Isolation and characterization of Plant Growth Promoting Bacteria (PGPB) from drought stress soil

A RESEARCH PROJECT

Submitted by

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VIVEKANAND COLLEGE, KOLHAPUR

(AN EMPOWERED AUTONOMOUS INSTITUTE)

YEAR 2024-2025

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This is to certify that Ms. **Apurva Uday Jadhav** studying in M. Sc. part II Microbiology at Vivekanand College, Kolhapur (Empowered Autonomous) has sincerely completed research project work entitled **"Isolation and characterization of Plant Growth Promoting Bacteria (PGPB) from drought stress soil"** during academic year 2024-25.

21/12/24

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ACKNOWLEDGEMENT

I wish to express my deep sense of appreciation to Dr. Komal K. Bhise, Assistant Professor, Department of Microbiology, Vivekanand College, Kolhapur (An Empowered Autonomous Institute) for her valuable support and expert guidance during the course of this study. She has been extremely understanding and cooperative and has always taken great interest in this work.

I wish to express my sincere thanks to Dr. G. K. Sontakke, Head, Department of Microbiology and Dr. R. R. Kumbhar, Principal, Vivekanand College, Kolhapur (An Empowered Autonomous Institute) for providing the laboratory facilities in the department to carry out the experimental work.

I express my thanks to Ms. V. V. Misal, Coordinator of PG Department of Microbiology and faculty members, Dr. S. D. Mali, Mr. S. D. Gabale, Ms. S. A. Pise and Ms. M. M. Nadkarni for their valuable suggestions and help during the work.

I convey my gratitude to Mrs. D. S. Shinde (Laboratory Assistant), Mr. S. K. Maskar and Mr. S. P. Mali (Laboratory Staff) of the department for their kind help in the laboratory.

I am thankful to the librarian and library staff for providing facilities of computer andreference books. My special thanks and gratitude to my entire classmates who have been constant source of inspiration and help during entire project work. I am highly obliged to authors past and present whose literature has been cited.

Finally, I thank my family members for their blessings and support because of which this work has proved satisfactory to me.

Place: Kolhapur

Date: 18/12/2024

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INTRODUCTION

Isolation and characterization of Plant Growth Promoting Bacteria (PGPB) from drought Stress Soil

Introduction

Agriculture plays a crucial role in sustaining human life and the global economy. It is the primary source of food production, ensuring food security for billions of people worldwide. Beyond food, agriculture provides raw materials for industries such as textiles, pharmaceuticals, and biofuels, contributing significantly to economic growth. In many developing nations, agriculture is the backbone of employment, offering jobs not only in farming but also in related sectors like transportation, food processing, and retail. Additionally, agriculture supports rural economies, promoting development and reducing urban migration. Sustainable agricultural practices help preserve biodiversity and protect natural resources, contributing to environmental health. Furthermore, agricultural exports play a vital role in global trade, generating foreign exchange and stabilizing economies.

Microorganisms play a crucial role in agriculture, often acting as unseen helpers in soil health, plant growth, and pest control. These tiny organisms, including bacteria, fungi, algae, and protozoa, contribute significantly to various agricultural processes, making them indispensable to sustainable farming practices.

1. Soil Fertility and Nutrient Cycling

Microorganisms are essential for soil fertility because they help decompose organic matter, releasing nutrients back into the soil that plants can absorb. Some key ways microorganisms enhance soil health include:

Decomposition: Microbes break down plant residues, animal waste, and other organic materials into simpler compounds. This process enriches the soil with nutrients, such as nitrogen, phosphorus, and potassium.

Nitrogen Fixation: Certain bacteria, like *Rhizobium* and *Azotobacter*, convert atmospheric nitrogen into a form that plants can absorb and use for growth. This process is essential because nitrogen is a vital nutrient, but plants cannot access it directly from the air.

Phosphorus Solubilization: Microorganisms like *Penicillium* and *Pseudomonas* can make phosphorus more available to plants by breaking down complex forms of this nutrient in the soil.

