Isolation of Plant Growth Promoting Bacteria from Rice Fields of Goa

A Dissertation for

Course code and Course Title: GBT 651- Dissertation

Credits: 16

Submitted in partial fulfilment of Master's Degree

M.Sc. in Biotechnology

by

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22P0470006

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DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, **"Isolation of Plant Growth Promoting Bacteria from Rice Fields of Goa"** is based on the results of investigations carried out by me in the Biotechnology Discipline at School of Biological Sciences and Biotechnology, Goa University under the Supervision of Mrs. Dviti Volvoikar and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will be not be responsible for the correctness of observations / experimental or other findings given the dissertation.

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Place: Goa University

COMPLETION CERTIFICATE

This is to certify that the dissertation report "**Isolation of Plant Growth Promoting Bacteria from Rice Fields of Goa**" is a bonafide work carried out by Ms. Joleta D'Costa under my supervision in partial fulfilment of the requirements for the award of the degree of Master of Science in the Biotechnology Discipline at the School of Biological Sciences and Biotechnology, Goa University.

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CONTENT

PREFACE

In the quest for sustainable agricultural practices, the spotlight shines brightly on plant growth-promoting rhizobacteria (PGPR), the unsung heroes dwelling in the soil. The rich biodiversity of Goa's soil offers a promising environment for the isolation and study of plant growth-promoting rhizobacteria (PGPR). This preface delves into the significance of PGPR in sustainable agriculture, highlighting their role in enhancing plant growth, nutrient uptake, and stress tolerance.

The primary objective of this study was to isolate Plant growth promoting rhizobacteria from the fields of Goa and exploiting them for their various plant growth promoting abilities. The main focus was isolating PGPR from rice fields which are less explored and can be useful in promoting plant growth and as an essential substitute for the chemical fertilizers which have a bad impact on our environment. Hence this topic sets the stage for the exploration of Goa's fields as a potential reservoir for novel PGPR strains, aiming to contribute to agricultural sustainability and productivity in the region and offering a glimpse into a greener, more resilient future for us.

ACKNOWLEDGEMENT

The best way to pursue happiness is to help other people. Nothing else will make you happier – George Lucas

After the successful completion of this dissertation, I am highly elated to thank all those who guided me in the right direction towards conceptualization and completion of my endeavour.

My warm and heartfelt thanks to our discipline for providing me with the necessary infrastructure and laboratory facilities. I would also like to convey a note of gratitude to our Dean Mr. Bernard F. Rodrigues, Vice Dean Dr. Sanjeev Ghadi, for their constant support and encouragement.

I would like to express my sincere gratitude to my project guide, Mrs. Dviti Volvoikar for her continuous guidance, cooperation, and encouragement throughout the project and for contributing her valuable time in to giving it a successful closure.

I would like to thank Goa University Science Instrumentation Centre for helping me to carry out the SEM analysis.

I would like to extend my gratitude to all the faculty members, office staff, Mrs. Sanjana and research scholars of the Biotechnology Discipline for their constant help, support and encouragement.

I am also thankful to Mr. Redualdo Serrao, Laboratory technician, Mrs. Sandhya Pankaj Singh, senior laboratory assistant and Mr. Ashish, Mr. Sameer and Mrs. Jaya, laboratory attendants, for providing the requirements associated with the project and helping me throughout.

Lastly, I would like to thank the almighty, my family and friends for their constant support and unconditional encouragement towards the fulfilment of my project.

TABLES

FIGURES

ABBREVIATIONS USED

Entity	Abbreviation Used
Citrate Utilization	CU
Carboxy Methyl Cellulose	CMC
Chrome azurol S	Cas
Degree Celsius	ºC
Distilled Water	DW
Gram(s)	G
Hydrogen Cyanide	HCN
Indole	I
Indole Acetic Acid	IAA
Methyl Red	MR
Microlitre(s)	µL
Millilitre(s)	mL
Minute(s)	Min
Nano Meter(s)	nm

Entity	Abbreviation Used
Number(s)	No.
Nutrient Agar	NA
Phosphate Buffer Saline	PBS
Plant Growth Promoting Properties	PGPP
Plant Growth Promoting Rhizobacteria	PGPR
Revolutions per minute	rpm
Room Temperature	RT
Scanning Electron Microscope	SEM
Serial	Sr.
Sterile Distilled Water	SDW
Sulfur Indole Motility	SIM
Vigor Index	VI
Vogues-Proskauer	VP

ABSTRACT

Plant growth promoting rhizobacteria can reduce the use of chemical fertilizer in agriculture as it has the potential to directly improve plant growth. The objective of my research is screening, characterization and selecting the the effective PGPR to be used as a bioinoculum for rice production. Four efficient strains namely JDDM14, JDDM53, JDDM67 and JDDM70 were screened and identified as the isolates with best plant growth promoting abilities.

The strain JDDM14 had nitogen fixing, phosphate solubilizing, ammonia production, indole acetic acid production, HCN production and siderophore production ability while strain JDDM70 had nitrogen fixing, phosphate solubilization, siderophore production and indole acetic acid production ability. Followed by these two strains were JDDM53 with phosphate solubilization, indole acetic acid production and HCN production ability and JDDM67 with nitrogen fixation, phosphate solubilization, siderophore production and HCN production ability.

Rice seed germination was carried out in combining the above mentioned two strains each and also by combining all four strains to check the most effective combination for rice field application. The combination of all the four strains was very much effective with good germination index hence proved to be an efficient substitute to chemical fertilizer.

CHAPTER 1

INTRODUCTION

1.1) Background

1.1.1) Rhizosphere

Rhizosphere is the zone of soil surrounding the roots while rhizobacteria implies to those bacteria residing in this rhizosphere Plant root in addition to taking up nutrient and water, they also secrete certain compounds in the soil known as root exudates which act as attractant to certain group of micro-organisms while repel the others. These compounds are taken up by the rhizospheric microbes as carbon and nitrogen source and in return the plant takes up compounds secreted by the microbes for its better growth and development (Ahemad & Kibret, 2014).

1.1.2) Plant Growth Promoting Rhizobacteria

Overuse of chemical fertilizers has been significantly impacted the rhizosphere microflora, which has decreased soil production and nutrient usage efficiency. A class of free-living bacteria known as plant growth-promoting rhizobacteria (PGPR) inhabits the rhizosphere and promotes root growth (Podile & Kishore, 2006). Plant Growth Promoting Rhizobacteria (PGPR) is a biocontrol agent that can produce plant growth hormones, solubilize phosphorus, and act as an alternative to artificial fertilizers. When applied as seed or crop inoculation, PGPR—free-living soil bacteria isolated from the rhizosphere—promote plant growth and yield through a variety of mechanisms, including the production of phytohormones, the provision of available nitrogen through biological nitrogen fixation, the availability of phosphorus through phosphate solubilization, and the suppression of phytopathogens. (Lavakush et al., 2012).

1.1.3) Mechanism of action of PGPR

Mechanism of action of PGPR is of two ways: Direct and Indirect Mechanism. Indirectly, PGPR promote plant growth through rhizosphere competition, induced systemic

resistance (ISR), and the biosynthesis of stress-related phytohormones like jasmonic acid (JA), cadaverine (Cad), or the enzyme related to ethylene catabolism. Directly, PGPR facilitate resource acquisition like nitrogen, phosphorus, and essential minerals via biological nitrogen fixation, phosphate solubilization, and iron sequestration by siderophore, respectively.

1.1.4) Rice

Nitrogen is the most important input needed to produce rice, which is the most important staple crop in the developing world. Utilizing diazotrophic bacteria that can release biologically fixed nitrogen for rice plant growth is necessary to make rice farming sustainable and less reliant on nitrogen from fertilizers(Verma, 2001). Chemical fertilizers are currently used extensively in rice production, which puts rice-producing areas at risk for environmental damage (Wartiainen et al., 2008). Nonetheless, in order to prevent environmental degradation and enhance environmental quality, agricultural systems have evolved. Adding biological agents to conventional chemical fertilization techniques is a common strategy used in integrated plant nutrient management systems to increase crop yields. In this sense, PGPR might play a part in creating crop production systems that are sustainable.

1.1.5) Application of PGPR

Nowadays a lot of farmers are dependent on chemical fertilizers for the better yield of rice. These fertilizers used are not only costly but also have an impact in the depletion of natural flora and also the natural properties of soil. Hence this plant growth promoting bacteria could be used as a biofertilizer which will not only reduce the expenses but also promote plant growth with no harm to the environment.

1.1.6) Biofertilizer

Biofertilizer is a substance which contains living microorganisms which, when connected to seed, plant surfaces, or soil, colonize the rhizosphere or the insides of the plant and advance development by expanding the supply or accessibility of essential supplements to the have plant (Basu et al., 2021).

1.1.7) Formulation of biocarrier

An important aspect of PGPR application in field setting is the development of a suitable carrier to carry the desired isolates in solid state which could preserve the microbial stability for a long period of time. Taking into account the consumers point, it should be delivered similar to that of a chemical fertilizer for seed treatment and soil application. Many studies have proved the stability of the bioinoculum with the carrier in the powdered state (Ramamoorthy et al., 2001).

