microorganisms (Memon 2023)

The bacterial cultures were inoculated in nutrient broth with 1% of glucose. The culture tubes were incubated at room temperature for 48 hours. The tubes grown were centrifuged at 8000 rpm for 10 min. The supernatant was treated

with thrice the volume of chilled ethanol (i.e. 4 ml of culture and 12 ml of ethanol). Precipitation observed indicated exopolysaccharide production.

Screening for siderophore producing microorganisms (Arora & Varma 2017)

All the isolates were spot inoculated on the CAS plates and incubated at 28°C for 5-7 days and observed for the formation of orange zone around the bacterial colonies.

Screening for ammonia producing microorganisms (Kyaw et al. 2021)

The bacterial isolates were cultured in 10 ml of peptone broth and incubated at 37°c for 48 hours. To check for ammonia production, add Nessler's reagent and identified by colour change from brown to yellow.

Screening for Nitrate reduction test (Dahal 2024)

Inoculate the nitrate broth with bacterial suspension. Incubated the tubes at optimal temperature for 37°C for 24 hours. Look for N2 gas before adding reagents. Then add 6-8 drops of nitrate reagent (Salfanilic acid) or 6-8 drops of (α– naphthylamine). Observe for colour change if no colour change adds zinc powder and observe for at least 3 minutes for red colour to develop.

Pot Assay

Unsterile soil was used for pot experiment. A single bacterial colony of bacterial isolates from mangroves was inoculated in the nutrient broth and incubated in a rotary shaker at 120 rpm for 2-4 days. After growth the broth was centrifuged at 8000 rpm for 10 min. Suspension was made for each of the culture pellets and used for pot experiment. For seed treatment of experimental cultures 40 mung seeds were soaked overnight in each

suspension; also, a mixture of all suspension was made in which seeds were soaked overnight. Control was maintained with same number of seeds soaked in water. Approximate 1 kg of soil was weighed for experimental and control to which the treated seeds and the seeds just soaked in water was introduced into each pot with appropriate labels. The plants were sprinkled with water daily as per the requirements.

For soil treatment same number of seeds were taken and soaked in water overnight. The weighed soil was mixed with the culture suspension thoroughly and the mung seeds were introduced to each pot. Plant Growth promotion of mangrove isolates was observed for 15 days considering parameters such as germination time and number, root length, leaf length, leaf number, shoot length and shoot number of each pot.

Biochemical Identification (Bergy's Manual of Determinative Bacteriology)

Spore forming bacteria

Make a smear on clean grease free slide and place the slide over a beaker of steaming water. Cover the slide with blotting paper or tissue with malachite green and let the slide steam for 3-5 minutes. After that remove the blotting paper or tissue paper and rinse the slide with water to remove any pieces of loose paper and dry the slide. Counterstain with safranin for 1 minute, then wash with water dry the slide and mount under the microscope and view with oil immersion lens.

Strict anaerobic bacteria

The culture was stab inoculated into the hugh leifson medium, keeping two tubes for one isolate. One tube with paraffin oil to make anaerobic condition

and one tube kept at aerobic condition. After 24-72 hrs check for the colour change from purple to yellow in both the tubes.

Catalase producing bacteria

Take a loopful of culture on the slide add a drop of 3% hydrogen peroxide and observe for bubble formation on the slide.

Starch hydrolysing bacteria

The bacterial isolates were streaked on the NA plate containing 0.5% starch powder, incubate the plates at RT for 24 hrs and observe for zone of clearance by adding iodine solution.

Glucose fermenting bacteria

The bacterial isolates were inoculated in NB containing 1% of glucose and 1 ml of phenol red, incubate the tubes at RT for 24 hrs and observe for colour change from red to yellow and also the gas production in durham's tube.

Oxidase producing bacteria

The loopful of culture were streak on oxidase disc and observe for blue coloration on the disc.

Motility test

The bacterial isolates were stab inoculate into the semi-solid agar medium and incubate for 24 hrs and observe for motility.

Citrate utilization bacteria

The bacterial isolates were streaked on slants containing the Simmon's citrate agar medium, incubate the tubes at RT for 24-72 hrs and observe for blue coloration on the slants.

Methyl Red-Voges-Proskauer test

The bacterial isolates were inoculated in the MR-VP broth incubate the tubes at RT for 48 hrs, separate the little amount of grown culture in other tube to check the methyl red test. Add barritt's reagent A (α-Naphthol) and Barritt's reagent B (Potassium hydroxide) to check the VP test and for MR test add methyl red indicator. Observe for red ring formation for MR test and brownish coloration for VP test.

CHAPTER 4

RESULTS

SAMPLING SITES

Sampling sites for plant growth rhizospheric bacteria from mangroves Goa would typically involve collecting soil or root and water samples from different mangroves area in Goa.

Fig 4.1: Sampling site at Diwar Ferry Terminal, Piedade, Goltim-Navelim VP, Goa

Coordinates: (15.504313°) (73.878697°)

Fig 4.2: Sampling sites at Chorao, Goa

Coordinates: (15.513077°) (73.870444°)

ISOLATION OF BACTERIAL CULTURES

Thirty-one morphologically distinct colonies from mangroves (water and sediment) samples were isolated on sterile nutrient agar plates. These isolates were then purified by spot inoculating on NA plates and were given culture codes as shown in fig 2. These isolates were characterized based on their morphology and Gram characteristics.

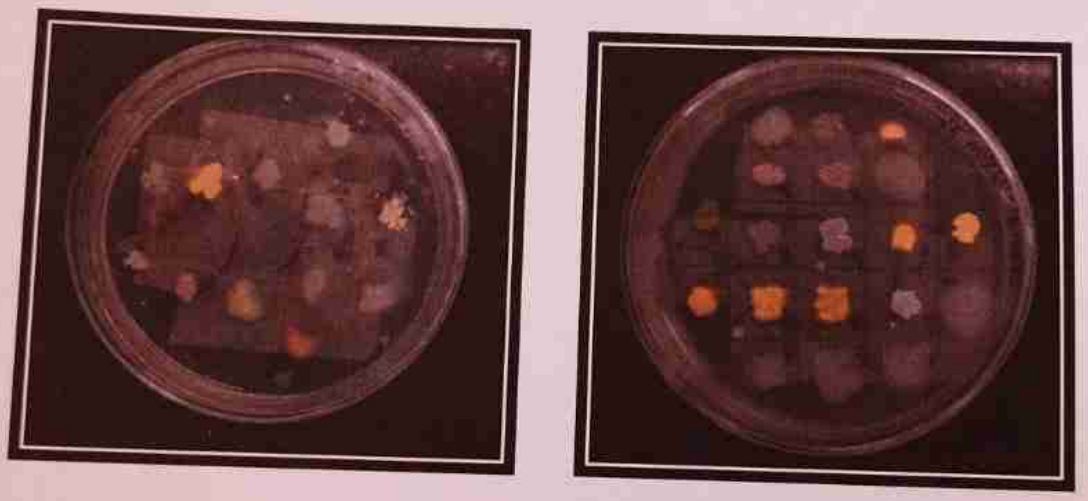


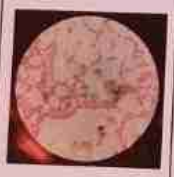
Fig 4.3: Bacterial isolates on Nutrient agar plates

COLONY CHARACTERISTICS OF SELECTED CULTURES

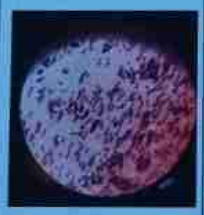
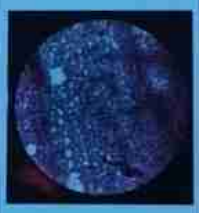
Nitrogen fixing bacteria

Out of 31 bacterial isolates , 9 isolates were selected as shown in fig 4.3. and given in table 4.9., that capable of transforming atmospheric nitrogen into fixed nitrogen.

Table 4.1: Colony characteristics of Nitrogen fixing cultures

Culture number	3	6	13	15	16
Size	3mm	2mm	1mm	2mm	2mm
Shape	Circular	Circular	Circular	Circular	Circular
Time	24 hours	24 hours	24 hours	24 hours	24 hours
Temperature	RT	RT	RT	RT	RT
Colour	Green	Yellow	Yellow	Yellow	White
Elevation	Flat	Flat	Flat	Raised	Raised
Margin	Entire	Entire	Entire	Entire	Entire
Opacity	Opaque	Opaque	Opaque	Opaque	Opaque
Consistency	Non sticky	Non sticky	Sticky	Sticky	Non sticky
Gram character	Gram negative cocci	Gram negative cocci	Gram negative cocci	Gram negative cocci	Gram positive cocci
Image					

Culture number	17	21	27	31
Size	2mm	1mm	1mm	2mm

Shape	Circular	Circular	Circular	Circular
Time	24 hours	24 hours	24 hours	24 hours
Temperature	RT	RT	RT	RT
Colour	Creamy	Yellow	Creamy white	Yellow
Elevation	Flat	Flat	Raised	Flat
Margin	Entire	Entire	Entire	Entire
Opacity	Opaque	Opaque	Opaque	Opaque
Consistency	Sticky	Sticky	Non sticky	Sticky
Gram character	Gram positive rods	Gram positive cocci	Gram negative cocci	Gram negative cocci
Image				

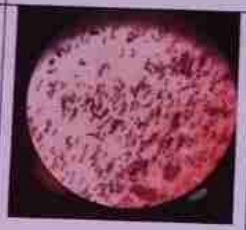
Phosphate solubilizing bacteria

Out of 31 bacterial isolates, 6 islotes were selected that capable of solubilizing inorganic phosphorus from insoluble compounds as shown in fig 4.5 and given in table 4.11and 4.12.

Table 4.2: Colony characteristics of Phosphate solubilizing cultures

Culture number	3	6	15
Size	3mm	2mm	2mm
Shape	Circular	Circular	Circular
Colour	Creamy white	Yellow	Yellow
Time	24 hours	24 hours	24 hours
Temperature	RT	RT	RT
Elevation	Flat	Raised	Flat
Margin	Entire	Entire	Entire
Opacity	Opaque	Opaque	Opaque
Consistancy	Sticky	Non sticky	Sticky
Gram character	Gram negative cocci	Gram positive cocci	Gram negative cocci
Image			

Culture number	16	17	30
Size	2mm	2mm	3mm
Shape	Circular	Circular	Circular
Colour	White	Creamy	Creamy white
Time	24 hours	24 hours	24 hours

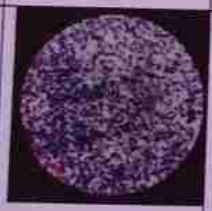
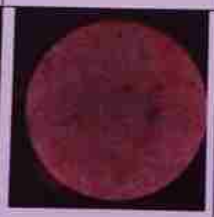
Temperature	RT	RT	RT
Elevation	Flat	Raised	Flat
Margin	Entire	Entire	Entire
Opacity	Opaque	Opaque	Opaque
Consistancy	Sticky	Non sticky	Sticky
Gram character	Gram positive cocci	Gram positive rods	Gram positive rods
Image			

IAA producing bacteria

Out of 31 bacterial isolates, 4 isolates were selected as high producers of IAA as shown in figure 4.7 and given in table 4.13. The absorbance of the selected isolates was measure at 540nm and Graph of IAA producing isolates v/s OD at 540 nm was drawn as shown in fig 4.6 and given in table 4.14.