2. Plant Growth Promotion

Some microorganisms directly promote plant growth by producing substances that enhance root development and nutrient uptake. For example:

Plant Growth-Promoting Rhizobacteria (PGPR): These beneficial bacteria, such as *Bacillus* and *Pseudomonas*, colonize plant roots and promote growth by producing hormones like auxins, which stimulate root elongation, and siderophores, which help plants absorb iron.

Mycorrhizal Fungi: Mycorrhizae are fungi that form symbiotic relationships with plant roots, extending their reach to access more water and nutrients, especially phosphorus. This relationship enhances plant growth and stress tolerance.

3. Disease and Pest Control

Microorganisms can also protect crops from diseases and pests, reducing the need for chemical pesticides. Some examples include:

Biocontrol Agents: Certain bacteria and fungi can control plant pathogens naturally. Trichoderma fungi, for instance, protect against root rot and other fungal diseases by outcompeting or directly attacking harmful pathogens.

Endophytes: These are bacteria or fungi that live inside plant tissues without harming the host. They can improve the plant's resistance to pests and diseases, producing compounds that deter herbivores and pathogens.

Insect Pathogens: Some microorganisms, like *Bacillus thuringiensis* Bt produce toxins that are deadly to specific insect pests but harmless to humans and other animals. Bt is widely used in organic farming as a natural insecticide.

4. Enhancing Soil Structure

Microorganisms like bacteria and fungi produce substances that help bind soil particles together, forming aggregates. This process improves soil structure, enhancing aeration, water infiltration, and root growth. For instance, fungal hyphae (long filaments) act like natural threads that bind soil particles, promoting a crumbly texture beneficial to plant roots.

5. Biofertilizers and Biopesticides

Microorganisms are used to create biofertilizers and biopesticides, which are eco-friendly alternatives to chemical fertilizers and pesticides.

Biofertilizers: These products contain living microorganisms that enrich soil nutrients. For example, *Azospirillum* bacteria in biofertilizers can help enhance nitrogen availability, while phosphate-solubilizing bacteria improve phosphorus uptake.

Biopesticides: These are derived from microorganisms that target specific pests without harming beneficial organisms. For example, *Beauveria bassiana* is a fungus used as a biopesticide to control insect pests.

Drought stress is a critical challenge in agriculture, with direct consequences for food security and soil health. Globally, climate change is exacerbating drought frequencies and intensities, leading to severe implications for arid and semi-arid agricultural regions (IPCC, 2021). Drought conditions deprive plants of water, limit essential nutrient uptake, reduce soil microbial diversity, and cause physical deterioration of soil structure (Farooq et al., 2009). For farmers and communities dependent on rain-fed agriculture, drought can lead to significant yield losses, food shortages, and economic strain (FAO, 2019). Traditional approaches to mitigating drought impacts, such as increased irrigation and chemical fertilizers, are often unsustainable. These methods rely on extensive water and energy resources and may degrade soil health in the long term (Sato et al., 2013).

An innovative, environmentally friendly approach to address drought stress in agriculture is the application of PGPB. PGPB are beneficial soil microbes that form symbiotic relationships with plant roots, helping to enhance plant growth and stress resilience through a variety of biochemical and physiological mechanisms (Vejan et al., 2016). Studies have shown that PGPB can improve crop performance and soil health in drought-affected regions by boosting root development, improving nutrient uptake, and promoting water-use efficiency (Nadeem et al., 2014; Sarma & Saikia, 2014). The use of PGPB thus represents a promising, low-cost, and sustainable solution for mitigating the impacts of drought on agriculture.

Drought-affected soils face specific challenges: they have reduced water-holding capacity, nutrient availability, and microbial diversity (Farooq et al., 2009). These limitations create unfavourable conditions for crops, leading to lower yields and increased vulnerability to climate variability. Effective soil management in drought-prone areas demands sustainable methods that can restore soil health and enhance plant resilience without heavy dependence on water or chemical inputs (Sato et al., 2013). This project aims to leverage PGPB as a solution to these challenges by exploring their potential to improve soil health, enhance plant drought tolerance, and increase crop productivity in water-limited environments.