1.2) Aim and Objectives

Aim

To isolate plant growth promoting rhizobacteria from the fields of Goa and to check for its efficacy as a biofertilizer

Objectives

- Isolation and identification of plant growth promoting Rhizobacteria
- Screening the isolates for plant growth-promoting abilities.
- Development of a standard carrier for PGPR inoculum
- To develop a bioinoculum consortium and to find an alternative to chemical fertilizer to promote plant growth in rice

1.3) Hypothesis/ Research Question

In the present research, the hypothesis includes:

- An isolate which exhibits maximum PGPR properties
- Desired Carrier using coconut oil cake
- Plant growth promotion of rice using the formulated bio inoculum

1.4) Scope

PGPR play an important role promoting plant growth. This study mainly focuses on isolation of PGPR which not only possesses maximum plant growth promoting properties but which can also contribute to sustainable agriculture by reducing the need for chemical fertilizers and pesticides, thus mitigating environmental impacts.

be an economically feasible biofertilizer rather than the use of chemical fertilizer which is not only expensive but also has a bad impact on the environment. The benefit of this finding could include:

PGPR strains isolated from fields that are well-adapted to local environmental conditions, increasing their potential for successful colonization and plant growth promotion.

Some PGPR strains could also exhibit biocontrol properties against plant pathogens and pests, providing opportunities for integrated pest management strategies.

Isolated PGPR strains can be further studied for their genetic and biochemical traits, leading to the development of novel biotechnological applications in agriculture.

Effective PGPR strains can be incorporated into farming practices through inoculants, biofertilizers, or other delivery methods, benefiting farmers and ecosystems.

Overall, the scope for isolating PGPR from fields is vast and holds great potential for improving crop productivity, soil health, and sustainability in agriculture.

CHAPTER 2

LITERATURE REVIEW

2.1) What is PGPR?

Rhizobacteria that are either directly or indirectly involved in promotion of plant growth are known as plant growth promoting rhizobacteria. Rhizobacteria promote plant growth release compounds such as mineral and organic acids, phosphatases, and gibberellins, which in turn enhance crop growth.(Adnan et al., 2020)

Most of the plant growth promoting rhizobacteria are known to be to be gram negative rods with very less portion of gram positive rods, cocci or pleomorphic (Ahemad & Kibret, 2014). Plant growth is benefited by PGPR because it lowers the numbers of harmful bacteria and root diseases in the rhizosphere(Podile & Kishore, 2006). Studies on Plant Growth-Promoting Rhizobacteria (PGPR) in non-legumes, like rice, have demonstrated positive effects through biological N2 fixation (Malik et al., 1997), better root growth (M.A. Baset Mia, 2012) with improved nutrient uptake (Yanni et al., 1997), the production of phytohormones (Chabot et al., 1996), and disease prevention (Ramamoorthy et al., 2001).

Bacillus megaterium DD-2, Bacillus aryabhattai DD-3 and *Bacillus subtilis DD-4* were isolated from Rice field of organic Farm in Jiamusi district in northeastern China and they showed PGPR properties and enhanced the growth of rice (Liu et al., 2022).

2.2) Mechanisms of plant growth promotion

2.2.1) Direct mechanisms

Acquiring Nutrients: Certain microbe-oriented molecules are reabsorbed by plants for growth and development, while a portion of these plant-derived small organic molecules

are further broken down by nearby microorganisms as sources of carbon and nitrogen.(Kang et al., 2010).

Nitrogen fixation: Nitrogen is an important element in the growth and development of plants. Our atmosphere comprises of about 78.8% of nitrogen even then it is unavailable for the plants. A certain group of microbes known as nitrogen fixers fix nitrogen into plant utilizable biological form of nitrogen by using a complex enzyme system known as nitrogenase (Ahemad & Kibret, 2014). Diazotrophs(Rhizobacteria that fix N2 in non-leguminous plants) are plant growth-promoting rhizobacteria that fix nitrogen in non-leguminous plants and can interact with their hosts non-obligatorily(Glick et al., 1999). The majority of biological nitrogen fixation is facilitated by the activity of diazotrophs' molybdenum nitrogenase (Bishop & Joerger, 1990). In a study done by Vessey, it is evident that *Azospirillum sp* and *Cyanobacteria* can stimulate the plant growth by their ability to fix atmospheric nitrogen (Vessey, 2003).

Phosphate solubilization: In both organic and inorganic forms, phosphorus (P), the second most significant nutrient that limits plant growth after nitrogen, is widely available in soils(Khan et al., 2009). Rhizobacteria can increase crop plant growth and yield by solubilizing inorganic P sources. It has been observed that PGPRs solubilize precipitated phosphates in plants, suggesting a potential mechanism for promoting plant growth in field settings(Verma, 2001). In field settings, rhizobacteria's capacity to solubilize precipitated phosphates and increase rice's phosphate availability may be a mechanism for promoting plant growth.(Verma, 2001). *Azotobacter chroococcum* is one of the commonly known P-solubilizing microbial species that is closely linked to a variety of agricultural crops, including potato, tomato, wheat, and radish(Kumar et al., 2001).

Siderophore Production: One of the most important nutrients for all life forms is iron. In aerobic environment, iron usually occurs as Fe^{3+} and form insoluble oxyhydroxide and hydroxides which cannot be taken up by plants nor micro-organisms. But bacteria have the ability to secrete iron chelators known as the siderophores. Siderophores are mostly water-soluble hence they increase the soluble metal concentration when bound to metal (Ahemad & Kibret, 2014). In a study researchers found that PGP characteristic that may have an impact on plant growth is the generation of siderophores, which bind to the accessible iron form (Fe3+) in the rhizosphere. Iron is rendered inaccessible to the phytopathogens by this process. The siderophore simultaneously safeguards the health of the plant.(Siddiqui, 2006)

Phytohormone production: By producing various phytohormones such as gibberellic acid, cytokinin, and IAA, PGPR can modify root architecture and stimulate plant development(Kloepper et al., 2007). Plant cell division, extension, and differentiation are influenced by IAA. It also promotes germination of seeds and tubers, speeds up the development of xylem and roots, regulates vegetative growth processes, starts the formation of lateral and adventitious roots, mediates responses to light, gravity, and florescence, and influences pigment formation, photosynthesis, the biosynthesis of different metabolites, and resistance to stress. By altering the plant auxin pool, IAA produced by rhizobacteria probably messes with the aforementioned physiological processes of plants. Additionally, bacterial IAA lengthens and increases the surface area of the roots, giving the plant better access to nutrients in the soil. Additionally, rhizobacterial IAA relaxes plant cell walls, which encourages more root exudation and supplies extra nutrients to support rhizosphere bacterial growth(Parray et al., 2016). Plant growth promoting rhizobacteria (PGPR) have been shown to produce IAA in a variety of ways depending on the species and strain additionally the availability of substrate,

development stage, and culture conditions have all been found to have an impact.(Sajjad Mirza et al., 2001). Rhizobacteria synthesizes IAA, a crucial hormone that promotes plant growth and causes root elongation, root hair, and lateral root proliferation.(Wang et al., 2016). It has been reported that a number of PGPR and certain pathogenic, symbiotic, and free-living rhizobacterial species produce gibberellic acid and IAA in the rhizospheric soil. As a result, they are important for increasing the number of root tips and root surface area in many different types of plants(Han et al., 2005). In a study done by Vessey, it is evident that *Aeromonas veronii* and *Enterobacter cloacae* stimulate plant growth in rice due to their ability if indole acetic acid production (Vessey, 2003).

ACC Deaminase Activity: Rhizobacteria that support plant growth and have the enzyme 1-aminocyclopropane-1-carboxylate (ACC) Deaminase promotes salt tolerance, lowers drought stress in plants, and lowers ethylene levels to aid in the growth and development of plants(Nadeem et al., 2007).

2.2.2) INDIRECT MECHANISM

Serving as biocontrol agents is the main indirect way that rhizobacteria promote plant growth, The main mechanisms of biocontrol activity in PGPR are nutrient competition, niche exclusion, induced systemic resistance, and the generation of antifungal metabolites (Lugtenberg & Kamilova, 2009). With indirect mechanisms, antifungal metabolites such as phenazines, pyrrolnitrin, 2,4-diacetylphloroglucinol, pyoluteorin, viscosinamide, and tensin have been reported to be produced by a variety of rhizobacteria(Bhattacharyya & Jha, 2012).

2.3) Characteristics of an ideal PGPR

If a rhizobacterial strain has particular characteristics that promote plant growth and has the ability to increase plant growth after inoculation, it is regarded as a putative PGPR. The following requirements should be met by the perfect PGPR strain:

- It ought to be extremely eco-friendly and rhizosphere-competent.
- After being injected, it should significantly multiply and colonize the plant roots.
- It ought to have the capacity to encourage plant growth.
- Its range of action should be extensive.
- It ought to get along with other rhizosphere bacteria.
- It must be resistant to a variety of physicochemical conditions, including heat, desiccation, radiation, and oxidants.
- It ought to exhibit enhanced competitive abilities in comparison to the current rhizobacterial communities.

(Basu et al., 2021)

2.4) Present research status:

Plant growth-promoting rhizobacteria have evolved a number of ways to immobilize, mobilize, or transform metals so they can no longer be absorbed by heavy metal ions and survive in a metal-stressed environment.(Parray et al., 2016)

The study by Parray demonstrated that bacterial inoculation of rice (Oryza sativa) improved salt tolerance by lowering toxicity from reactive oxygen species, which in turn improved cell viability by lowering the plant cell membrane index, cell caspase-like protease activity, and programmed cell death.(Parray et al., 2016)

Numerous investigators have exhibited the beneficial function of rhizobacteria in mitigating the adverse effects of salinity on crop growth in both laboratory and field settings.(Parray et al., 2016).

Phosphate Solubilizing Bacteria (PSB) have been inoculated into various crops to increase soil phosphorus content and improve plant growth and yield(Lavakush et al., 2012).