Table 4.3: Colony characteristics of IAA producing microorganisms

Culture number	7	8	25	27
Size	3mm	2mm	2mm	1mm
Shape	Circular	Circular	Circular	Circular

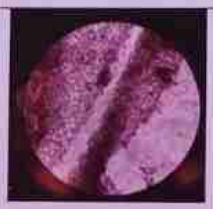
Colour	Creamy white	Creamy white	White	Creamy white
Time	24 hours	24 hours	24 hours	24 hours
Temperature	RT	RT	RT	RT
Elevation	Raised	Raised	Flat	Raised
Margin	Entire	Entire	Entire	Entire
Opacity	Opaque	Opaque	Opaque	Opaque
Consistency	Sticky	Sticky	Sticky	Non sticky
Gram character	Gram positive rods	Gram negative cocci	Gram positive rods	Gram negative cocci
Image				

EPS producing bacteria

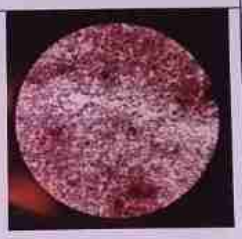
Out of 31 bacterial isolates, 6 isolates were selected as high producers of EPS as shown in fig 4.8 and fig 4.9 and given in table 4.15.

Table 4.4: Colony characteristics of EPS producing microorganisms

Culture number	8	10	13

Size	2mm	3mm	1mm
Shape	Circular	Circular	Circular
Colour	Creamy white	White	Yellow
Time	24 hours	24 hours	24 hours
Temperature	RT	RT	RT
Elevation	Raised	Flat	Flat
Margin	Entire	Entire	Entire
Opacity	Opaque	Opaque	Opaque
Consistency	Sticky	Non sticky	Sticky
Gram character	Gram negative cocci	Gram negative rods	Gram negative cocci
Image			

Culture number	19	22	28
Size	2mm	2mm	1mm
Shape	Circular	Circular	Circular
Colour	Creamy white	Orange	Pinkish
Time	24 hours	24 hours	24 hours
Temperature	RT	RT	RT

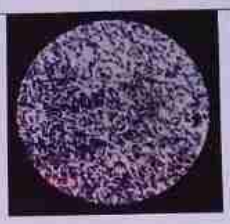
Elevation	Raised	Raised	Flat
Margin	Entire	Entire	Entire
Opacity	Opaque	Opaque	Opaque
Consistency	Non sticky	Non sticky	Non sticky
Gram character	Gram positive rods	Gram positive rods	Gram positive rods
Image			

Siderophore producing bacteria

Out of 31 bacterial isolates, 5 isolates were selected as best siderophore producing microorganisms as shown in fig 4.10 and given in table 4.16.

Table 4.5: Colony characteristics of siderophore producing microorganisms

Culture number	**2**	**4**	**12**
Size	2mm	1mm	3mm
Shape	Circular	Circular	Circular
Colour	Yellow	Pinkish	Creamy white
Time	24 hours	24 hours	24 hours
Temperature	RT	RT	RT
Elevation	Raised	Flat	Raised
Margin	Entire	Entire	Entire

Opacity	Opaque	Opaque	Opaque
Consistency	Sticky	Non sticky	Non sticky
Gram character	Gram negative cocci	Gram positive Cocci	Gram positive rods
Image			

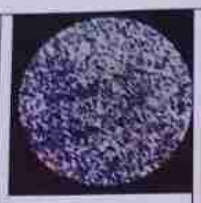
Culture number	**18**	**31**
Size	1mm	2mm
Shape	Circular	Circular
Colour	Yellow	Yellow
Time	24 hours	24 hours
Temperature	RT	RT
Elevation	Raised	Flat
Margin	Entire	Entire
Opacity	Opaque	Opaque
Consistency	Sticky	Non sticky
Gram character	Gram negative cocci	Gram negative cocci
Image		

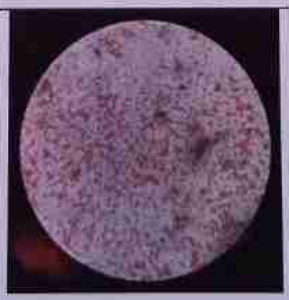
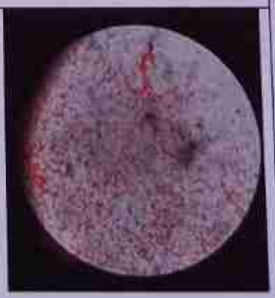
Nitrate reducing bacteria

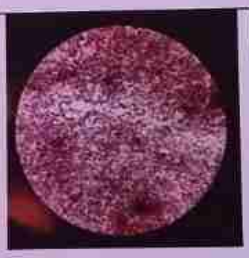
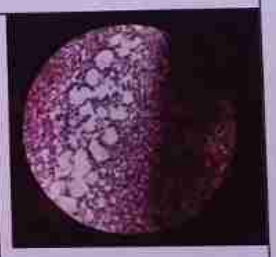
Out of 31 bacterial isolates, 10 isolates were selected as nitrate reducers as shown in fig 4.11 and given in table 4.17, which is capable of reducing nitrate to nitrite.

Table 4.6: Colony characteristics of Nitrate reducing microorganims

Culture number	7	8	11	13
Size	3mm	2mm	1mm	1mm
Shape	Circular	Circular	Circular	Circular
Colour	Creamy white	Creamy white	White	Yellow
Time	24 hours	24 hours	24 hours	24 hours
Temperature	RT	RT	RT	RT
Elevation	Raised	Raised	Raised	Flat
Margin	Entire	Entire	Entire	Entire
Opacity	Opaque	Opaque	Opaque	Opaque
Consistency	Sticky	Sticky	Non sticky	Sticky
Gram character	Gram positive rods	Gram negative cocci	Gram negative cocci	Gram negative cocci

Image				

Culture number	**15**	**18**	**23**
Size	2mm	1mm	2mm
Shape	Circular	Circular	Circular
Colour	Yellow	Yellow	Yellow
Time	24 hours	24 hours	24 hours
Temperature	RT	RT	RT
Elevation	Flat	Raised	Flat
Margin	Entire	Entire	Entire
Opacity	Opaque	Opaque	Opaue
Consistency	Sticky	Sticky	Non sticky
Gram character	Gram negative cocci	Gram negative cocci	Gram negative rods
Image			

Culture number	27	28	30
Size	1mm	1mm	3mm
Shape	Circular	Circular	Circular
Colour	Creamy white	Pinkish	Creamy white
Time	24 hours	24 hours	24 hours
Temperature	RT	RT	RT
Elevation	Raised	Flat	Flat
Margin	Entire	Entire	Entire
Opacity	Opaque	Opaque	Opaque
Consistency	Non sticky	Non sticky	Sticky
Gram character	Gram negative cocci	Gram positive rods	Gram positive rods
Image			

Ammonia producing microorganisms

Out of 31bacterial isolates, 2 isolates selected as high ammonia producing microorganisms as shown in fig 4.12 and given in table 4.18.

Table 4.7: Colony characteristics of ammonia producing microorganisms

Culture number	14	27
Size	2mm	1mm

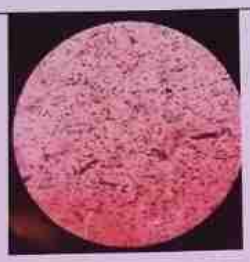
Shape	Circular	Circular
Colour	Yellow	Creamy white
Time	24 hours	24 hours
Temperature	RT	RT
Elevation	Flat	Raised
Margin	Entire	Entire
Opacity	Opaque	Opaque
Consistency	Sticky	Non sticky
Gram character	Gram negative rods	Gram negative cocci
Image		

GA_3 producing bacteria

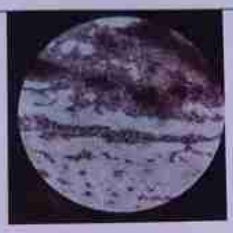
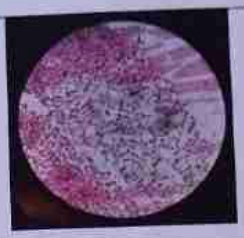
Out of 31 bacterial isolates, 4 isolates were selected as high GA_3 producing microorganisms. The extraction was shown in fig 4.14 and the Graph of GA_3 v/s OD at 254nm as shown in fig 4.13 and given in table 4.19.

Table 4.8: Colony characteristics of GA_3 production

Culture number	14	15
Size	2mm	2mm
Shape	Circular	Circular
Colour	Yellow	Yellow
Time	24 hours	24 hours

Temperature	RT	RT
Elevation	Flat	Flat
Margin	Entire	Entire
Opacity	Opaque	Opaque
Consistency	Sticky	Sticky
Gram character	Gram negative rods	Gram negative cocci
Image		

Culture number	**22**	**26**
Size	2mm	1mm
Shape	Circular	Circular
Colour	Orange	White
Time	24 hours	24 hours
Temperature	RT	RT
Elevation	Raised	Flat
Margin	Entire	Entire
Opacity	Opaque	Opaque
Consistency	Non sticky	Sticky
Gram character	Gram positive rods	Gram positive cocci

Image		

SCREENING OF NITROGEN FIXING MICROORGANISMS

The bacterial isolates showed growth on Ashby's Mannitol agar media, out of 31 isolates, 9 bacterial isolates grew well on the media as show in Table 4.9 therefore this twelve isolates were nitrogen fixing microorganisms.

Table 4.9: Bacterial isolates showing nitrogen fixing microorganisms

Culture number	Nitrogen fixation
R1	+
R2	+ +
R3	+ +
R4	+
R5	-
R6	+ +
R7	-
R8	-
R9	+
R10	-
R11	+
R12	-

R13	+ + +
R14	-
R15	+ +
R16	+ + +
R17	+ + +
R18	-
R19	+
R20	-
R21	+ + +
R22	-
R23	+
R24	+
R25	+
R26	+
R27	+ + +
R28	-
R29	-
R30	+
R31	+ + +

Key: - No growth; +,++Less growth; +++ Good growth

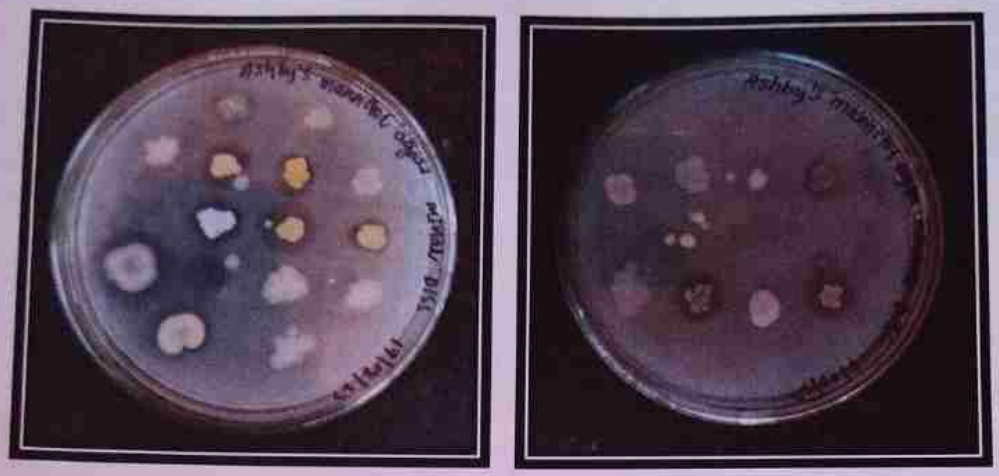


Fig 4.4: Bacterial isolates showing growth on Ashby's mannitol agar media

Table 4.10: Bacterial isolates showing Nitrogen fixing ability

Culture no.	Growth diameter	Zone of clearance
R1	0.4	0.5
R2	0.5	0.7
R3	**0.3**	**0.8**
R4	0.2	0.3
R6	**0.4**	**0.8**
R9	0.3	0.5
R11	0.3	0.5
R13	**0.6**	**0.9**
R15	0.4	0.7
R16	**0.8**	**1.2**

R17	**0.6**	**1.4**
R19	0.3	0.6
R21	**0.5**	**0.9**
R23	0.3	0.4
R24	0.4	0.5
R25	0.2	0.3
R26	0.3	0.5
R27	**0.7**	**0.9**
R30	0.2	0.4
R31	**0.6**	**1.1**

SCREENING OF PHOSPHATE SOLUBILIZING MICROORGANISMS

The bacterial isolates showed growth on Pikovskaya agar media, out of 31 isolates, 6 bacterial isolates grew well on the media as shown in Table 4.11. therefore this six isolates were selected for further studies.