6. Mechanisms of PGPB in Drought Mitigation

Phytohormone Production: Certain PGPB strains produce plant hormones, such as indole-3-acetic acid (IAA), gibberellins, and cytokinins, which stimulate root growth and increase water uptake. This is particularly beneficial in dry soils, where deep-rooted plants are better able to access subsurface water (Spaepen et al., 2007; Ali et al., 2014).

ACC Deaminase Activity: Drought stress triggers ethylene production in plants, which can inhibit root growth and overall plant health. Many PGPB produce ACC deaminase, an enzyme that reduces ethylene levels, allowing plants to grow more effectively under water-limited conditions (Glick, 2014).

Siderophore Production: Siderophores are iron-chelating compounds produced by PGPB that increase iron bioavailability to plants, which is crucial for photosynthesis and stress resistance (Ma et al., 2011).

Exopolysaccharide (EPS) Production: Certain PGPB produce exopolysaccharides, which help in soil aggregation and improve water retention. EPS-producing bacteria enhance soil structure and create a microenvironment around the roots that retains moisture, providing a buffer against drought (Sandhya et al., 2009).

Nutrient Solubilization: Some PGPB can solubilize nutrients like phosphate and potassium, making them more accessible to plants. Under drought conditions, nutrient solubilization improves plant nutrient uptake, supporting better growth and yield (Rodriguez et al., 2004).

Each of these mechanisms not only boosts plant health under water stress but also contributes to overall soil fertility, enhancing the resilience of both the plants and the soil ecosystem.

7. Importance of PGPB in Drought Management

Environmental Sustainability: PGPB reduce the need for chemical fertilizers and excessive irrigation, which helps to protect natural resources and lower the environmental footprint of farming practices (Adesemoye et al., 2009).

Improved Soil Health: PGPB applications support the biodiversity of beneficial soil microbes, improving soil structure and nutrient cycling, which are essential for long-term soil health (Beneduzi et al., 2012).

Enhanced Crop Resilience: Through improved water-use efficiency and nutrient acquisition, PGPB make plants more resilient to climate-induced stresses, helping to stabilize crop yields under drought conditions (Nadeem et al., 2014).


REVIEW OF
LITERATURE

Review of Literature

1. Drought Stress in Agricultural Soils

Drought stress is one of the primary abiotic factors limiting agricultural productivity globally. Reduced water availability in soils leads to stunted plant growth, nutrient deficiencies, and diminished crop yields. According to recent studies, drought conditions not only affect plant physiology but also degrade soil structure and diminish microbial biodiversity, further aggravating the challenge of achieving sustainable crop yields under water scarcity (Farooq et al., 2009). This has led to a growing interest in identifying sustainable, cost-effective solutions to mitigate drought effects on crops, especially in light of climate change, which is expected to increase the frequency and intensity of droughts (IPCC, 2021).

2. Plant Growth-Promoting Bacteria (PGPB):

Plant Growth-Promoting Bacteria (PGPB) are a group of naturally occurring, beneficial microorganisms known for their ability to enhance plant growth and stress tolerance. Researchers have classified PGPB into free-living, symbiotic, and endophytic categories based on their relationship with plants (Glick, 2012). PGPB support plant growth through various mechanisms, including nutrient solubilization, hormone modulation, and pathogen inhibition. Over recent decades, PGPB have gained recognition as an eco-friendly alternative to synthetic inputs in agriculture, offering sustainable benefits such as improved crop resilience to abiotic stresses like drought (Vejan et al., 2016).

3. Mechanisms of PGPB in Drought Tolerance

Studies highlight several mechanisms through which PGPB help plants withstand drought conditions. These include:

Production of Phytohormones:

Certain PGPB strains produce phytohormones like indole-3-acetic acid (IAA), gibberellins, and cytokinin, which play essential roles in root growth, water uptake, and overall plant development under stress. IAA, for instance, stimulates root elongation, allowing plants to access deeper water sources in drought-prone soils (Spaepen et al., 2007).