According to early research on systemic tolerance to drought, pepper plants' ability to withstand drought was improved by PGPR Bacillus licheniformis K11 inoculation.(Parray et al., 2016)

Several investigations have been published on the relationship between siderophore-mediated Fe-uptake and the promotion of plant growth following siderophore-producing rhizobacterial inoculations(Rajkumar et al., 2010). (Sharma et al., 2003) evaluated the impact of Pseudomonas strain GRP3, which produces siderophores, on Vigna radiata's iron nutrition. After 45 days, the plants' chlorotic symptoms decreased, and when compared to controls, the iron, chlorophyll a, and chlorophyll b content of the strain GRP3-inoculated plants increased.

In the study done recently, three isolates of growth-promoting bacteria *Bacillus megaterium DD-2, Bacillus aryabhattai DD-3*, and *Bacillus subtilis DD-4* were isolated and identified from rice rhizosphere soil. All of the isolates could dissolve phosphorus and potassium. Every isolate demonstrated the capacity to produce siderophores, gibberellic acid, and IAA, all of which have the potential to greatly accelerate rice growth(Liu et al., 2022).

The potent PGPR strains for biofertilizers are said to be *Allorhizobium, Azorhizobium*, *Bradyrhizobium, Mesorhizobium, Rhizobium*, and *Sinorhizobium*(Vessey, 2003).

Bacterial isolate SJ-5 significantly increased shoot and root length, shoot and root dry weight, and lateral root number when applied as a bioinoculant to soyabean plant (Jain et al.,2016).

In a recent study researchers isolated a strain called *Pantoea dispersa K4NRR2AY011* which gave positive results for PGPR propereties and when applied to cowpea plants showed an increase of 43.15% and 19.91% in VI compared to the control (Shet & Garg, 2022).

Isolate ABSK9, ABSK29, ABSK186 identified as *Pseudomonas multiresinivorans, Microbacterium esteraromaticum* and *Bacillus subtilis* when added individually and in combination with farmyard manure significantly improved rice plant growth parameters (Bhambure et al., 2018).

In a study carried out by De Souza et al., all of the bacterial isolates in an investigation were found to be capable of producing indolic compounds, siderophores, and solubilizing phosphate. Through the synthesis of siderophores, which are created by aerobic bacteria and fungi growing in low-iron environments, PGPR can either directly or indirectly affect plant growth. According to data, rhizospheric bacteria's excretion of siderophores may improve plant nutrition or prevent phytopathogens from accessing Fe, thereby promoting plant health. In this study a total of 284 isolates (84%) out of 336 isolates were found to produce siderophores and also similar numbers of bacteria that produce siderophores have been reported by other studies. The most frequent mechanism of action linked to PGPR to boost host plant nutrient availability is the solubilization of P in the rhizosphere. Only 101 (30%) of our 336 isolates were found to be phosphate solubilization capable. Out of all the isolates in the current study, strains of *Burkholderia* and *Enterobacter* showed the most traits that promoted plant growth. Furthermore, 96 isolates out of 336

were able to simultaneously produce IC, siderophores, and solubilize phosphates(De Souza et al., 2013).

2.5) Biofertilizer

The term "biofertilizers" was later known an item (carrier or fluid based) containing living or dormant organisms (microscopic organisms, actinomycetes, organisms, green growth) alone or in combination, which offer assistance in fixing the atmospheric nitrogen or solubilizers soil supplements in expansion to the discharge of development advancing substances for upgrading plant development and yield. Furthermore, biofertilizers can proliferate, take part in the cycling of nutrients, and aid in crop production for sustainable farming when applied as seed or as soil inoculants. Compared to their chemical counterparts, microbial inoculants have a number of advantages. They are reliable, environmentally friendly sources of renewable nutrients needed to preserve the biology and health of the soil. Additionally, they fight abiotic stresses and demonstrate antagonistic activity against a number of agricultural pathogens. A wide range of microbial taxa have been employed in the commercial sector as effective biofertilizers due to their capacity to extract nutrients from the soil, fix atmospheric nitrogen, promote nutrient solubilization, and function as biocontrol agents (Basu et al., 2021).

2.6) Carrier

Research on the survival of *Rhizobium* bacteria in agricultural by-product carriers, such as sawdust, peanut shell, and paddy husk, has demonstrated that paddy husk is a more cost-effective and alternative carrier than sawdust and peanut shell. According to reports,

press mud and bagasse were used as a carrier for *Azospirillum* species after being composted using vermicomposting techniques (Yasmin et al., 2016).

CHAPTER 3

METHODOLOGY

3.1) SAMPLING

The sampling for the plant growth promoting bacteria was carried out at the following areas:

- The Jaya rice variety sample was collected from a rice field in Macasana Goa
- The salt tolerant rice variety sample (local name: Asgo) was collected from a rice field at Loutolim Goa.

3.2) ISOLATION

3.2.1) Jaya Rice Variety

Rice rhizosphere soil attached to the roots of rice plants was collected and 1g of soil was dissolved in 9ml of 0.85%, then serially diluted in 0.85% saline up to 10^{-7} dilutions. The last three dilutions i.e 10^{-5}, 10^{-6} and 10^{-7} were spread plated on nutrient agar medium to obtain isolated colonies.

3.2.2) Salt tolerant Rice Variety

Rice rhizosphere soil attached to the roots of rice plant was collected and 1g of soil was dissolved in 9ml of 0.85%, then serially diluted in 0.85% saline up to 10^{-7} dilutions. The last three dilutions i.e 10^{-5}, 10^{-6} and 10^{-7} were spread plated on nutrient agar medium in the presence of 5% and 8% salt to obtain isolated colonies.

Isolated colonies with different characteristic were then pure cultured. A total of 70 isolates were pure cultured and maintained for further use.

3.3) SCREENING FOR PLANT GROWTH PROMOTING BACTERIA

The 70 isolates obtained were then screened for various plant growth promoting attributes including nitrogen fixation, phosphate solubilization, siderophore production, IAA production, HCN production and ammonia production.

3.3.1) Nitrogen Fixation

To determine the nitrogen fixers the isolates were streaked on Jensen's nitrogen free agar medium. A total of 70 isolates were streaked on the Jensen's nitrogen free media. Growth of the isolates on this media indicates them being nitrogen fixers.

3.3.2) Phosphate solubilization

The ability of the isolates to solubilize the insoluble phosphate and to make it available for plant growth was determined by spot inoculating the isolates on Pikovsksya's agar medium. A total of 70 isolates were spot inoculated on plates containing Pikovskaya's agar medium. Positive result was determined on the basis of halo zone formation around the isolates.

3.3.3) Siderophore Production

Isolates were spot inoculated onto Chrome azurol S dye incorporated in kings B medium, and the plates were incubated at 28°C for 48–72 h. Development of a yellow, orange or violet halo around the bacterial colony was considered to be positive for siderophore production.

3.3.4) Indole Acetic acid production

An aliquot of each bacterial suspension was cultured in a liquid NB medium supplemented with 1 g/L L-tryptophan, then incubated at 28 °C on a rotary shaker for 4 days. The cultures were then centrifuged for 20 min at 5000 rpm. Later 1 mL of the cell-

free culture supernatant was mixed with 2 mL of Salkowski's reagent and color development was observed. The pink-red color formation indicates IAA production, while a yellow color formation or no colour change indicates a negative result (Jabiri et al., 2023).

3.3.5) HCN Production

To determine the ability of the isolates to produce hydrogen cyanide the isolates were streaked on nutrient agar slants including 4.4gL^{-} glycine and a filter paper dipped in 0.5% picric acid and 2% sodium carbonate was placed in the tubes containing nutrient agar slants. A total of 70 isolates were screened for HCN production. Change in the colour of the filter paper from yellow to orange red indicated positive result.

3.3.6) Ammonia Production

To determine the ability of isolates to produce ammonia, the isolates were inoculated in 10 ml of peptone broth and incubated for 72 hours at room temperature. 0.5 ml of Nessler's reagent was added to each tube and development of deep yellow to brown colour indicated positive result (Mohammed & Lemma, 2023).

3.4) ISOLATES SELECTED BASED ON SCREENING FOR PLANT GROWTH PROMOTING PROPERTIES

Out of the 70 isolates, 4 isolates i.e JDDM14, JDDM53, JDDM67 and JDDM70 were selected based on their maximum plant growth promoting properties. The 4 isolates were used for further study.

3.5) COLONY CHARACTERISTICS

JDDM14, JDDM53, JDDM67 and JDDM70 isolate were streaked on nutrient agar plates and incubated for 24 hours at room temperature. Later they were observed and their colony characteristics were noted. In addition, gram staining and motility of the isolates was also determined.

3.5.1) Gram staining: A loop was touched to the isolate colony and a smear of the same was made on the slide having a drop of saline. The slide was air dried and flooded with crystal violet stain for 1 minute. The slide was washed with water followed by flooding the slide with gram's iodine for 1 minute. The slide was then washed with water and rinsed with gram's decolouriser. Then the slide was flooded with safranin for 45 seconds. Washed and then air dried and then observed under 100X magnification.

3.5.2) Test for motility: SIM media was prepared, autoclaved and poured in tubes to make stabs. A nichrome needle was dipped in broth containing the culture and aseptically inoculated into the stab. The tubes were then incubated for 24 hours and observed for diffused growth along the inoculated line to check for motility.