Table 4.11: Bacterial isolates showing zone of clearance

Culture no.	Zone of clearance
R1	+
R2	+
R3	+
R4	+
R5	+
R6	+
R7	-

R8	-
R9	+
R10	-
R11	+
R12	+
R13	+
R14	-
R15	+
R16	+
R17	+
R18	-
R19	-
R20	-
R21	+
R22	-
R23	-
R24	-
R25	+
R26	+
R27	-
R28	-
R29	-
R30	+
R31	-

Key: - No zone of clearance; + Zone of clearance

Table 4.12: Bacterial isolates showing Phosphate solubilization efficiency

Culture no.	Solubilization Diameter	Growth diameter	Phosphate solubilization efficiency (%)
R1	0.9	0.5	180
R2	**0.8**	**0.4**	**200**
R3	0.9	0.6	150
R4	0.5	0.2	250
R5	1	0.8	125
R6	**0.8**	**0.4**	**200**
R9	1.1	0.7	157
R11	**1.3**	**0.5**	**260**
R12	**0.9**	**0.4**	**225**
R13	**0.7**	**0.3**	**233**
R15	1	0.6	166
R16	1.3	0.8	162
R17	1.5	0.9	166
R21	0.8	0.7	114
R25	**1.2**	**0.6**	**200**
R26	1.1	0.6	183
R30	1.3	0.8	162

Fig 4.5: Bacterial isolates showing phosphate solubilizaton

Screening of IAA producing microorganisms

One of 31 isolates obtained, 9 isolates showed positive results for the production of IAA. After adding Salkowski's reagent to the supernatant of IAA producing culture it turned pink as shown in the fig 4.7. and absorbance was measured at 540 nm. Out of 31 bacterial culture, 8 were selected that has higher absorbance shown in Fig 4.6. Hence culture 24 has found highest activity and lowest in culture culture 7 as shown in table 4.14. Graph of absorbance was plotted for 8 selected cultures as shown in Fig 4.6. More the absorbance more the IAA produced.

Table 4.13: Bacterial isolates showing IAA producing microorganisms

Culture no.	IAA production
R1	-
R2	-
R3	-
R4	-

R5	+
R6	-
R7	+
R8	+
R9	-
R10	-
R11	+
R12	-
R13	-
R14	-
R15	-
R16	-
R17	-
R18	-
R19	-
R20	-
R21	-
R22	-
R23	+
R24	+
R25	+
R26	-
R27	+
R28	-
R29	-
R30	-

R31	-

Key: + Presence of IAA; - Absence of IAA

Table 4.14: Bacterial isolates showing absorbance at 540nm for IAA production

Culture no.	Absorbance at 540nm
R7	0.22375
R8	**0.5855**
R11	0.2375
R12	0.2871
R23	**0.6021**
R24	**0.6252**
R25	**0.5415**
R27	0.4617

Fig 4.6:Graphical reperesentation of bacterial isolates showing highest absorbance value at 0.6252nm in culture number 24 for IAA production.

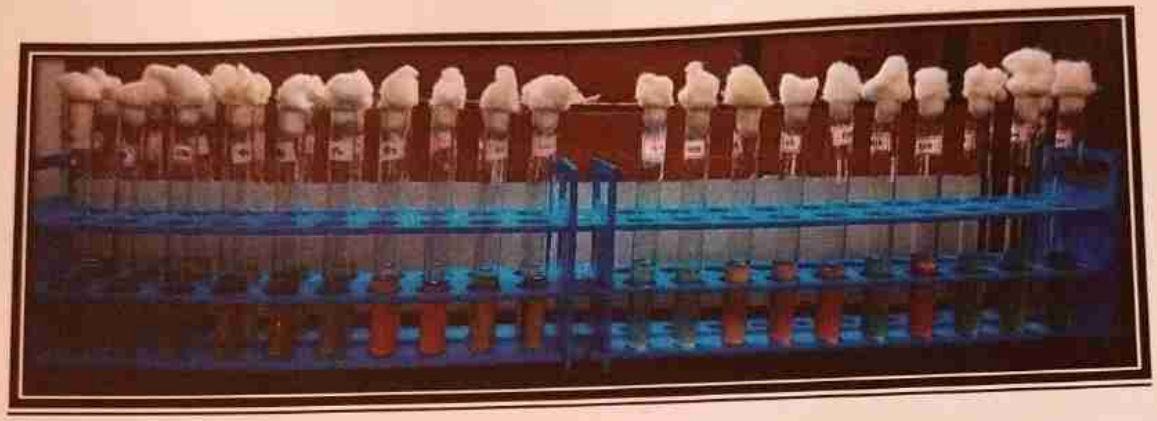


Fig 4.7: Isolates showing pink colouration after adding Salkowski reagent

Screening of EPS producing microorganisms

Among 31 bacterial isolates 7 isolates were selected as best EPS producers as shown in Table. Isolate 8,10,13,19,22,23 and 28 produced high amount of precipatate.

Table 4.15: Bacterial isolates showing presence and absence of precipitation

Culture no.	Precipitation
R1	-
R2	-
R3	-
R4	-
R5	+
R6	+
R8	-
R9	+
R10	-

R11	+
R12	+
R13	+
R14	+
R15	+
R16	+
R17	+
R18	+
R19	+
R20	-
R21	+
R22	+
R23	+
R24	+
R25	+
R26	+
R27	-
R28	+
R29	-
R30	+
R31	-

Key:+ Presence of precipitate ; - Absence of precipitate

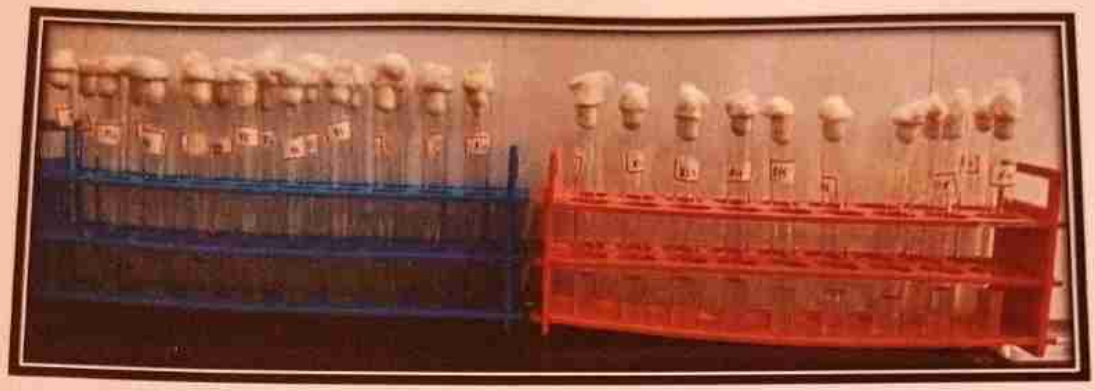


Fig 4.8: isolates showes EPS production after adding thrice the amount of chilled ethanol

Fig 4.9: Image of precipitate formation for EPS production

Screening of siderophore producing microorganisms

Among 31 bacterial isolates , 14 isolates showed positive results for the production of siderophore as shown in Table. Isolate 2,4,12,18 and 31 shows

more orange colour zone of clearance around the colonies as shown in fig 4.10.

Table 4.16: Bacterial isolates showing presence or absence of siderophore producing microorganisms

Culture number	Diameter of zone of clearance (mm)
R1	10
R2	11
R3	10
R4	**12**
R5	9
R6	10
R7	10
R8	10
R9	-
R10	-
R11	10
R12	**12**
R13	-
R14	9
R15	-
R16	10
R17	-
R18	**13**
R19	-
R20	-

R21	-
R22	-
R23	-
R24	-
R25	-
R26	-
R27	-
R28	-
R29	-
R30	-
R31	**12**

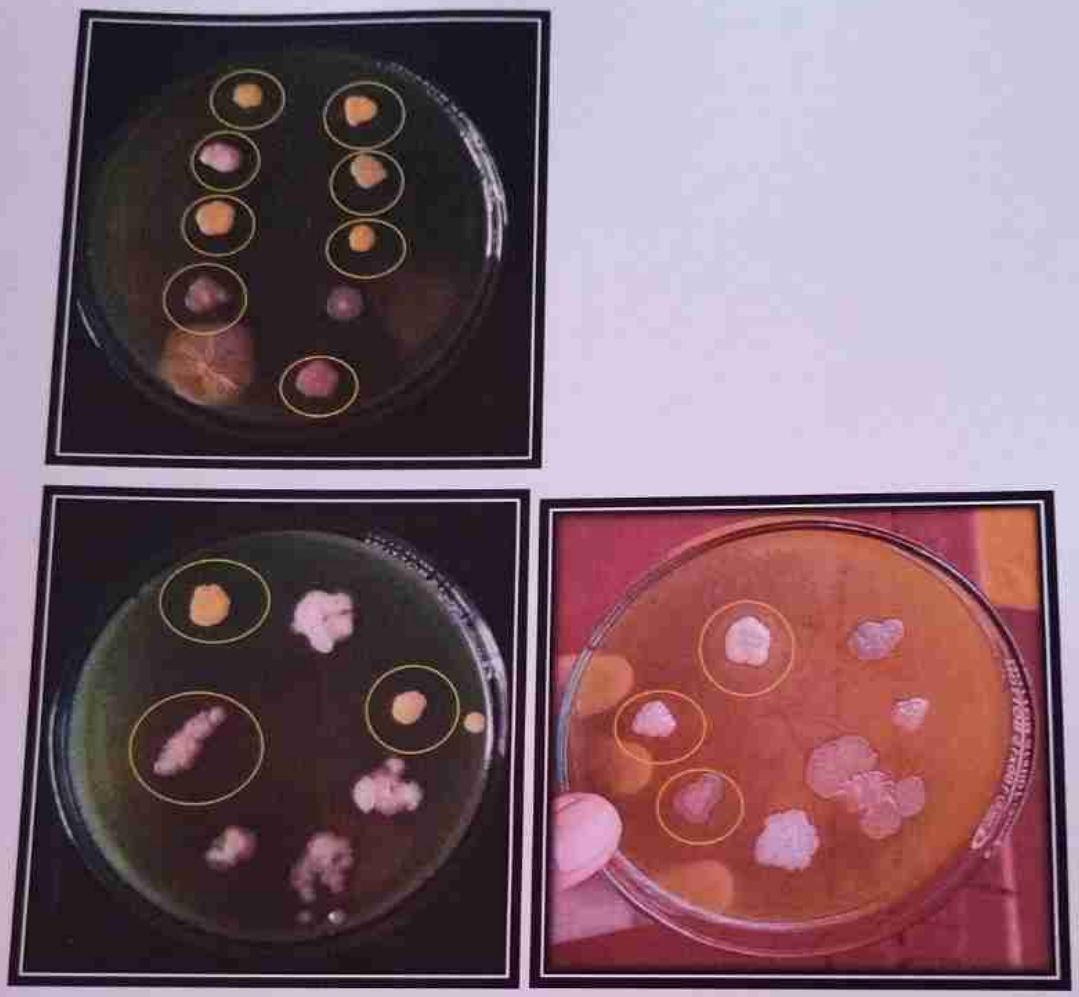


Fig 4.10 : Bacterial isolates showing siderophore production

Screening of nitrate reducer microorganisms

Nitrate reduction was observed by color change to red by adding griess reagent.