❖ IAA Production Ability

Indole-3-acetic acid (IAA) production is a key trait in plant growth-promoting microorganisms, especially bacteria and fungi associated with plant roots. IAA is a form of auxin, a plant hormone that plays an essential role in plant growth and development, including root elongation, cell differentiation, and stress response. The ability of microorganisms to produce IAA can significantly impact plant health, making it a valuable area of study for agricultural applications.

➢ Importance of IAA-Producing Microorganisms in Agriculture

Enhanced Root Growth: Microbial IAA can stimulate plant root elongation and branching, allowing plants to absorb nutrients and water more efficiently.

Stress Tolerance: IAA-producing microbes can help plants endure abiotic stresses, such as drought and salinity, by enhancing root structure and function.

Plant Growth Promotion: Microorganisms with IAA-producing capabilities are commonly used as biofertilizers to promote sustainable agriculture, reducing the need for synthetic chemicals.

➢ Examples of IAA-Producing Microorganisms

1)*Rhizobium spp*

2)Azospirillum brasilense

3)Pseudomonas fluorescens

❖ Gibberellins:-

A class of plant hormones, are crucial for regulating a wide range of growth and developmental processes. They were first discovered in the fungus *Gibberella fujikuroi*, which infected rice plants and caused exaggerated growth. Gibberellins (GAs) are found throughout the plant kingdom and play a key role in growth regulation, influencing processes from seed germination to flowering and fruit development.

1. Functions of Gibberellins in Plants

Seed Germination: Gibberellins promote the breakdown of stored starches into sugars, providing energy for the growing embryo. GA also helps in mobilizing nutrients within the seed by stimulating enzymes like amylase.

Stem Elongation: One of the hallmark effects of gibberellins is promoting stem elongation by stimulating cell division and elongation, making them essential for overall plant height.

Flowering: GAs play a significant role in inducing flowering in many plants, particularly in long-day plants and biennials. They help transition the plant from vegetative to reproductive growth.

Leaf Expansion: Gibberellins stimulate cell expansion in leaves, contributing to leaf size and the overall photosynthetic area of the plant.

Fruit Development: GAs influence fruit set and growth. They are widely used in agriculture for seedless fruit production, like grapes, where GA application promotes larger fruit size and uniformity.

Dormancy: Gibberellins help break bud and seed dormancy, allowing plants to resume growth after periods of dormancy or unfavourable conditions.

2. Agricultural Applications of Gibberellins

Gibberellins are used commercially to regulate growth and improve yield in various crops:

Seedless Fruits: GAs are applied to promote the development of seedless grapes and other fruits.

Growth Promotion: In crops like sugarcane, GAs are used to boost growth rates and sugar content.

Malting in Barley: GAs are used to enhance enzyme production in barley, a key step in the beer-making process.

Overcoming Dormancy: Applied to seeds, bulbs, and tubers to stimulate germination and sprouting in commercial agriculture.

❖ ACC Deaminase Activity:

ACC deaminase is an enzyme produced by certain soil microorganisms that plays an important role in helping plants cope with various environmental stresses, including drought, salinity, and heavy metal toxicity. Here's a detailed overview of ACC deaminase activity and its significance:

1. Role and Mechanism of ACC Deaminase

ACC deaminase catalyses the breakdown of ACC, which is the immediate precursor of the plant hormone ethylene. Ethylene is produced in plants as a response to stress and has various functions in growth and development. However, high ethylene levels, especially under stress conditions, can inhibit root growth and compromise plant health.

The reaction catalysed by ACC deaminase is as follows:

$$\text{ACC} \longrightarrow \text{Ammonia } (NH_3) + \text{alpha-ketobutyrate}$$

$$C_4H_7NO_2 \longrightarrow C_4H_6O_3 + NH_3$$

Through this reaction, ACC deaminase-producing bacteria can lower ACC levels, reducing ethylene synthesis in plants. This, in turn, alleviates stress and allows the plant to maintain normal growth patterns.

2. Sources of ACC Deaminase

ACC deaminase is found in several PGPB, particularly those associated with the rhizosphere (the root zone), where they interact with plant roots. Common genera of bacteria that produce ACC deaminase include:

Pseudomonas,Rhizobium,Azospirillum,Burkholderia,Bacillus,Enterobacter

These bacteria can form beneficial associations with plants, often attaching to or colonizing root surfaces where they can interact directly with root-produced ACC.