3.6) SEM ANALYSIS

The isolates JDDM14, JDDM53, JDDM67 and JDDM70 were inoculated in nutrient broth and incubated at 28ºC for 48 hours. The culture broth was then centrifuged at 10,000rpm for 8 minutes. The supernatant was discarded and the pellet was washed with phosphate buffer saline twice followed by resuspending it in 2 ml of phosphate buffer saline. A loop full culture was taken and a smear was made on the glass slide and allowed to airdry. The smear was then fixed with 2.5% glutaraldehyde solution overnight. Next day the slide was washed with phosphate buffer saline followed by successive washes

with 20%, 40%, 60%, 80% and 100% ethanol. The slide was then air dried and was given for sputter coating followed by analysis.

3.7) HALOTOLERANCE OF THE ISOLATES

Nutrient agar plates with different concentration of salt (0,2%,4%,6%,8%,10%,12%,14% and 16%) were prepared. The isolates JDDM14, JDDM53, JDDM67 and JDDM70 were spot inoculated on these plates and were monitored for their growth at the respective salt concentration.

3.8) BIOCHEMICAL TESTS

3.8.1) Indole test

The HiMViC^{TM} Test kit was taken and in well 1, 50µl of culture was added and incubated for 24 hours. Then 1-2 drops of Kovac's reagent was added. Development of reddish pink colour within 10 seconds indicated a positive result while no colour change indicated negative result.

3.8.2) Methyl Red test

The HiMViC^{TM} Test kit was taken and in well 2, 50µl of culture was added and incubated for 24 hours. Then 1-2 drops of methyl red reagent were added. Development of red colour indicated positive result whereas development of yellow colour or no change in colour indicated negative result.

3.8.3) Voges Proskaeur's test

The HiMViC™ Test kit was taken and in well 3, 50µl of culture was added and incubated for 24 hours. Then1-2 drops of Baritt reagent A and 1-2 drops of Baritt reagent B was added. Positive result is indicated with the development of the pinkish red colour while negative result is indicated when there is no change in colour of the reagent.

3.8.4) Citrate utilization test

The HiMViC™ Test kit was taken and in well 4,50µl of culture was added and incubated for 24 hours. Change in colour of the media from green to blue indicated positive result.

3.8.5) Sugar utilization test

The HiMViC™ Test kit was taken and 50µl of culture was added to well 5, 6, 7, 8, 9, 10, 11 and 12 and incubated for 24 hours. Change in colour of the media from pinkish red to yellow indicated the utilization of sugars.

3.9) ENZYME ACTIVITY

3.9.1) Amylase production

Starch hydrolysis by the isolate was determined by spot inoculating the isolates on starch agar. The plates were then incubated at room temperature for 48 hours and then flooded with iodine solution and were kept undisturbed for 10 minutes. The plates were then observed for halo zones around the isolate indicating its ability to hydrolyse starch (Oo et al., 2020).

3.9.2) Cellulase production

Carboxy-methylcellulose with yeast extract medium plates were prepared. The isolates were spot inoculated on these plates and incubated at room temperature for 5 days. The plates were then flooded with congo red solution and the positive result was indicated by the formation of a halo zone around the colony (Mohammed & Lemma, 2023).

3.9.3) Chitinase production

To determine the ability of the isolates to produce chitinase, they were spot inoculated on colloidal chitin agar media. The plates were then incubated for three days at room temperature and checked for halo zone formation around the colony to indicate chitinase production by the isolate (Mohammed & Lemma, 2023).

3.9.4) Protease production

Skim milk agar plates were prepared. The isolates were spot inoculated on these plates and incubated at room temperature for 48 hours and checked for halo zones around the colony indicating the ability of the isolate to produce protease enzyme (Mohammed & Lemma, 2023).

3.10) EVALUATION OF BIOCOMPATIBILITY OF THE ISOLATES FOR FORMING CONSORTIUM BASED BIOFERTILIZER

For checking the biocompatibility of the isolates, the selected isolates JDDM14, JDDM53, JDDM67 and JDDM70 were cross streaked against each other on nutrient agar plate and incubated for 24 hours at room temperature for growth.

3.11) BIOINOCULUM FORMULATION

The coconut oil cake was washed with tap water and then dried at 105ºC overnight. Using a mixer grinder the coconut oil cake was then powdered and autoclaved three times. The cultures JDDM14, JDDM53, JDDM67 and JDDM70 were inoculated in 50 ml flasks respectively and incubated for 24 hours for growth of the culture. OD of the culture was taken at 600nm after 24 hours and the culture dilutions were carried out of each culture to obtain the final OD as 0.6. Ones the OD of 0.6 was achieved the culture was centrifuged at 9500 rpm for 10 minutes. The supernatant was discarded and the pellet was washed with sterile distilled water for three times, followed by resuspending the pellet in 1X phosphate buffer saline. The culture was then mixed with the sterile coconut oil cake powder at 3:2 v/w. The bioinoculum prepared was then stored at 4ºC until used.

3.12) IN VITRO SEED GERMINATION TEST

Rice seed were washed with tap water, then surface sterilized with 70% ethanol for 5 minutes followed by 0.2% sodium hypochlorite solution for 5 minutes. Rice seeds were then rinsed in sterile distilled water three times. Different combinations of the bioinoculum were made as indicated in Table3.1.

Table 3.1: Different combinations of bioinoculum for invitro germination test

Test	1	2	3	4	5	6	7
Different combinations of bioinoculum	Sterile Distilled Water	JDDM14 + JDDM53	JDDM14 + JDDM67	JDDM14 + JDDM70	JDDM53 + JDDM67	JDDM53 + JDDM70	JDDM67 + JDDM70

The sterilized rice seed were soaked in them respectively and were kept for 2 hours under shaking conditions. Control for the same was kept by soaking the sterilized rice seeds in sterile distilled water. The bioinoculum coated seeds were then aseptically transferred to sterile petriplates containing wet non-absorbent cotton at the rate of 14 seeds per plate. Thes plates were then incubated for 6 days at room temperature with spraying of sterile water at regular intervals. The experiment was performed in triplicates per treatment. After 6 days germination rate, the shoot length and the root length of the rice seeds was recorded and the vigor index was calculated using the formula:

Vigor Index= Germination rate x Seedling length (root length + shoot length)

(Tamreihao et al., 2016).

3.13) POT TRIALS

10 plastic pots of the same size were taken filled with 2.5 kg field soil each. The pot trials were carried out in duplicates per treatment. The 6 days old seedlings from plates were transferred to the pots as indicate in table 3.2. at the count of 6 seeds per pot. The pots were watered until the soil surface was covered up to one centimetre with 200ml of tap water per pot daily and the Vigor index of the 6-day and 18-day old rice plant was calculated using the formula Vigor Index= Germination rate x plant length (shoot length+ root length) (Liu et al., 2022).

Table 3.2: Treatments applied to each pot for pot trials					
Pots	1	2	3	4	5
Treatments	Control	Bioinoculum coated seeds	Bioinoculum added to soil	Bioinoculum coated seeds + Bioinoculum added to soil	Chemical fertilizer
Treated Rice Seeds	-	+	-	+	-

- = untreated rice seeds

+ = treated rice seeds with the bioinoculum treatment (JDDM14 + JDDM53 + JDDM67 + JDDM70).

CHAPTER 4

ANALYSIS AND CONCLUSION

4.1. RESULTS AND DISCUSSION

4.1.1) Sampling

Sampling site for Jaya Rice Variety was a rice field at Macasana Goa as depicted in Figure 4.1.1.

Sampling sit for salt tolerant rice variety was a rice field at Loutolim Goa as shown in Figure 4.1.2

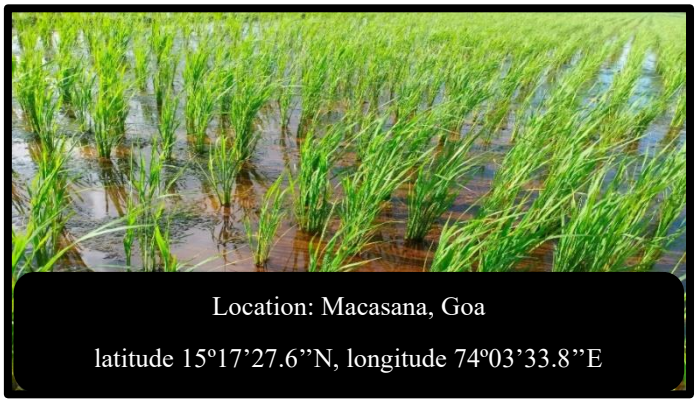


Figure 4.1.1: Sampling site (Jaya rice variety)

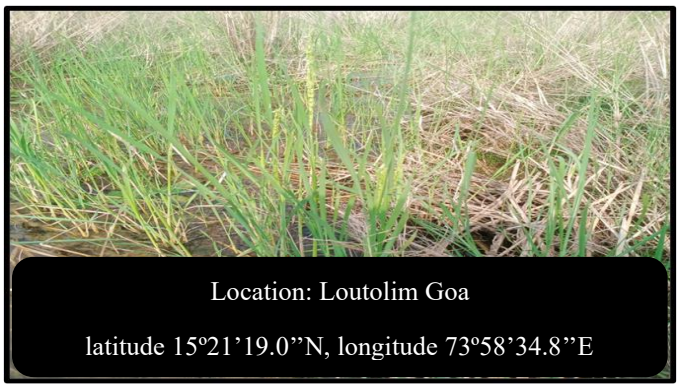


Figure 4.1.2: Sampling site for salt tolerant rice variety (Assgo)

Table 4.1.1: Parameters of the sampling sites		
Sample site	Parameters	
	Temperature	pH
Jaya rice variety	35ºC	6
Assgo salt tolerant rice variety	35ºC	9

4.1.2) Isolation

In this study the isolates were isolated from the rhizosphere of the rice seedling collected from sampling sites. A total of 70 isolates were obtained and were screened for plant growth promoting properties. The Figure 4.1.3 and Figure 4.1.4 depicts the isolates obtained after sampling.