Among 31 bacterial isolates , 10 isolates showed positive results for the nitrate reduction as shown in Table 4.17.

Table 4.17: Bacterial isolates showing nitrate reducing activity

Culture no.	Nitrate reduction
R1	-
R2	-
R3	-
R4	-
R5	-
R6	-
R7	+
R8	+
R9	-
R10	-
R11	+
R12	-
R13	+
R14	-
R15	+
R16	-
R17	-
R18	+
R19	-
R20	-
R21	-
R22	-
R23	+
R24	-

R25	-
R26	-
R27	+
R28	+
R29	-
R30	+
R31	-

Key: + Presence of nitrate reducers ; - Absence of nitrate reducers

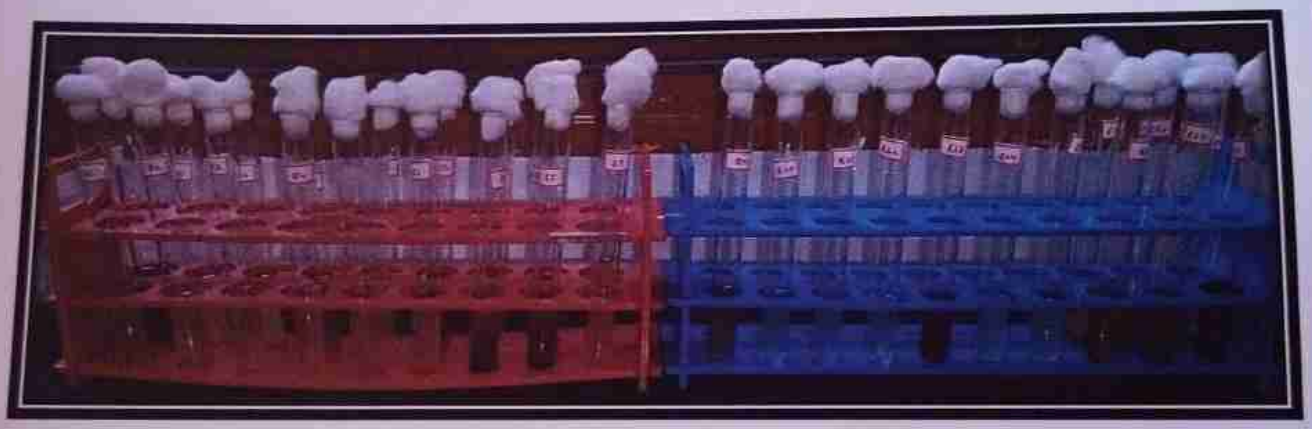


Fig 4.11: Bacterial isolates showing nitrate reducers

Screening of ammonia producing bacteria

The ammonia producing microorganisms was checked by adding Nesseler's reagent. Among all 31 cultures all bacterial isolates shows positive results for ammonia producers.

Table 4.18: Bacterial isolates showing ammonia producers

Culture no.	Ammonia producers
R1	+
R2	+
R3	+
R4	+
R5	+
R6	+
R7	+
R8	+
R9	+
R10	+
R11	+
R12	+
R13	+
R14	+
R15	+
R16	+
R17	+
R18	+
R19	+
R20	+
R21	+
R22	+
R23	+
R24	+

R25	+
R26	+
R27	+
R28	+
R29	+
R30	+
R31	+

Key: + Ammonia producers

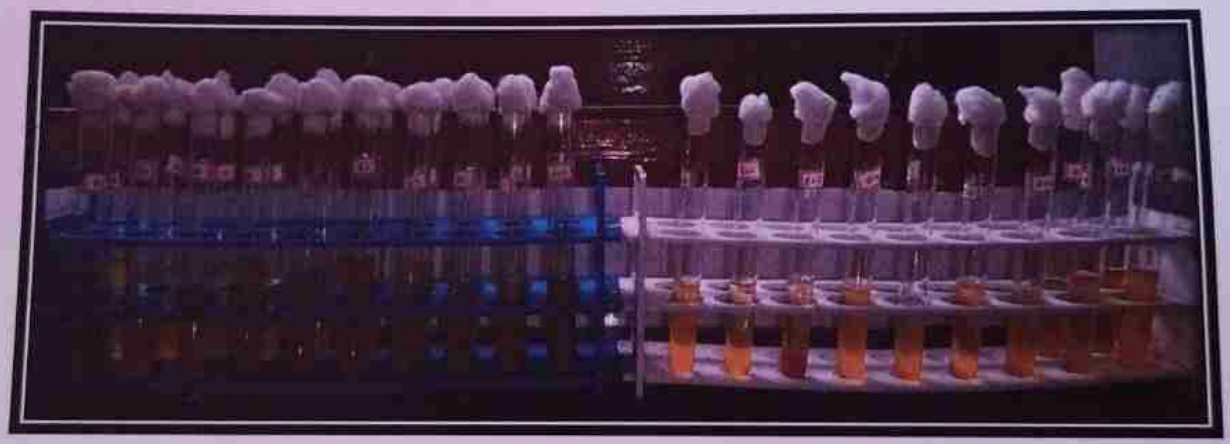


Fig 4.12: Bacterial isolates showing ammonia producers

Screening of GA_3 producing microorganisms

Out of 31 bacterial isolates, 4 were selected that produced higher Gibberellic acid on extraction shown in Fig 4.14 . Hence the activity was found more in culture 22 and lowest in culture 14 as shown in the table 4.19. Graph of absorbance was plotted for 4 selected cultures as shown in Fig 4.13. More the absorbance more the amount of GA_3 is produced.

Table 4.19 : Bacterial isolates showing absorbance at 254nm for GA_3 production

Culture no.	Absorbance at 254nm
Control	0.1935
R1	0.1539
R2	0.2648
R3	0.1686
R4	0.1748
R5	0.1743
R6	0.2323
R7	0.1922
R8	0.1485
R9	0.2092
R10	0.1721
R11	0.2201
R12	0.2700
R13	0.2767
R14	**0.2858**
R15	**0.3223**
R16	0.2183
R17	0.1918
R18	0.1925
R19	0.2664
R20	0.2372
R21	0.2706
R22	**0.3516**
R23	0.2658

R24	0.1969
R25	0.2948
R26	**0.3164**
R27	0.1977
R28	0.2176
R29	0.1651
R30	0.1988
R31	0.1531

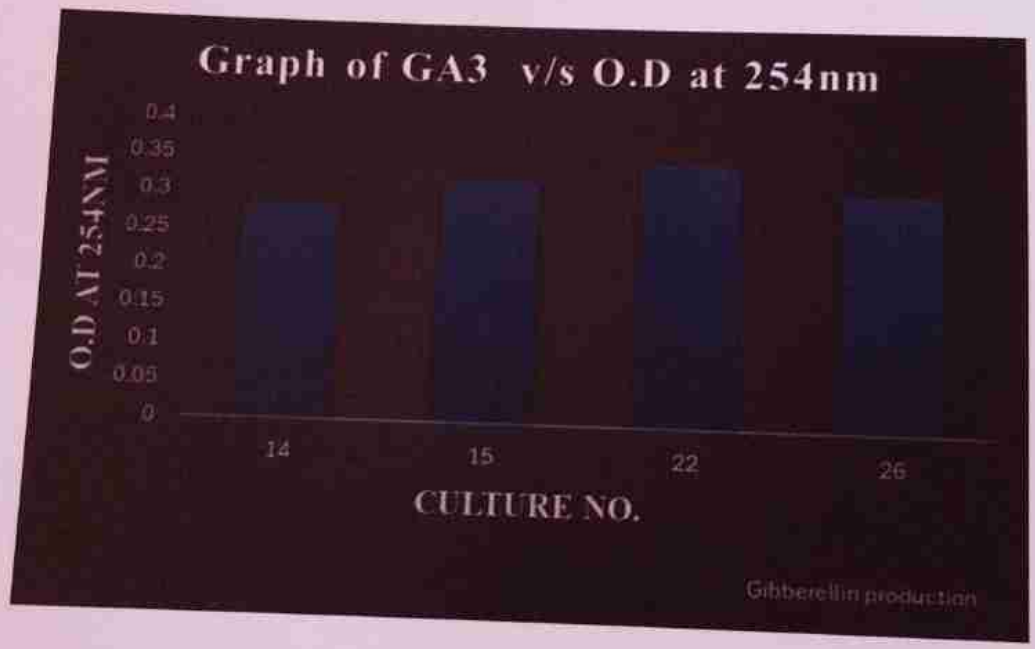


Fig 4.13: Graphical representation of bacterial isolates showing highest absorbance value at 0.3516nm in culture 22 for GA_3 production.

Fig 4.14: Extraction using ethyl acetate

Screening for enzyme producing microorganisms

31 Bacterial siolates were checked for enzyme activity. The ezyme detected were Amylase, Lipase and Cellulose. No growth was observed on the experimental plates, Hence the enzymes were not produced.

Fig 4.15: Test for amylase activity

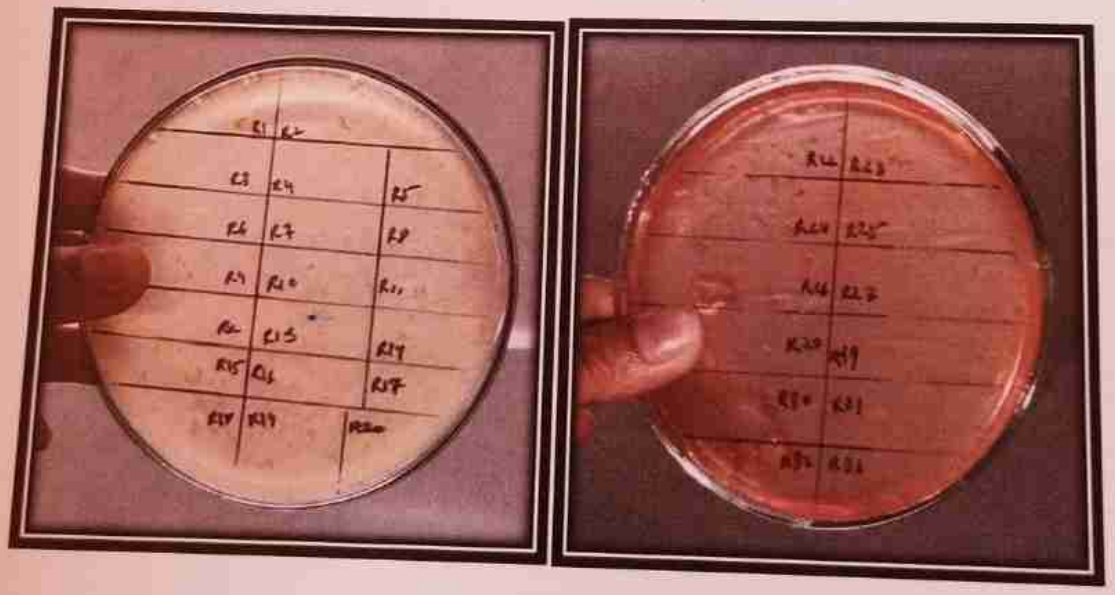


Fig 4.16: Test for lipase activity

Fig 4.17: Test for cellulase activity

Pot Assay

It is concluded that culture number 3, 6, 13, 27, 31, 7, 8, 11, 28, 30, 14, 18, 23 were giving (N2), 15, 16, 17 (IAA Producers) and 22 and 26 were produce (GA3 Production) were considered for pot assay (Refer to figure 4.18 and figure 4.19).