3. Impact on Plant Growth Under Stress Conditions

Ethylene, while essential in small amounts, can inhibit root growth when produced in excess. This is particularly problematic under stress conditions, such as drought, salinity, or flooding, where ethylene levels tend to rise. High ethylene can lead to:

- Reduced root elongation
- Premature leaf senescence (aging)
- Decreased chlorophyll content, affecting photosynthesis
- Increased susceptibility to further environmental stresses

By lowering ethylene levels, ACC deaminase helps plants to:

Promote Root Elongation: With lower ethylene, roots can grow deeper into the soil, which is especially beneficial under drought conditions.

Enhance Stress Tolerance: Plants with lower ethylene levels tend to be more resilient to stress conditions, as they can allocate resources to growth rather than just stress responses.

Improve Nutrient and Water Uptake: A healthy root system enhances the plant's ability to absorb nutrients and water, even under challenging environmental conditions.

4. Application in Agriculture

ACC deaminase-producing bacteria have become a valuable tool in sustainable agriculture. These microbes are often used in biofertilizers and bio stimulants aimed at improving crop productivity, especially in stress-prone areas. Some key agricultural benefits include:

Drought Resistance: In arid or semi-arid regions, ACC deaminase-producing bacteria can support plants by minimizing the adverse effects of drought on growth.

Soil Health and Reduced Chemical Inputs: By promoting plant growth naturally, these bacteria reduce the need for synthetic fertilizers and other chemicals, supporting soil health.

Yield Stabilization: In conditions of environmental stress, such as high salinity or heavy metal toxicity, these bacteria help stabilize crop yields by maintaining plant health.

5. Research and Advancements

Recent research has focused on isolating and genetically improving ACC deaminase-producing strains to enhance their effectiveness in stress management. Advances include:

Genetic Engineering: Creating microbial strains with enhanced ACC deaminase activity to better support plant growth under extreme stress.

Formulating Consortia: Combining ACC deaminase-producing bacteria with other beneficial microbes, such as phosphate-solubilizing bacteria, to create more effective biofertilizers.

❖ Siderophore Production:

Microorganisms: Many bacteria and fungi produce siderophores to survive in iron-limited environments. Pathogens like *Escherichia coli, Pseudomonas aeruginosa, and Aspergillus fumigatus* are well-known for their siderophore production, which helps them to acquire iron and thrive in hostile environments, including within host organisms.

Mechanism: Siderophores chelate iron with high affinity and transport it into the cells through specific receptors. This mechanism not only aids in iron acquisition but also contributes to an organism's survival and virulence, particularly in pathogenic microbes.

Applications:

Agriculture: Beneficial bacteria like *Pseudomonas fluorescens* produce siderophores that promote plant growth by making iron more bioavailable in the rhizosphere.

Biomedicine: The inhibition of siderophore synthesis or uptake in pathogenic bacteria is being explored for its potential in new antimicrobial therapies.

❖ Exopolysaccharide (EPS) Production:

Exopolysaccharides (EPS) are high-molecular-weight polysaccharides secreted by microorganisms, including bacteria, fungi, and algae, into their surrounding environment. EPS play a crucial role in microbial interactions with plants, soil, and other organisms. They contribute to microbial adhesion, biofilm formation, and environmental stress tolerance, such as in drought conditions. In the context of PGPB, EPS are particularly beneficial for plant health, especially under stress.

1. Structure and Composition of EPS

• Chemical Structure: EPS are complex, often consisting of repeated units of monosaccharides (like glucose, galactose, mannose, rhamnose) and sometimes non-carbohydrate components (like proteins, lipids, or acetyl groups) that provide unique properties.

• Types of EPS:

• Homo-EPS: Composed of repeating units of a single type of sugar (e.g., dextran, levan).

• Hetero-EPS: Composed of two or more types of sugars (e.g., xanthan, alginate).

• Molecular Weight and Complexity: The molecular weight of EPS can vary significantly and often affects their physicochemical properties, such as viscosity, gel formation, and water-holding capacity.