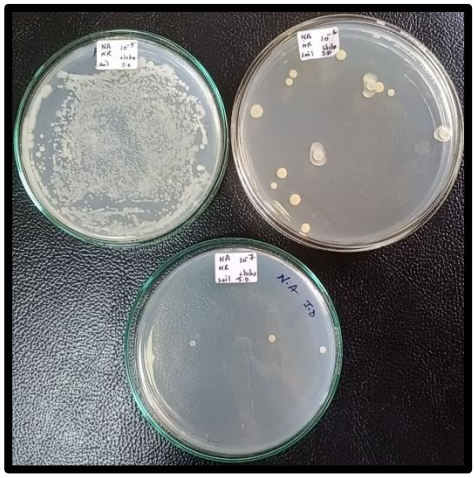


Figure 4.1.3: Isolated rhizobacteria from the field of Jaya rice variety

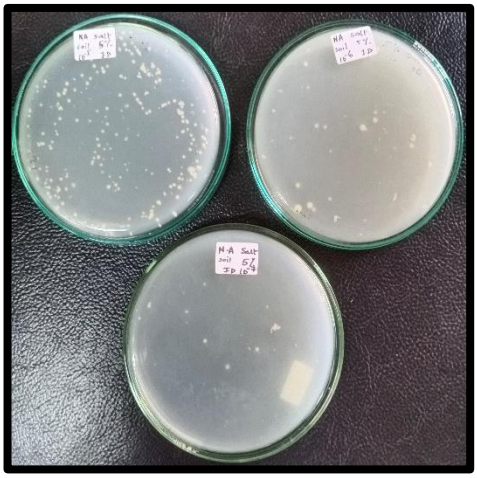


Figure 4.1.4: Isolated rhizobacteria from the field of salt tolerant rice variety

4.1.3) Screening for PGPR Properties

Table 4.1.2: The table below shows the result of screening of isolates for different plant growth promoting properties. The isolate JDDM14 had nitrogen fixing, phosphate solubilizing,

ammonia production, indole acetic acid production, HCN production and siderophore production ability while isolate JDDM70 had nitrogen fixing, phosphate solubilization, siderophore production and indole acetic acid production ability. Followed by these two isolates were JDDM53 with phosphate solubilization, indole acetic acid production and HCN production ability and JDDM67 with nitrogen fixation, phosphate solubilization, siderophore production and HCN production ability.

Table 4.1.2: Screening of isolates for various plant growth promoting properties

Isolate	Nitrogen Fixation	Phosphate Solubilization	Siderophore Production	Indole Acetic acid Production	HCN Production	Ammonia Production
JDDM01	-	-	-	+	-	-
JDDM02	-	-	-	-	-	-
JDDM03	-	-	-	-	-	-
JDDM04	-	-	-	-	-	-
JDDM05	-	-	-	-	-	-
JDDM06	-	-	-	-	-	-
JDDM07	+	-	-	-	-	-
JDDM08	+	-	-	-	-	-
JDDM09	-	-	-	-	-	-
JDDM10	+	-	-	-	-	-
JDDM11	-	-	-	-	-	-
JDDM12	-	-	-	-	-	-
JDDM13	+	-	-	-	-	-
JDDM14	+	+	+	+	+	+

JDDM15	+	-	-	-	-	-
JDDM16	+	-	-	-	-	-
JDDM17	+	-	-	-	-	-
JDDM18	-	-	-	-	-	-
JDDM19	-	-	-	-	-	-
JDDM20	+	-	-	-	-	-
JDDM21	+	-	-	-	-	-
JDDM22	-	-	-	-	-	-
JDDM23	-	-	-	-	-	-
JDDM24	-	-	-	-	-	-
JDDM25	+	-	-	-	-	-
JDDM26	-	-	-	-	-	-
JDDM27	+	-	-	-	-	-
JDDM28	-	-	-	-	-	-
JDDM29	-	-	-	-	-	-
JDDM30	+	-	-	-	-	-
JDDM31	-	-	-	-	-	-
JDDM32	+	-	-	+	-	-
JDDM33	-	-	-	-	-	-
JDDM34	+	-	-	+	-	-
JDDM35	-	-	-	-	-	-
JDDM36	-	-	-	-	-	-
JDDM37	+	-	-	+	-	-
JDDM38	+	-	-	-	-	-

JDDM39	-	-	-	-	-	-
JDDM40	-	-	-	-	-	-
JDDM41	+	-	-	-	-	-
JDDM42	-	-	-	-	-	-
JDDM43	-	-	-	-	-	-
JDDM44	+	-	-	-	-	-
JDDM45	+	-	-	-	-	-
JDDM46	-	-	-	-	-	-
JDDM47	+	-	-	-	-	-
JDDM48	+	-	-	+	-	-
JDDM49	-	-	-	-	-	-
JDDM50	-	-	-	-	-	-
JDDM51	-	-	-	-	-	-
JDDM52	+	-	-	-	-	-
JDDM53	+	+	-	+	+	-
JDDM54	-	-	-	-	-	-
JDDM55	-	-	-	-	-	-
JDDM56	-	-	-	+	+	-
JDDM57	-	-	-	-	-	-
JDDM58	-	-	-	-	-	-
JDDM59	+	-	+	-	-	-
JDDM60	+	-	-	+	-	-
JDDM61	-	-	-	+	+	-
JDDM62	+	-	-	+	-	-

JDDM63	-	-	-	-	-	-
JDDM64	+	-	-	-	-	-
JDDM65	-	-	-	+	-	-
JDDM66	-	-	-	-	-	-
JDDM67	+	+	+	-	+	-
JDDM68	-	-	-	+	-	-
JDDM69	-	-	-	-	-	-
JDDM70	+	+	+	+	-	-

+ = Positive, - = Negative

4.1.3.a) Nitogen Fixation

The Figure 4.1.5 depicts the ability of certain isolates to grow on nitrogen free media indicating their ability to fix atmospheric nitrogen. Since the media used lacks a nitrogen source, an essential need for the microorganisms to grow only those isolates which can fix atmospheric nitrogen are able to grow on this media. In this study a total of 30 out of 70 i.e 43% of the isolates showed nitrogen fixation ability. A similar result was obtained by (Habibi et.al, 2014) in which it was reported that 43.4% of the isolates obtained from rice rhizosphere were nitrogen fixers.

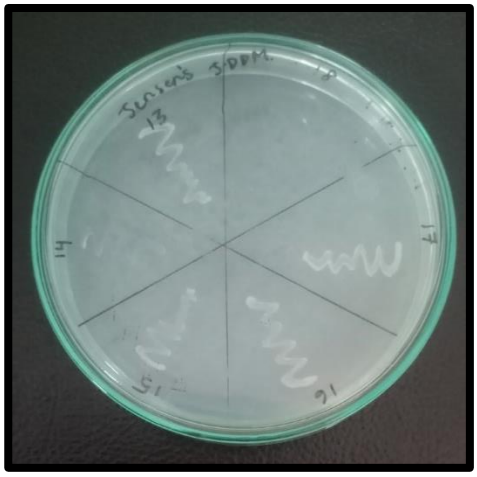


Figure 4.1.5: Isolates streaked on Nitrogen Free Agar plate

4.1.3.b) Phosphate Solubilization

The Figure 4.1.6 depicts the halo zones produced the isolates JDDM14, JDDM53, JDDM67 and JDDM70 indicating their ability to solubilize insoluble phosphate and make it available to plants for their better growth.

The rhizosphere being a home to phosphate solubilizing bacteria, and one common way to help convert insoluble forms of P into forms that plants can use is through the secretion of organic acids and phosphatases. Out of the 70 isolates only 4 isolates were found to be phosphate solubilization capable which accumulates to only about 6%. There is evidence of a comparable low quantity of phosphate-solubilizing bacteria by (De Souza et al., 2013).

Figure 4.1.6: Isolates spot inoculated on Pikovskaya's agar plate

4.1.3.c) Siderophore Production

The Figure 4.1.7 depicts the halo zone produced by the isolate JDDM67 indicating its ability to produce siderophores and sequester the iron present in the surrounding. While the isolate JDDM64, JDDM65 and JDDM66 do not produce siderophores.

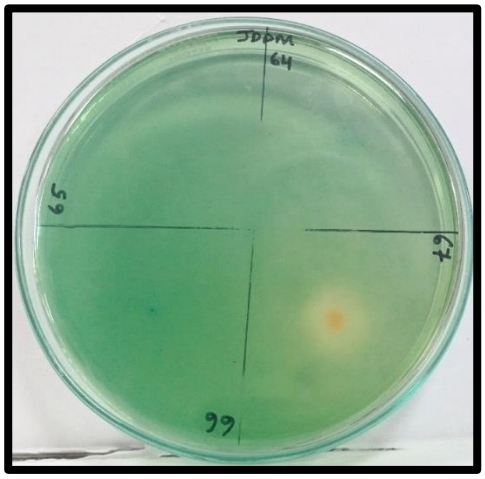


Figure 4.1.7: Isolates spot inoculated on Cas agar plate

According to data, rhizospheric bacteria's excretion of siderophores may improve plant nutrition or prevent phytopathogens from accessing Fe, thereby promoting plant health. In the present study 4 out of 70 isolates were able to produce siderophores, similar numbers of bacteria that produce siderophores have been reported by other studies. For instance, De Souza et al assessed the variety of cultivable siderophore-producing bacteria in the rhizosphere of tobacco and discovered that isolates that produced siderophores in a liquid medium with limited iron (De Souza et al., 2013).