The time taken for each plant to germinate was maximum 2-3 days of which pot with culture of N2, IAA were germinate faster as compared to the containing other culture and the one with mixed culture. The growth parameter of each experimental pot were compared with control and was seen that each plant grew well in comparison with control. Pot containing cultures N2 , IAA and Mixed germinated faster as that of control. Likewise pot containing cultures GA3 grew a little slower than the other pots. 20 seeds were germinated which was maximum in pot containing IAA and mixed culture and the rest pots gave better results than the control. The root length with 2.5

cm showed best in pot IAA production as compared to others and pot Nitrogen fixation showed short root length as compared to control. The experimental pots showed poor leaf lengths as compared to control. The leaf number was same for all in comparison with control. The shoot length was seen maximum in pot phosphate producers with 11.8 cm in height as compared to other experimental pots and control. The shoot number was seen same in all experimental and control pot. For soil treatment the time taken for each plant to germinate was maximum 3-4 days of which pot with cultures of IAA producers and Nitrogen fixation germinate faster in 3 days as compared to the other experimental pots. The growth parameters of each experimental pot were compared with control and was seen that each plant grew well with some parameters in comparison with control. Pot containing IAA producers and Nitrogen fixation germinated faster but slower than control. Likewise pot containing GA3 grew much slower than the other pots. 20 seeds germinated maximum in pot IAA producers and the rest pots gave better results than the control. The root length with 5 cm showed best in pot GA3 production as compared to others and mixed culture pot showed short root length as compared to control. The experimental pots showed poor leaf lengths as compared to control. The leaf number was same for all in comparison with control. The shoot length was seen maximum in pot phosphate producers with 10 cm in height as compared to other experimental pots and control. The shoot number was seen same all the experimental and control pot.

Fig 4.18: Soil treated pots

Fig 4.19: Seed treated pots

Biochemical indentification

Biochemical identification for Gram positive rods

Out of 31 bacterial isolates 7 isolates identified as Gram positive rods. Further biochemical test were performed.

Spore Forming Bacteria

Out of 7 isolates only two isolate shows green colouration of bacterial cell which means it producing spores.

Table 4.20: Isolates selected for spore formation

Culture number	Spore formation
R17	**Spore formed**
R19	**Spore formed**
R22	No Spore formed
R25	No Spore formed
R26	No Spore formed
R28	No Spore formed
R30	No Spore formed

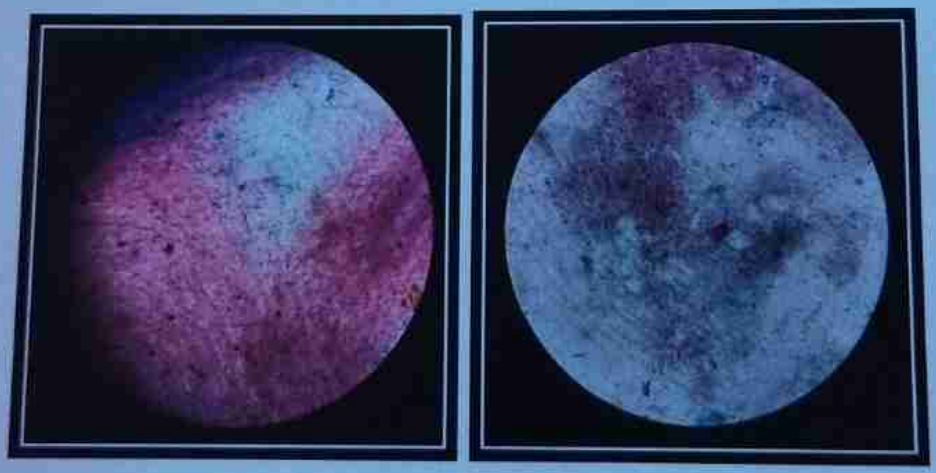


Fig 4.20: Bacterial isolates showing spore formation

Strict anaerobic bacteria

Out of 7 isolates all shows yellow in both tubes with oil and without oil. Hence all 7 isolates are strict aerobic.

Table 4. 21: Bacterial isolates showing strict aerobes

Culture number	Aerobic/Anaerobic bacteria	
	With oil	Without oil
R17	+	+
R28	+	+
R30	+	+
R26	+	+
R25	+	+
R22	+	+
R19	+	+

Key: + Aerobic Bacteria

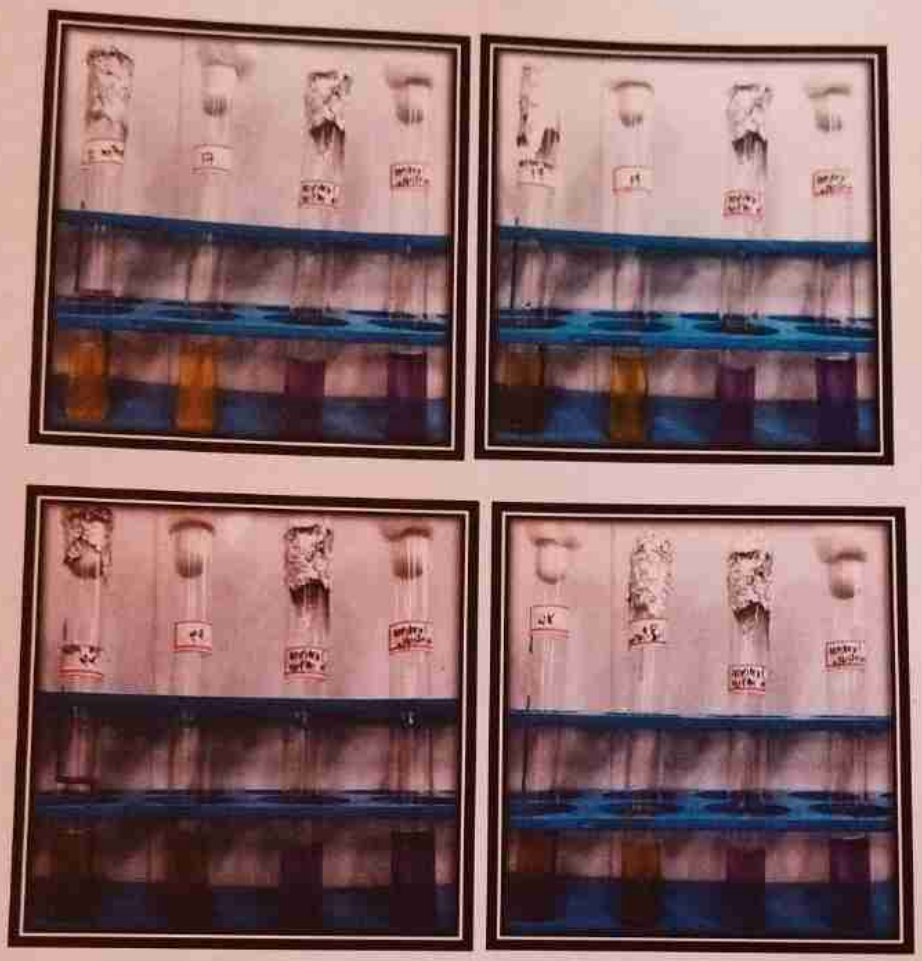


Fig 4. 21: Isolates showing Strict aerobic growth

Catalase producing bacteria

Out of all 7 culture all 7 culture shows bubble formation for catalase test.

Table 4. 22: Bacterial isolates showing catalase test

Culture number	Catalase test
R17	Positive ; bubble formation
R28	Positive ; bubble formation
R30	Positive ; bubble formation

R26	Positive ; bubble formation
R25	Positive ; bubble formation
R22	Positive ; bubble formation
R19	Positive ; bubble formation

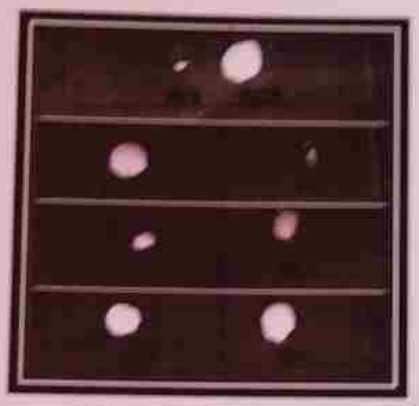


Fig 4. 22:Isolates showing catalase production

Starch hydrolysing bacteria

Out of 7 isolates only three isolates shows starch hydrosis by showing

Of clearance around the colonies.

Table 4. 23: Bacterial isolates showing starch hydrolysis test

Culture number	Zone of clearance
R17	-
R28	-
R30	-
R26	+
R25	-
R22	+

R19	+

Key: + Zone of clearance; - No zone of clearance

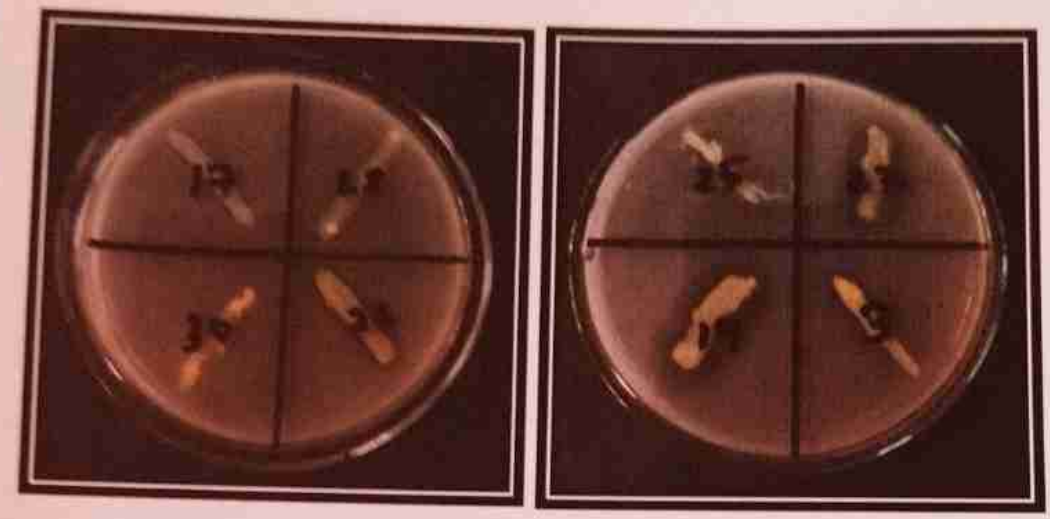


Fig 4. 23: Isolates showing zone of clearnce after adding iodine solution

Glucose fermentating bacteria

Out of 7 isolates all 7 isolates shows yellow coloration after 24 hours of incubation.Hence all isolates ferments glucose with no gas formation.