2. Role of EPS in Plant Stress Tolerance

Drought Tolerance: Under drought conditions, EPS-producing PGPB are particularly beneficial:

Moisture Retention in Rhizosphere: EPS increase the water-holding capacity of the soil around plant roots, maintaining moisture availability and creating a more stable microenvironment.

Reduction of Hydraulic Conductance: EPS form a viscous layer in the rhizosphere that minimizes rapid water loss, slowing down water evaporation from the soil.

Salt Stress Alleviation: EPS can help plants tolerate salinity by binding and sequestering ions in the soil, reducing the uptake of toxic ions by plants.

Ion Chelation: EPS have sites that can bind ions, lowering the concentration of salts like sodium in the immediate root zone and reducing osmotic stress on plants.

3. EPS as a Barrier Against Pathogens and Heavy Metals

Physical Barrier: EPS form a protective layer around microbial cells, making it difficult for pathogens to penetrate and infect host plants.

Heavy Metal Chelation: EPS can bind heavy metals like lead, cadmium, and arsenic, preventing their uptake by plant roots and thus reducing toxicity.

Immobilization of Toxic Metals: By chelating metals, EPS reduce the bioavailability of these toxins, protecting both microbial cells and associated plants from their harmful effects.

5. Influence of EPS on Soil Structure and Fertility

Soil Aggregation and Stability: EPS secreted by soil microbes, particularly in the rhizosphere, bind soil particles and contribute to the formation of stable soil aggregates.

Improved Aeration and Root Growth: By stabilizing soil structure, EPS help improve soil aeration and allow better root growth, which is essential for healthy plant development.

Organic Matter Accumulation: EPS contribute to the accumulation of organic matter in soils, enhancing soil fertility and microbial activity.

Carbon Sequestration: EPS are carbon-rich compounds, which contribute to the organic carbon pool in soil, potentially aiding in carbon sequestration.

6. Agricultural Applications of EPS-Producing Bacteria

Drought-Resistant Biofertilizers: EPS-producing bacteria are being explored for use as biofertilizers, especially in drought-prone areas. These bacteria enhance soil water retention, promote root growth, and improve plant resilience to water scarcity.

Soil Stabilizers: EPS-producing bacteria may be used in soil stabilization projects to improve soil structure in degraded or sandy soils, making them more suitable for agriculture.

Phytoremediation: EPS-producing bacteria can be applied in phytoremediation, particularly in contaminated soils with heavy metals or high salt levels. By immobilizing toxins, they help plants grow in adverse conditions.

7. Examples of EPS-Producing PGPB

1) *Rhizobium and Sinorhizobium*
2) *Pseudomonas spp.*
3) *Azospirillum*
4) *Bacillus spp.*

❖ Enhanced Nutrient Solubilization:

PGPB can solubilize nutrients such as phosphate, making them available for plants. Improved nutrient uptake is especially critical under drought conditions, where nutrient mobility in the soil is often reduced (Rodriguez et al., 2004).

These mechanisms collectively improve the drought resilience of soils and crops, making PGPB a promising tool for sustainable agriculture in water-limited environments.

❖ Phosphate solubilizing ability :-

Phosphate solubilization refers to the process by which certain microorganisms, mainly bacteria and fungi, convert insoluble forms of phosphate into soluble forms that plants can absorb. This ability is particularly significant for agriculture, as phosphate is a crucial nutrient for plant growth, yet is often present in soils in forms that plants cannot readily access. Here are some details about the process and its significance:

➢ Mechanism of Phosphate Solubilization

1. Organic Acid Production: Many phosphate-solubilizing microorganisms (PSMs) produce organic acids, such as gluconic acid, citric acid, and lactic acid, which acidify the soil microenvironment. These acids chelate cations bound to phosphate, thereby releasing the phosphate into a soluble form.
2. Enzyme Secretion: Certain enzymes, like phosphatases, are secreted by these microbes, which helps in breaking down organic forms of phosphate into inorganic phosphate that plants can take up.
3. Acidification: PSMs often lower the pH of their surroundings by producing protons, further aiding the dissolution of insoluble phosphate minerals.