4.1.3.d) Indole Acetic Acid production

The Figure 4.1.8 depicts the colour change of the broth containing respective isolates from cream to pink. This indicates the ability of isolate JDDM14 and JDDM53 to produce indole acetic acid.

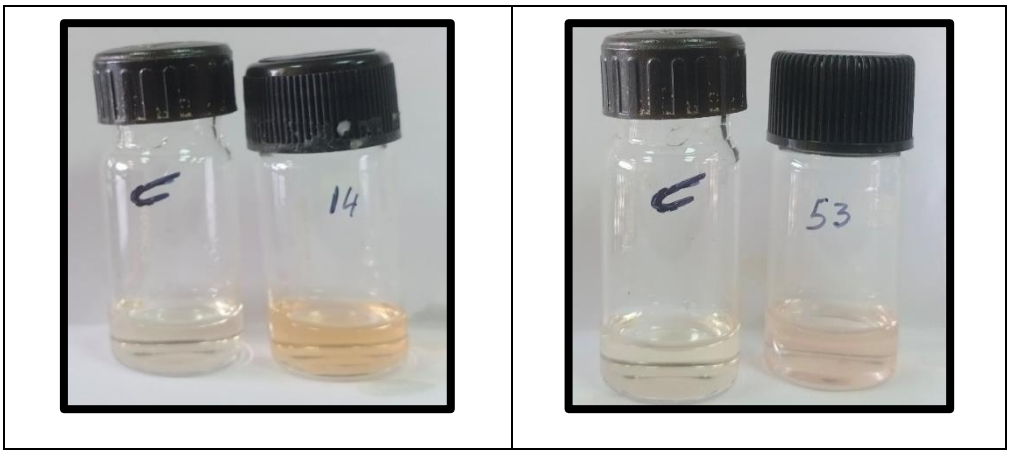


Figure 4.1.8): Change in colour of the broth in test on addition of Salkowski's reagent

The synthesis of Indole Acetic Acid is a crucial characteristic of PGPR that can affect plant growth. Most often, it is thought that IAA-producing PGPR lengthen and grow their roots more, which increases their surface area and allows the plant to absorb more nutrients from the soil. The ability to produce IAA was the most prevalent characteristic

among the isolates in the current study, as 20% of the isolates demonstrated this ability when the precursor L-tryptophan was present. Regarding IAA production, our findings were consistent with those of (Ahmad et al., 2008), who also observed that a comparable number of their isolates exhibited IAA production as one of the common trait that promoted plant growth.

4.1.3.e) Hydrogen cyanide Production

The above figure depicts the change in colour of the filter paper dipped in 0.5% picric acid and 2% sodium carbonate solution from light yellow to orange in the tubes containing isolate JDDM56, JDDM61 and JDDM67 indicating HCN production by the isolates when compared to control in which there is no change in the colour of the filter paper. Out of the 70 isolates only 5 isolates were able to produce HCN hence these isolates have the potential to act as a biocontrol agent against pathogens.

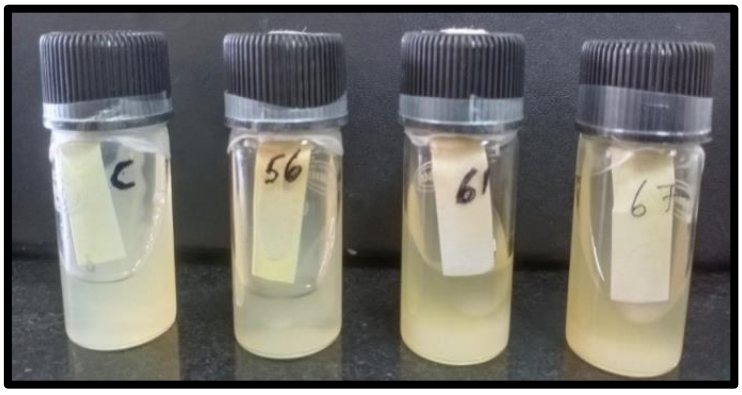


Figure 4.1.9): HCN production detection tubes

4.1.3.f) Ammonia Production

The Figure 4.1.10 depicts the change in colour of the solution to brown in tube containing the isolate JDDM14 when compared to control on addition of Nessler's reagent indicating the ability of the isolate to produce ammonia.

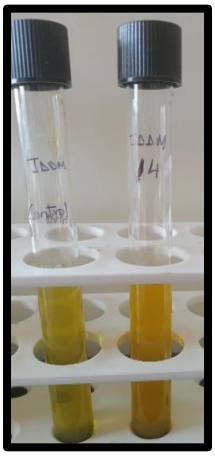


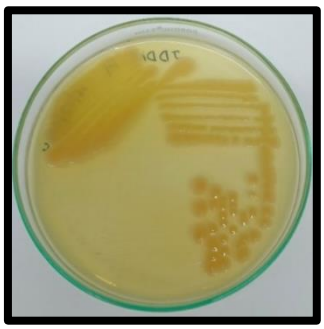
Figure 4.1.10): Tubes for Ammonia Production

4.1.4) Isolates selected based on screening

Out of the 70 isolates 4 isolates were selected based on their various plant growth promoting properties mentioned in the Table 4.1.3:

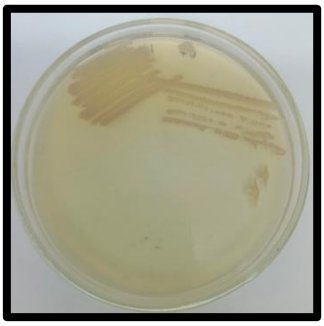
Table 4.1.3: Selected Isolates Based on Screening for plant growth promoting properties

Selected Isolates	**Nitrogen Fixation**	**Phosphate Solubilization**	**Siderophore Production**	**Indole Acetic acid Production**	**HCN Production**	**Ammonia Production**
JDDM14	+	+	+	+	+	+
JDDM53	+	+	-	+	+	-
JDDM67	+	+	+	-	+	-
JDDM70	+	+	+	+	-	-

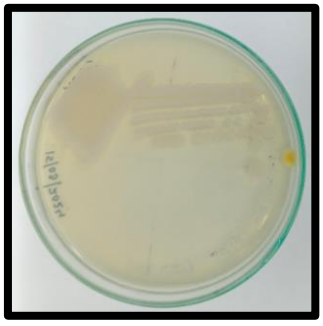


Isolate JDDM14

Isolate JDDM53



Isolate JDDM67



Isolate JDDM70

Figure 4.1.11: Selected isolates based on screening

4.1.5) Colony Characteristics

Table 4.1.4 shows colony characteristics of the 4 selected isolates.

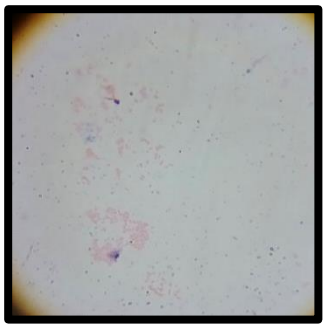
Table 4.1.4: Colony characteristics of the selected isolates

Colony Characteristics	Isolates			
	JDDM14	JDDM53	JDDM67	JDDM70
Size	Large	Large	Small	Large
Colour	Light brown	Creamish	Beige	White
Shape	Circular	Circular	Irregular	Circular
Elevation	Raised	Flat	Flat	Flat
Opacity	Opaque	Opaque	Opaque	Opaque

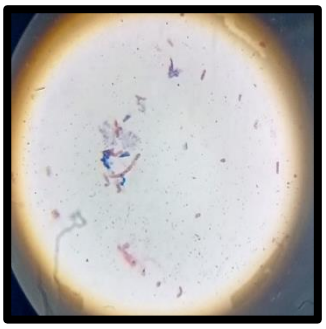
Consistency	Butyrous	Butyrous	Mucoid	Brittle
Margin	Entire	Entire	Undulate	Entire
Gram character	Gram negative rods	Gram negative rods	Gram negative rods	Gram negative rods
Motility	Motile	Motile	Motile	Motile

4.1.5.a) Gram Staining

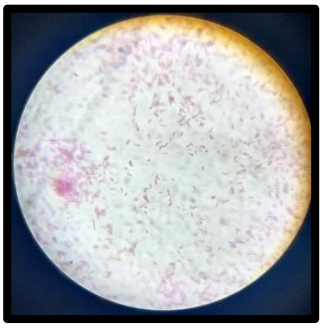
The Figure 4.1.12 depicts the gram staining character of the selected isolates wherein all the isolates are gram negative with short rods.