Table 4. 24: Bacterial isolates fermenting glucose

Culture number	Glucose fermentation
R17	+
R28	+
R30	+
R26	+
R25	+
R22	+
R19	+

Key : + Glucose fermentation with acid production and no gas formation

Fig 4. 24: Bacterial isolates showing glucose fermentation

Biochemical identification for Gram negative rods

Out of 31 bacterial isolates 4 isolates were identified as Gram negative rods. Further biochemical test were performed.

Oxidase producing bacteria

Out of 4 bacterial isolates 2 isolates gives positive results for by showing color changes to dark purple.

Table 4. 25: Isolates showing oxidase production

Culture number	Oxidase production
R11	-
R23	+
R14	+
R10	-

Key: - No oxidase formation; + oxidase formation

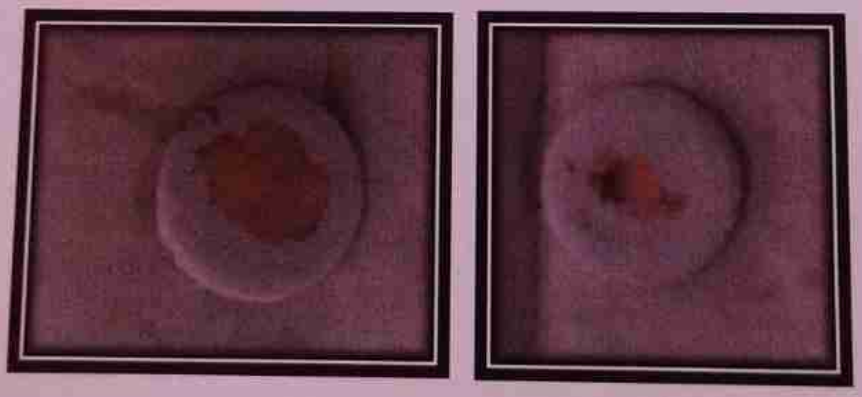


Fig 4. 25: Bacterial isolates showing negative results

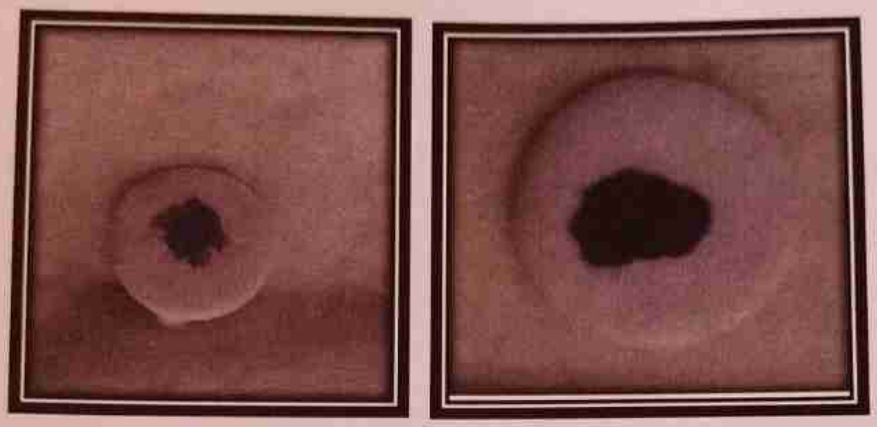


Fig 4. 26: Bacterial isolates showing positive results

Glucose fermentation

Out of 4 bacterial isolates all 4 shows positive results for glucose by changung color from red to yellow and only culture number 14 shows gas production.

Table 4. 26: Bacterial isolates showing glucose fermentation

Culture number	Glucose fermentation
R11	+ +
R23	+
R14	+
R10	+

Key: + + Glucose fermentation with acid production and with gas formation; + Glucose fermentation with acid production and no gas formation

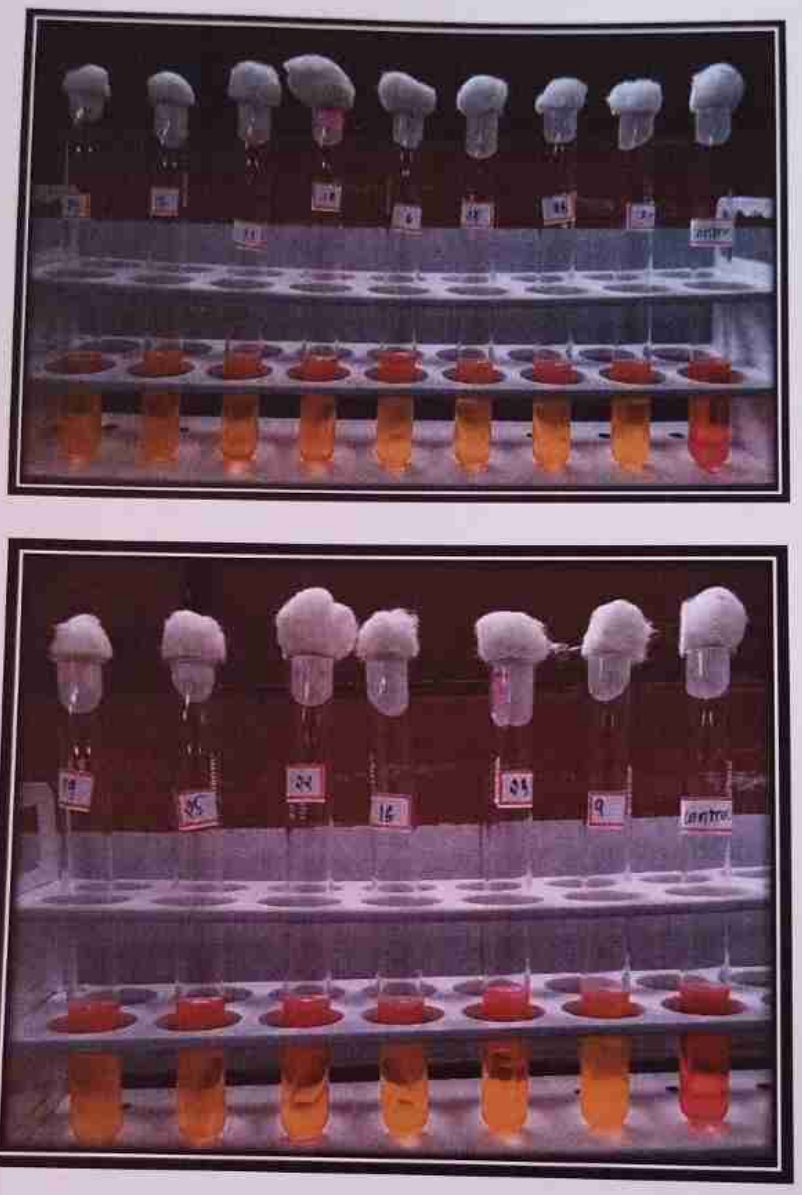


Fig 4. 27: Bacterial isolates showing glucose fermentation

Motility test for bacteria

Out of 4 bacterial isolates all 4 bacterial isolates are non motile.

Table 4. 27: Bacterial isolates showing no motility

Culture number	Motile / Non motile
R11	Negative

R23	Negative
R14	Negative
R10	Negative

Fig 4. 28: Bacterial isolates showing non motile

Lactose fermenting bacteria

Out of 4 bacterial isolates 3 are lactose fermenting bacteria.

Table 4. 28: Bacterial isolates showing lactose fermentation

Culture number	Lactose fermentation
R11	+
R23	-
R14	+ +
R10	+

Key: + + Lactose fermentation with acid production and with gas formation; + Lactose fermentation with acid production and no gas formation; - No Lactose fermentation

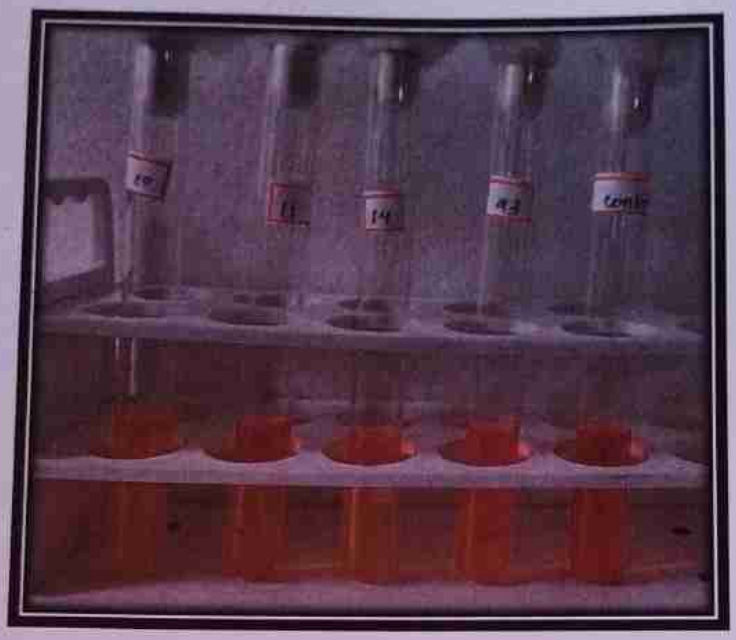


Fig 4. 29: Bacterial isolates showing lactose fermentation

Biochemical identification for Gram positive cocci

Out of 31 bacterial isolates only 3 bacterial isolates were identified as Gram positive cocci. Futher bichemical tests were performed.

Catalase producing bacteria

Out of 3 bacterial isolates all 3 isolates shows catalase positive by showing bubble formation.

Table 4. 29: Bacterial isolates showing catalase test

Culture number	Catalase production
R6	+
R9	+
R16	+

Key: + Bubble formation occur

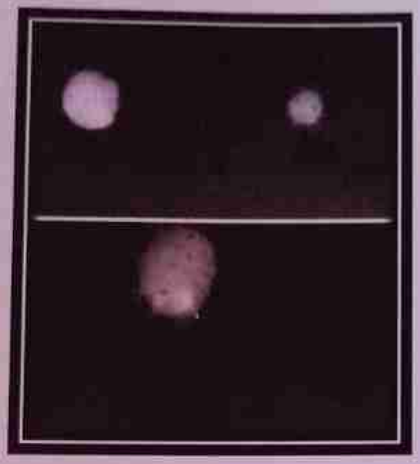


Fig 4.30: Isolates showing bubble formation after adding hydrogen peroxide

Glucose fermentating bacteria

Out of 3 bacterial isolates all 3 were fermenting glucose by changing color from red to yellow.