➢ Types of Phosphate-Solubilizing Microorganisms

Bacteria: Genera such as *Pseudomonas, Bacillus,* and *Rhizobium* are known for their phosphate-solubilizing abilities.

Fungi: Fungi like *Aspergillus* and *Penicillium* are also recognized for their effectiveness in phosphate solubilization.

Actinomycetes: Though less common, *Actinomycetes* also contribute to phosphate solubilization.

➢ Importance in Agriculture

Improved Crop Yield: By making phosphate more accessible to plants, PSMs enhance plant growth and productivity.

Reduced Fertilizer Usage: Phosphate-solubilizing microbes can reduce the dependency on chemical fertilizers, promoting more sustainable agricultural practices.

Soil Health: These microbes also contribute to soil biodiversity and improve soil health over time.

❖ Nitrogen fixation Ability

Nitrogen fixation is a crucial biological process in which atmospheric nitrogen (N_2) is converted into ammonia (NH_3), making nitrogen accessible to plants in a form they can use for growth. Plants need nitrogen as a nutrient, but they cannot directly absorb atmospheric nitrogen. Therefore, certain microorganisms, mainly bacteria, perform this fixation process and significantly contribute to soil fertility and sustainable agriculture.

➢ Mechanism of Nitrogen Fixation

Enzyme Nitrogenase: Nitrogen-fixing bacteria produce the enzyme nitrogenase, which catalyses the conversion of nitrogen gas to ammonia. This enzyme functions under anaerobic conditions, as it is highly sensitive to oxygen.

Symbiotic Nitrogen Fixation: In this type, bacteria, particularly *Rhizobium* and *Bradyrhizobium*, form symbiotic relationships with leguminous plants. They infect the root nodules, where they fix nitrogen in exchange for carbohydrates from the plant.

Free-Living Nitrogen Fixers: Some bacteria, like *Azotobacter* and *Clostridium*, do not require a host plant to fix nitrogen and instead function independently in the soil.

Associative Nitrogen Fixation: Other bacteria, such as *Azospirillum*, form loose associations with the roots of non-leguminous plants, enhancing nitrogen fixation in the rhizosphere.

➢ Types of Nitrogen-Fixing Microorganisms

- Symbiotic Bacteria: *Rhizobium, Bradyrhizobium*, and *Frankia* (which associates with actinorhizal plants) are well-known nitrogen fixers in symbiotic relationships.

Free-Living Bacteria: *Azotobacter*, *Clostridium*, and *Klebsiella* are examples of bacteria that fix nitrogen independently.

Cyanobacteria: Photosynthetic bacteria, such as *Anabaena* and *Nostoc*, fix nitrogen in aquatic environments and soils.

➢ Importance in Agriculture

Enhanced Crop Yield: By increasing the nitrogen available to plants, nitrogen-fixing organisms promote plant growth and productivity.

Reduction in Nitrogen Fertilizers: With the help of nitrogen-fixing bacteria, farmers can reduce the amount of nitrogen fertilizer required, which lowers costs and minimizes environmental pollution.

Soil Health: Nitrogen-fixing bacteria improve soil structure and fertility, contributing to long-term soil health and sustainability.

❖ Effectiveness of PGPB in Drought-Affected Soils

Numerous studies have demonstrated the effectiveness of PGPB in improving crop growth and yield under drought conditions. For instance, research by Sarma and Saikia (2014) demonstrated that the application of drought-tolerant Pseudomonas and Bacillus strains improved rice and wheat growth by enhancing root elongation and nutrient uptake. Similarly, a study on wheat under water-deficit conditions showed that PGPB application improved plant biomass, chlorophyll content, and water-use efficiency (Nadeem et al., 2014). These results suggest that PGPB can play a critical role in mitigating the adverse effects of drought on agriculture.