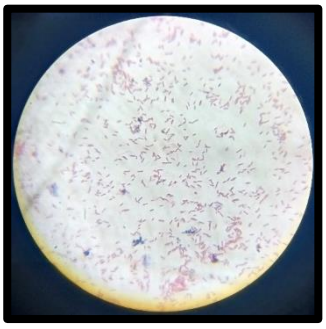
Isolate JDDM14



Isolate JDDM53



Isolate JDDM67

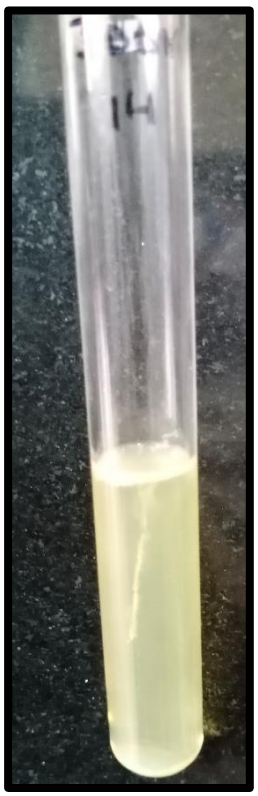


Isolate JDDM70

Figure 4.1.12: Gram staining of the isolates

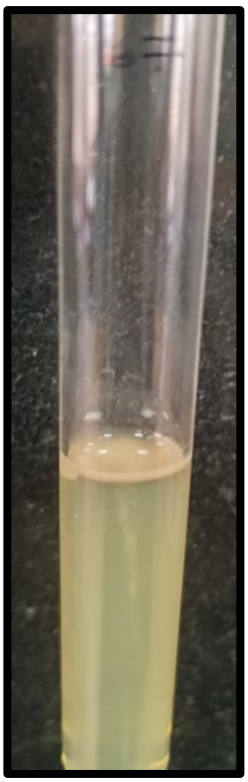
4.1.5.b) Motility

All four isolates when stabbed in the SIM media showed diffused growth from the line of inoculation indicated that the isolates were motile as seen in Figure 4.1.13



Isolate JDDM14

Isolate JDDM53



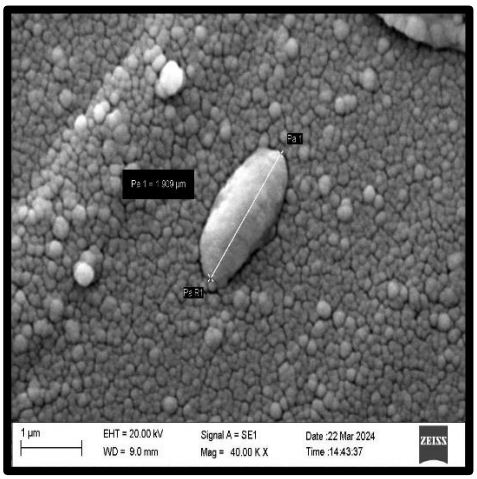
Isolate JDDM67

IsolateJDDM70

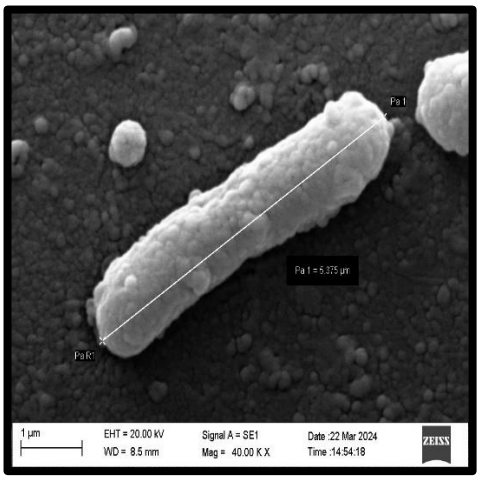
Figure 4.1.13: Motility test of the isolates

4.1.6. SEM Analysis

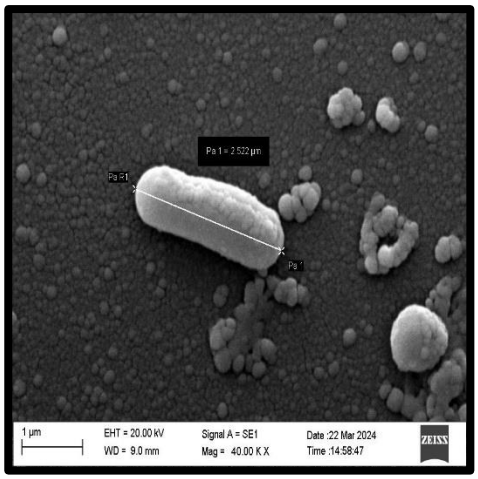
SEM Analysis of the isolate was carried out and their 3D images were obtained. The morphology of the four isolates was examined. The shape of the isolates was identified to be rod shaped as shown in Figure 4.1.14.



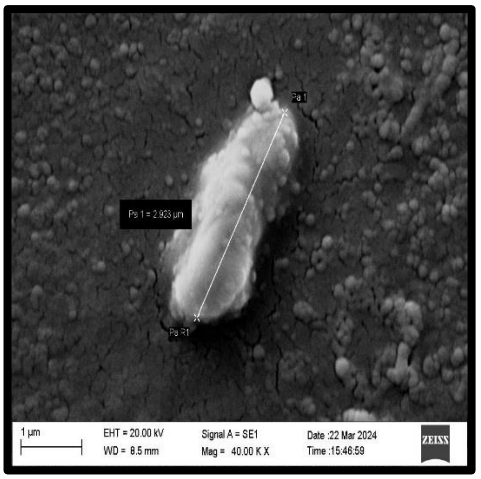
Isolate JDDM14



Isolate JDDM53



Isolate JDDM67



Isolate JDDM70

Figure 4.1.14: SEM images of the selected isolates

4.1.7) Halotolerance of the Isolates

In this study, all the four selected isolates were evaluated for halotolerance. It was observed that all the isolates were able to grow at salt concentration at 6%. JDDM53 and JDDM67 were able to tolerate at 10% salt. However, when the concentration reached to

14% only isolate JDDM53 was observed to grow at this high salt concentration (Table 4.1.5.)

Table 4.1.5: Growth of isolates at different salt concentration									
Isolate	Concentration of Salt								
	0	2%	4%	6%	8%	10%	12%	14%	16%
JDDM14	+	+	+	+	-	-	-	-	-
JDDM53	+	+	+	+	+	+	+	+	-
JDDM67	+	+	+	+	+	+	-	-	-
JDDM70	+	+	+	+	-	-	-	-	-

4.1.8) Biochemical Test

The biochemical characterization of the selected four isolates was carried out. Biochemical tests such as indole, methyl red, Voges' Proskauer's, citrate utilization and sugar utilization were done and the data obtained is noted down in Table 4.1.6.

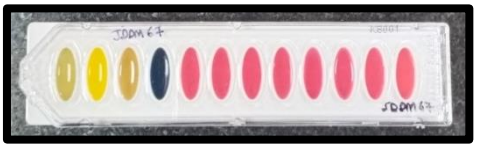
Table 4.1.6: Biochemical characteristics of the selected isolates				
Test	**Isolates**			
	JDDM14	**JDDM53**	**JDDM67**	**JDDM70**
Indole	**-**	**-**	**-**	**-**
Methyl red	+	+	**-**	**-**
Voges Proskauer's	**-**	**-**	**-**	**-**
Citrate utilization	+	+	+	+
Glucose	+	+	**-**	+
Adonitol	**-**	**-**	**-**	**-**

Arabinose	-	+	-	-
Lactose	-	-	-	-
Sorbitol	-	-	-	-
Mannitol	+	+	-	+
Rhamnose	-	-	-	-
Sucrose	-	+	-	+

From the above table it is evident that all the isolates were positive for citrate utilization while JDDM14 and JDDM53 were positive were positive for Methyl Red. The same results are depicted in the Figure4.1.15

Isolate JDDM14

Isolate JDDM53



Isolate JDDM67

Isolate JDDM70

Figure 4.1.15: Test results obtained by inoculating isolates on Biochemical strip

4.1.9. Enzyme Activity

The four selected isolates were screened for 4 different enzyme production. The result of the screening is summarized in the Table 4.1.7. Isolate JDDM14 and JDDM70 produced the amylase enzyme, JDDM53 and JDDM70 isolates were unable to produce any. In a similar vein, it is noted that JDDM14, JDDM53 and JDDM70 isolates produced protease enzyme, but JDDM67 was unable to produce this enzyme. All the isolates were unable to produce cellulase and chitinase.

Table 4.1.7: Enzymes Produced by the isolates

Isolates	Enzymes			
	Amylase	Cellulase	Chitinase	Protease
JDDM14	+	-	-	+
JDDM53	-	-	-	+
JDDM67	-	-	-	-
JDDM70	+	+	-	+

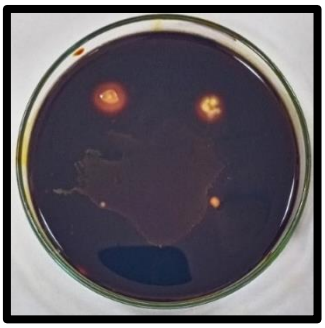


Figure 4.1.16.a): Isolate showing zone of clearance on addition of iodine indicating positive result for Amylase enzyme

Figure 4.1.16.b): Isolates showing zone of clearance on Skim agar indicating positive result for protease enzyme

Figure 4.1.16: Enzyme activity of the isolates

4.1.10. Evaluation of biocompatibility of the isolates for forming consortium based biofertilizer

The isolates were cross streaked against each other and were found to grow in the presence of each other without affecting the growth of the others and the same is depicted in the Figure 4.1.17

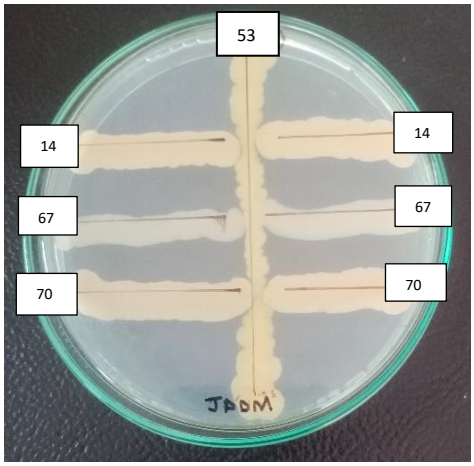


Figure 4.1.17: Isolates cross streaked on nutrient agar plates

4.1.10. In vitro seed germination

The invitro seed germination was carried for the bioinoculum treated rice seeds. The control for the same was kept as rice seeds soaked in sterile distilled water. Table 4.1.8 shows the rice seed germination images before and after 6 days.