Table 4.30: Bacterial isolates showing glucose fermentation

Culture number	Glucose fermentation
R6	+
R9	+
R16	+

Key: + Glucose fermentation with acid production and no gas formation

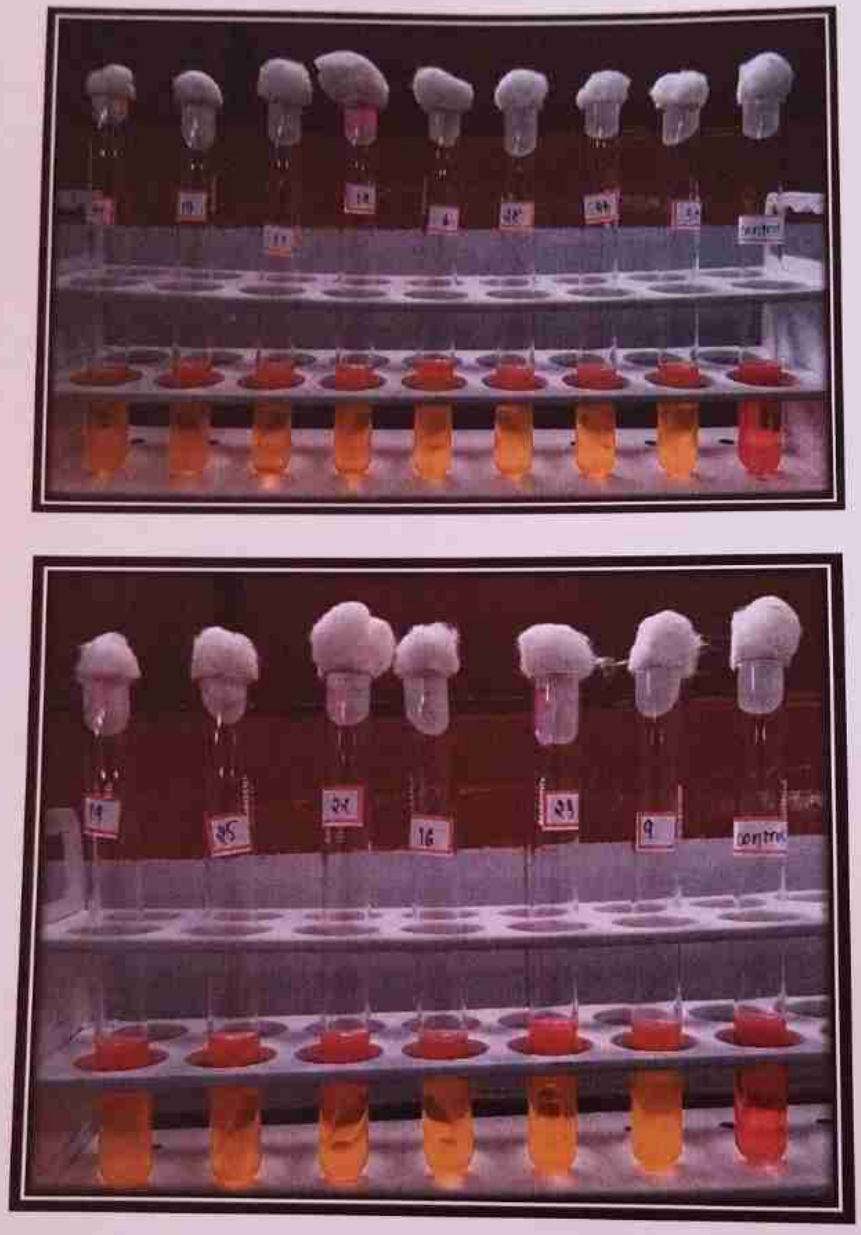


Fig 4.31: Bacterial isolates showing glucose fermentation

Biochemical identification for Gram negative cocci

Out of 31 bacterial isolates only 8 isolates identified as Gram negative cocci. Further biochemical tests were performed.

Starch hydrolysing bacteria

All the 8 bacterial isolates shows no zone of clearance. Hence it indicate that this 8 isolates were not hydrolysing the starch.

Table 4.31: Bacterial isolates showing starch hydrolysis activity

Culture number	Zone of clearance
R3	-
R13	-
R15	-
R27	-
R31	-
R7	-
R8	-
R18	-

Key: - No zone of clearance

Fig 4.32: Bacterial isolates showing no zone of clearance around the colonies

MR-VP test

Out of 8 bacterial isolates all 8 isolates shows no colour change for VP test ; hence it indicates negative results.

Out of 8 bacterial isolates 6 isolates shows red colouration ring formation at the top.

Table 4.32: Bacterial isolates showing Voges proskaur test

Culture number	VP test
R3	-
R13	-
R15	-
R27	-
R31	-
R7	-
R8	-
R18	-

Table 4.33: Bacterial isolates showing Methyl red test

Culture number	MR test
R3	-
R13	+
R15	+
R27	+
R31	+
R7	+
R8	+
R18	-

Key: + Red ring formed; - No red ring formed

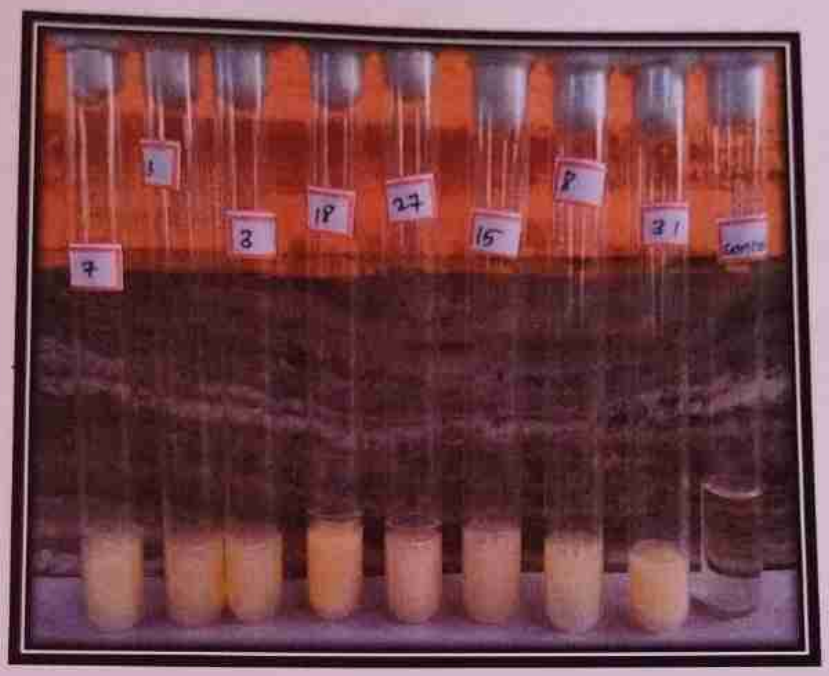


Fig 4.33: Bacterial isolates showing no red cherry colouration occur

Fig 4.34: Bacterial isolates showing red colouration ring formation

Citrate utilization bacteria

Out of 8 bacterial isolates only 5 isolates were identified as citrate producers. Showing colour change from Green to blue.

Table 4.34: Bacterial isolates showing citrate utilization

Culture number	Citrate producers
R3	+
R13	+
R15	+
R27	+
R31	+
R7	-
R8	-

R18	-

Key: + Citrate utilization; - No Citrate utilization

Fig 4.35: Isolates showing citrate activity

Catalase producing bacteria

All the 8 bacterial isolates were showing bubble formation after adding hydrogen peroxide; inicating catalase positive.

Table 4.35: Bacterial isolates showing catalase test

Culture number	Citrate producers
R3	+
R13	+
R15	+
R27	+
R31	+
R7	+
R8	+

R18	+

Key: + Catalase production

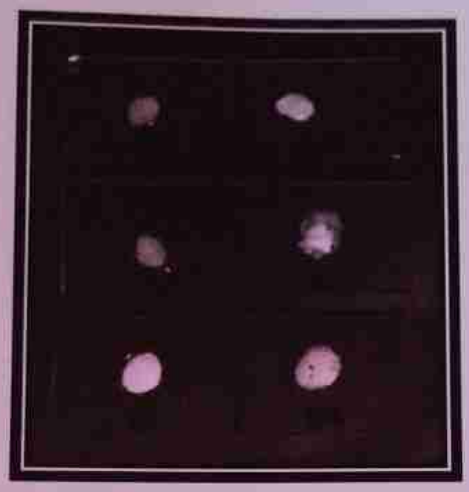


Fig 4.36: Bacterial isolates showing bubble formation

Identification of bacterial culture

Bacterial isolates were identified using a Bergy's manual.

Gram positive rods

Culture no.: R17

1.

↓

Spore forming (+)

↓

***Bacillus* spp. / *Clostridium* spp.**

↓

Strict Anaerobes (+)

***Clostridium* spp.**

2.

Culture no.: 19

↓

Spore forming (+)

↓

***Bacillus* spp. / *Clostridium* spp.**

↓

Strict Anaerobes (+)

***Clostridium* spp.**

3.

Culture no.: 22

↓

Spore formation (-)

***Corynebacterium* spp.**

***Lactobacillus* spp.**

***Mycobacterium* spp.**

↓

Catalase (+)

***Corynebacterium* spp.**

↓

Starch hydrolysis (+)

Corynebacterium kutsceri

4.

Culture no.:25

↓

Spore formation (-)

Corynebacterium spp.

Lactobacillus spp.

Mycobacterium spp.

↓

Catalase (+)

Corynebacterium spp.

↓

Starch hydrolysis (-)

Corynebacterium xerosis

5.

Culture no.: 26

↓

Spore formation (-)

Corynebacterium spp.

Lactobacillus spp.

Mycobacterium spp.

↓

Catalase (+)

Corynebacterium spp.

↓

Starch hydrolysis (+)

Corynebacterium kutsceri

6.

Culture no.: 28

↓

Spore formation (-)

Corynebacterium spp.

Lactobacillus spp.

Mycobacterium spp.

↓

Catalase (+)

Corynebacterium spp.

↓

Starch hydrolysis (-)

Corynebacterium xerosis

7.

Culture no.: 28

↓

Spore formation (-)

Corynebacterium spp.

Lactobacillus spp.

Mycobacterium spp.

↓

Catalase (+)

Corynebacterium spp.

↓

Starch hydrolysis (-)

Corynebacterium xerosis

Gram negative rods

1.

Culture no.: 11

↓

Oxidase test (-)

Enterobacteriaceae

↓

Lactose fermentation (+)

Citrobacter diversus, Citrobacter freundii, Enterobacter aerogenes, Enterobacter cloacae, Enterobacter amnigenus, Enterobacter intermedius, Erwinia carotovora, Erwinia chrysanthemi, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Serratia fonticola, serratia rubidaea

2.

Culture no.: 23

↓

Oxidase (+)

Aeromonas, Pseudomonas, Vibrio

↓

Glucose fermentation (+)

***Vibrio* spp., *Aeromonas* spp.**

3.

Culture no.: 14

↓

Oxidase (+)

Aeromonas, Pseudomonas, Vibrio

↓

Glucose fermentation (+)

Vibrio spp., *Aeromonas* spp.

4.

Culture no.: 10

Oxidase (-)

Enterobacteriaceae

↓

Lactose fermentation (+)

Citrobacter diversus, Citrobacter freundii, Enterobacter aerogenes, Enterobacter cloacae, Enterobacter amnigenus, Enterobacter intermedius, Erwinia carotovora, Erwinia chrysanthemi, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Serratia fonticola, serratia rubidaea

Gram positive cocci

1.

Culture no.: 6

↓

Catalase (+)

Micrococcus spp., Staphylococcus spp.

2.

Culture no.: 9

↓

Catalase (+)

Micrococcus spp., Staphylococcus spp.

3.

Culture no.: 16

↓

Catalase (+)

Micrococcus spp., Staphylococcus spp.

Gram negative cocci

Culture number 3, 13, 15 and 27 identifed as gram negative cocci , hence it identified as *Neisseria* or *Veillonella* sp

CHAPTER 5

DISCUSSION AND CONCLUSION

DISCUSSION

Nine bacterial isolates were examined as best nitrogen fixers as compared to 31 isolates. In their study, Liu et al. 2012) found that the isolates showing nitrogen fixation were the members of the genera Pseudomonas, Agrobacterium, Brevundimonas, Stenotrophomonas, Paenibacillus, and *Bacillus*. They reported, 44.4% of isolates belonging to the *Gammaproteobacteria Pseudomonas*, suggesting that the soil habitats found in the rhizosphere of mangrove plant acted as favorable ecological niches. Baskar and Prabakaran (2014) reported variation in nitrogen fixation rates observed among different species of bacteria associated with nitrogen fixation is also inhabited by several physical factor like light, temperature, and seasonal variations. In comparison to both these studies showed good results as compared to current study. Six bacterial isolates were examined as found to be best phosphate solubilizers among isolated 31 isolates. Culture 11 was the best solubilizer with 1.3mm clearance zone and 260% of phosphorus solubilization efficiency.

Vazquez et al. (1999) in their study showed the total phosphorus in the sediment of mangrove ecosystem was much higher than phosphorus in seawater and mainly composed of insoluble phosphorus and to lesser extent organic phosphorus. Marzban (2016) reported 13 colonies exhibiting P solubilizing activity among 86 colonies isolated. Four colonies (PSB1, PSB3, PSB10 and PSB12) shows maximum P solubilizing potential on agar medium, comparison maximum determined for PSB1 and PSB3 which were 3.5 and 2.6, respectively. In comparing my data with this two research studies my data shows better compared with one of the research paper.

In the present study, gibberellin production was highest in culture 22 with 0.3516nm absorbance. In a study report by Sharma et al (2017), GA_3 was extracted with ethyl acetate and purified and characterized by TLC and estimated via high performance liquid chromatography. The product extracted was found to be 116.1-485.8µg/ml. While Gunjal and Kapadnis (2013) reported highest gibberellin production to be 5.57±0.03µg/ml. The Gibberellins have been reported to play significant role in increasing the root surface area and number of root tips in many plants.

Maximum precipitate of EPS was found to be in cultures 8,10,13,19,22,23 and 28, showing that these cultures produce higher amount of polysaccharides. Ara et al (2023) focused on investigation of Exopolysaccharide (EPS) producing bacteria, they isolated thirteen isolates. 16S rDNA sequence analysis was conducted and were indentified as *Micrococcus* sp., *Bacillus subtilis*, *B. strearothermophilus*. Gunjal and Kapadnis (2013) investigated *Burkholderia gladioli* was found that it produced about 0.98 of EPS. More exopolysaccharide production was seen in these two research papers compared to the data obtained.

IAA was determined maximum in culture number 24 i.e 0.6252 at absorbance 540nm. Nathan et al. (2017) study, *T.viride* VKF3, a mangrove isolate showed high production of IAA and it was confirmed through Thin layer chromatography and High performance liquid chromatography. In Ratnanigsih et al. (2023) study investigates 171 isolates from the rhizosphere of a pineapple, of which 73 isolates produce indole acetic acid, the lowest IAA was detected on CHTJ 3E with the value of 1.8mg L-1 whereas the highest was achieved by control TPK 5B with the value of 43 mg L^{-1} . Comparing of both these two studies neither TLC nor HPLC was used in my data.

Siderophore production determined maximum in culture number 18 having 13mm clear zone. Gupta and Gopal (2008) found that out of ten PGPR isolates only six were found to produce siderophore. *Pseudomonas fluorescens* followed by *Enterobacter* sp., *Pseudomonas* sp., *Enterobacter* sp., *Azispirillum brasilense* and *Brevibacillus brevis*. Gull and Hafeez (2012) investigated 28 *Pseudomonas* strains for siderophore production, among 28 strains tested 14 were found to be siderophore producers. These strains were evaluated for their biocontrol potential against *Rhizoctonia salani* using various dual culture assays.

In the present study, all 31 bacterial isolates showed ammonia production. Weise et al. (2013) demonstrated the potential of ammonia emission by rhizobacteria and its consequences for the growth and development of *Arabidopsis thaliana* in volatile mediated co-cultivations with bacteria. Abdelwahed et al. (2022) investigated the ability of PGPR to produce ammonia by ammonia microplate estimation assay; among 9 bacterial isolates, only two, *Bacillus inaquosorum* and *Bacillus mojavensis*, produced the highest ammonia concentration of about 371 and 370µM. Kyaw et al. (2021) investigated all selected bacterial isolates; 20 isolates were seen to produce chitinase, and the highest discoloration was found in isolate SAK6 with a zone the diameter of 75mm. For β-glucanase enzyme the highest producer strain was BP2, MAP5, B3, B5, S3, S4 and WK5. For amylase among 39 isolates 24 isolates shows positive. 27 isolates produce pectinase enzyme while 37 out of 39 isolates produce cellulase. However, in the present study, none of the isolates showed enzyme activities tested.

For pot assay, nitrogen fixing isolates and IAA producing isolates showed faster growth with treatment of seeds and soil. Sezen et al (2016)

investigated the plant growth promoting potentials of isolates bacteria were tested in both pot experiment and field trials, the growth of wheat was influenced by bacterial inoculation in pot experiment; AS1, AS3, AS4 and AS8 were most effective strains and they stimulate shoot growth of wheat as 24.32%, 20.94%, 13.51% and 33,78% these four isolates enhanced the growth in all measured parameters. Results of field experiment showed that PGPR inoculants were lower than that of nitrogen application, inoculation significantly enhanced both biomass and grain yields and protein content. Strain AS8 provided 18.27% and 17.58% biomass and grain yields more than uninoculated control. In comparison of these research study my data gave good results with respect to parameter aimed.

CONCLUSION

- The bacteria isolated from the mangroves showed beneficial effects on plant growth promoting rhizosphere. PGPR improves the soil fertility by increasing plant nutrients by providing nitrogen, phosphate, gibberellin, exopolysaccharide, indole production, siderophore production and enzyme producing activity by bacteria available in soil.
- Total 31 morphologically distinct colonies were isolated on sterile nutrient agar and isolates were characterized based on their morphology and Gram character.
- Culture number 3, 6, 13,15, 16, 17, 21, 27 and 31 were the best nitrogen fixing organisms.
- The highest phosphate solubilization was found in culture number 11 having 13mm clear zone and 260% of phosphate solubilization efficiency as compared to others.
- The production of Gibberellin was more for culture number 22 i.e. 0.3516 at absorbance 254nm.
- The amount of EPS produced more for culture number 8,10,13,19,22,23 and 28.
- Screening of IAA was determined maximum in culture number 24 i.e 0.6252 at absorbance 540nm.
- Screening of siderophore was determined maximum in culture number 18 having 13mm clear zone.
- Out 31 bacterial culture all isolates shows good growth for ammonia production.

- For pot assay nitrogen fixing isolates and IAA producing isolates shows maximum and faster growth with treatment of seeds and treatment with soil.
- Based on biochemical characterization cultures were identified as *Clostridium* species, *Corynebacterium kutsceri*, *Corynebacterium xerosis*, *Vibrio* spp., *Aeromonas* spp., *Micrococcus* spp., *Staphylococcus* spp., *Neisseria sp and Veillonella* sp.
- Rhizobacteria are best alternatives to use for plants instead of chemical fertilizers and pesticides as it enhances plant growth due to their many modes of action that start with the ability to colonize both the intracellular and extracellular rhizosphere niche in their search of carbon source. Thus, this technology based on PGPR use, should be integrated into agricultural production strategies of all countries to a healthy and sustainable agriculture.

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APPENDIX

1. **Nutrient agar**

Ingredients	Grams/Litre
Peptone	5.000
Sodium chloride	5.000
HM peptone B#	1.500
Yeast extract	1.500
Agar	15.000
Final pH (at 25°)	7.4±0.2

- Equivalent to Beef extract

2. **Pikovskaya agar**

Ingredients	Grams/Litre
Yeast extract	0.500
Dextrose	10.000
Calcium phosphate	5.000
Ammonium sulphate	0.500
Potassium chloride	0.200
Magnesium sulphate	0.100
Manganese sulphate	0.0001
Ferrous sulphate	0.0001
Agar	15.000

3. **Ashby's mannitol agar**

Ingredients	Grams/Litre
Mannitol	20.000
Dipotassium hydrogen phosphate	0. 200

Magnesium sulphate	0. 200
Sodium chloride	0.100
Potassium sulphate	0.100
Calcium carbonate	5.000
Agar	15.000
Final pH (at 25°C)	7.4±0.2

4. Skim Milk powder

Ingredients	**Grams/Litre**
Casein enzymic hydrolyste	5.000
Yeast extract	2.500
Skim milk powder	1.000
Glucose	1.000
Agar	10.500
Final pH (at 25°C)	7.0±0.2

5. Salkowski Reagent

Ingredients	**Grams/Litre**
0.5 M Ferric chloride	15 ml
Distilled water	500 ml
Conc. Hydrogen sulfate	300 ml

6. Nutrient broth

Ingredients	**Grams/Litre**
Peptone	10.000
Beef extract	10.000
Sodium chloride	5.000

pH after sterilization	7.3±0.1

7. Luria Bertani Agar

Ingredients	**Grams/Litre**
Tryptone	10.000
Yeast extract	5.000
Sodium chloride	10.000
Agar	15.000
Final pH (at 25°C)	7.5±0.2

8. Peptone Water

Ingredients	**Grams/Litre**
Peptone	10.000
Sodium chloride	5.000
Final pH (at 25°C)	7.2±0. 2

9. Nitrate broth

Ingredients	**Grams/Litre**
Peptic digest of animal tissue	5.000
Beef extract	3.000
Potassium nitrate	1.000
Final pH (at 25°C)	7.0±0.2

10.Huge Leifson medium

Ingredients	**Grams/Litre**
Peptone	2.000

Sodium chloride	5.000
Dipotassium phosphate	0.300
Glucose	10.000
Bromothymol blue	0.050
Agar	2.000
Final pH (at 25°C)	6.8±0.2

11. Simmon's Citrate Agar

Ingredients	**Grams/Litre**
Magnesium sulphate	0.200
Ammonium dihydrogen phosphate	1.000
Dipotassium phosphate	1.000
Sodium citrate	2.000
Sodium chloride	5.000
Bromothymol blue	0.080
Agar	15.000
Final pH (at 25°C)	6.8±0.2

12.MR-VP Broth

Ingredients	**Grams/Litre**
Buffered peptone	7.000
Dextrose	5.000
Dipotassium phosphate	5.000
Final pH (at 25°C)	6.9±0. 2

13. Barritt's reagent A

Ingredients	**Grams/Litre**

a-naphtholin	6g
95% ethyl alcohol	100ml

14. Barritt's reagent A

Ingredients	**Grams/Litre**
Potassium hydroxide	16 g
Distilled water	100ml

15.Gram's iodine

Ingredients	**Grams/Litre**
Iodine	1g
Potassium iodide (KI)	2g
Distilled water	300 ml

16.Crystal violet

Ingredients	**Grams/Litre**
Solution A	
Crystal violet (90% dye content)	2g
Ethanol, 95%	20 ml
Solution B	
Ammonium oxalate	0.8g
Distilled water	80 ml

Mix solutions A and B. Store 24 h and filter through coarse filter paper.

17. Kovac's reagent

Ingredients	Grams/Litre
p-Dimethylaminobenzaldehyde	5g
Isoamyl alcohol	75 ml
Hydrochloric acid	25 ml

18. Methyl red

Ingredients	Grams/Litre
Methyl red	0.200 gm
Ethyl alcohol	60.000 ml
Distilled water	40.000 ml

19. Phenol red

Ingredients	Grams/Litre
Phenol red sodium salt	0.04 gm
Distilled water	100 ml

20. Malachite green

Ingredients	Grams/Litre
Malachite green crystals	0.5g
Distilled water	10 ml

Stir until crystals are dissolved, filter to remove residual crystals.

21. Chrome azurol S

Ingredients	Grams/Litre
CTAB (stock)	6 ml
Distilled water	40 ml

Ferric chloride (stock)	1.5 ml
Hydrochloric acid	6.5 ml
Anhydrous piperazine	4.3g