Table 4.1.8: Invitro rice seed germination

	Day 0	Day 6
Control		
Bioinoculum JDDM14 + JDDM53 + JDDM67 + JDDM70		

4.1.10.b: Germination Vigor index

From the Table 4.1.9 it is evident that the germination rate of the rice seeds with both the control and with the bioinoculum treatment (JDDM 14 + JDDM53 + JDDM67 + JDDM70) were 100%. Hence the rice seeds germinated with the bioinoculum treatment (JDDM 14 + JDDM53 + JDDM67 + JDDM70) was then used for pot trials. The vogor index of the germinated seed was also calculated. The highest vigor index calculated was of the rice seeds in control followed by rice seeds in the bioinoculum (JDDM 14 +

JDDM53 + JDDM67 + JDDM70). The rice seeds germinated with the other treatments as shown in Table 4.1.9. were excluded due to less vigor index.

Table 4.1.9: Germination rate and vigor index of germinated rice seeds on application of bioinoculum treatments

Various Treatments given to the rice grains	Germination rate %	Vigor Index
Control	100	740
JDDM 14 + JDDM53	71.4	278.5
JDDM 14 + JDDM67	78.6	346
JDDM 14 + JDDM70	71.4	236
JDDM53 + JDDM67	71.4	350
JDDM53 + JDDM70	64.2	237
JDDM67 + JDDM70	78.6	314
JDDM 14 + JDDM53 + JDDM67 + JDDM70	100	649.5

4.1.11. Pot Trials

The Figure 4.1.17 and Figure 4.1.18 depicts the difference in rice plants of pot trials based on the inoculum used. There is a clear increase in the height of the rice plants in pot 2 which contains seeds coated with the bioinoculum as compared to pot 1 i.e control when checked on Day 18.

Figure 4.1.18: Pots with rice seeds on day 0

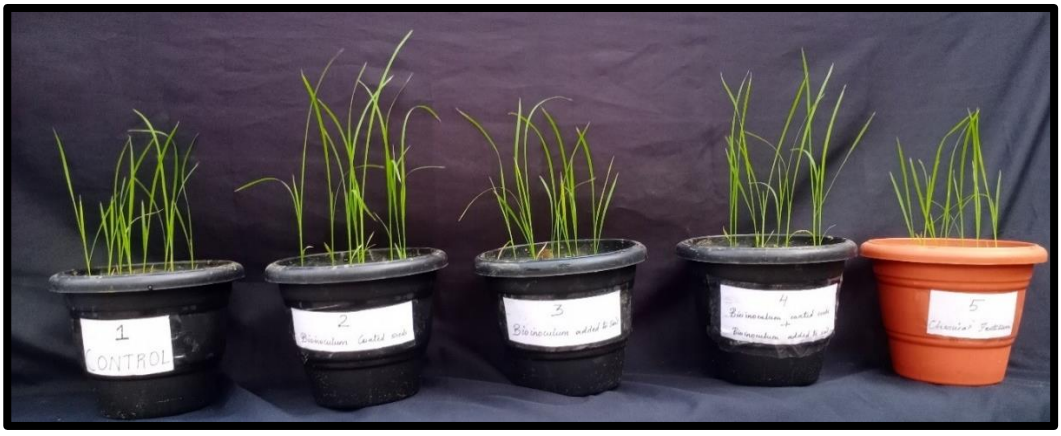


Figure 4.1.19: Pots with germinated rice seeds on day 18

4.1.12. Vigor Index

From the Table 4.1.10 it is evident that the rice plants in pot 2 with bioinoculum coated seeds had an 80% increse in vigor index from day1 to day 18, followed by pot 4 with bioinoculum coated seeds and bioinoculum directly added to soil had an 72% increase in vigor index. The rice plants treated with chemical fertilizer had an 65% increase in vigor index whereas control had 60% increase in vigor index which is comparatively low as compared to other treatments. The same is depicted in the Figure 4.1.20.

Table 4.1.10: Germination rate and vigor index of rice seeds used for pot trials			
Pots	Germination rate %	Vigor Index	
		Day 1	Day 18
Control	100	740	2600
Bioinoculum coated seeds	100	649.5	3163.5
Bioinoculum added to soil	100	740	2880
Bioinoculum coated seeds + Bioinoculum added to soil	100	649.5	2875
Chemical fertilizer	100	740	2750

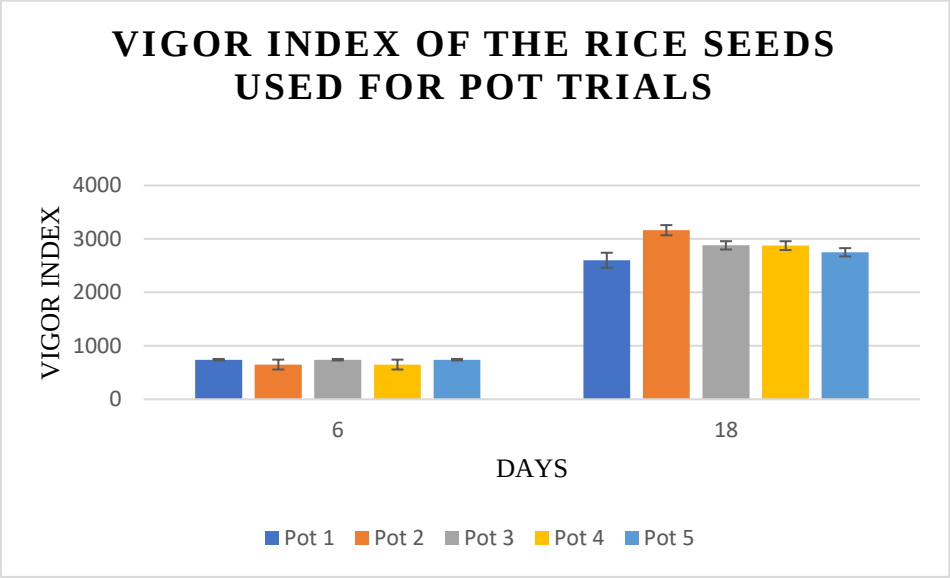


Figure 4.1.20: Graphical representation of the results obtained with pot trials

From the Figure 4.1.20. it is evident that the vigor index of rice seedling from pot 2 is highest among all the pots. Hence the bioinoculum formulated has the potential to act as a biofertilizer as an alternative to chemical fertilizer. In addition, the best method of application of bioinoculum to field is by coating the seeds with the bioinoculum rather than adding the bioinoculum directly to the field.

4.2. CONCLUSION

The PGPR is not only benefiting the plant by direct plant growth promotion but also acts as a biocontrol agent against plant pathogens. Certain PGPR strains including JDDM14, JDM53, JDDM67 and JDDM70 mixtures have shown a synergistic effect on the plant growth and as compared to the individual application and control. Mass producing such strains and binding them with suitable carrier is beneficial in crop production. Additionally developing a desired carrier could assist in better delivering of the bioinoculum to the field setting. From this research carried out it is concluded that instead of using a single PGPR strain, using a combination of strains is much more impactful on greater spectrum of PGPR properties, thus proving as a substitute for chemical fertilizer and leading to better plant growth as compared to the control.

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APPENDIX I

MEDIA

Nutrient Agar (pH7.4)

INGREDIENTS	GRAMS/LITRE
Peptone	5
Sodium Chloride	5
Yeast Extract	2
Agar Powder	15

Nutrient Broth (pH 7.4)

INGREDIENTS	GRAMS/LITRE
Peptone	5
Sodium Chloride	5
Yeast Extract	2
Beef Extract	1

Jensen's N free media (pH 7)

INGREDIENTS	GRAMS/LITRE
Sucrose	2
Dipotassium phosphate	1
Magnesium Sulphate	0.5
Sodium Chloride	0.5
Ferrus Sulphate	0.1
Sodium Molybdate	0.005
Calcium carbonate	2
Agar	15

Pikovskaya's Agar (pH 7)

INGREDIENTS	GRAMS/LITRE
Yeast Extract	0.5
Dextrose	10
Calcium Phosphate	5
Ammonium Sulphate	0.5
Potassium Chloride	0.2
Magnesium Sulphate	0.1
Manganese Sulphate	0.002
Ferrous Sulphate	0.002
Agar	15

King's B Agar (pH 7)

INGREDIENTS	GRAMS/LITRE
Proteose Peptone	20
Dipotassium Hydrogen Phosphate	1.5
Magnesium Sulphate Heptahydrate	1.5
Agar	20

APPENDIX II

REAGENTS AND SOLUTIONS

Cas Dye

INGREDIENTS	MILLIGRAMS/MILLILITRE
Chrome Azurol S Dye	18.15 in 4ml DW
Hexa Decyl Tri Methyl	21.87 in 3 ml DW
Ferric Chloride	16.221 in 3 ml DW

Picri Acid Solution

INGREDIENTS	GRAMS/LITRE
Picric Acid	5
Sodium Carbonate	20

Gram's staining kit:

- **Crystal violet (Primary stain)**
- **Gram's Iodine (Mordant)**
- **Decolorizer (95% ethanol or 1:1 acetone with ethanol)**
- **Safranin (Counterstain)**

INSTRUMENTS

- **Autoclave**
- **Cooling Centrifuge**
- **Scanning Electron Microscope**