❖ PGPB-Plant Interactions in Drought Conditions

The interactions between PGPB and plants under drought stress have been well-documented.(Vurukonda et al. 2016) observed that PGPB inoculation in maize led to enhanced root architecture and biomass accumulation, helping plants survive prolonged water scarcity. Furthermore, studies indicate that PGPB can induce systemic resistance (ISR) in plants, enhancing their tolerance to various abiotic stresses, including drought. The ISR response, along with other PGPB-induced metabolic changes, makes plants more resilient by preparing them to better respond to water stress (Kang et al., 2014).

PGPB are beneficial microbes that form symbiotic or associative relationships with plants, enhancing plant growth and tolerance to various environmental stresses, including drought. Under drought conditions, PGPB help plants by improving water uptake, stimulating growth, enhancing root architecture, and modulating stress responses. The mechanisms through which PGPB aid plants under drought stress are diverse and complex, involving both direct and indirect pathways.

1. Mechanisms of Plant Growth-Promoting Bacteria in Drought Tolerance

Production of Phytohormones: PGPB produce phytohormones like auxins (IAA), cytokinin, gibberellins, and abscisic acid (ABA). These hormones play key roles in modulating plant responses to drought:

- Auxins: PGPB-produced IAA enhances root growth and branching, allowing plants to access deeper water reserves.
- Cytokinin: Help delay leaf senescence and maintain cellular function under stress.
- Gibberellins: Stimulate growth processes, though their levels are often balanced with ABA during drought to prevent excessive growth.
- ABA: Acts as a stress hormone, regulating stomatal closure to reduce water loss and enhance drought tolerance. Some PGPB can increase ABA levels in plants, contributing to better water-use efficiency.
- Enhanced Root Architecture and Development: By producing auxins and other growth-promoting compounds, PGPB improve root growth and architecture. Enhanced root systems provide:
- Greater Root Surface Area: Increases water and nutrient absorption, helping plants survive in low-moisture soils.
- Deeper Root Penetration: Allows plants to reach water sources deeper in the soil profile, which is essential under drought.
- Production of EPS: PGPB can secrete exopolysaccharides, which help in:
  Soil Aggregation: EPS bind soil particles, improving soil structure and water retention around roots.
- Moisture Retention: EPS retain moisture near the root zone, creating a microenvironment that maintains humidity, which is critical for plant survival in drought conditions.
- Osmotic Adjustment and Production of Compatible Solutes: Some PGPB produce osmolytes, such as proline, glycine betaine, and trehalose, which help in:
- Maintaining Cellular Turgor: These osmolytes help maintain cell water balance and protect cellular structures from dehydration.
- Reducing Oxidative Stress: Osmolytes serve as antioxidants, neutralizing reactive oxygen species (ROS) generated under drought stress.
- Induction of Antioxidant Enzyme Activity: Drought stress causes oxidative damage in plants due to increased ROS production. PGPB can boost the plant's antioxidant

defence mechanisms by:

- Enhancing Enzymes Like Superoxide Dismutase (SOD), Catalase (CAT), and Peroxidase (POD): These enzymes detoxify ROS, reducing cellular damage.
- Producing Antioxidants: PGPB can produce antioxidants such as glutathione, which helps protect plant cells from oxidative stress induced by drought.
- Nitrogen Fixation: Certain PGPB, especially those in the rhizobia group, can fix atmospheric nitrogen, converting it into a form that plants can use.
- Improved Nitrogen Availability: In drought-stressed soils, where nutrient availability is often limited, nitrogen-fixing bacteria provide a steady nitrogen supply, aiding in protein synthesis and stress tolerance.
- Reduced Dependency on Soil Nutrients: Nitrogen fixation by PGPB enables plants to maintain growth even when soil nitrogen is limited under drought conditions.
- Phosphate Solubilization and Nutrient Mobilization: Drought conditions often limit the availability of nutrients, particularly phosphates, in the soil.
- PGPB Solubilize Phosphate: Through the production of organic acids and phosphatases, PGPB can solubilize and mobilize phosphorus, making it available for plant uptake.
- Enhanced Nutrient Uptake: PGPB help in mobilizing other nutrients like potassium and iron, which are essential for stress responses and overall plant health.

2. Suppression of Ethylene Production

ACC Deaminase Activity: Many PGPB produce ACC deaminase, an enzyme that breaks down ACC, the precursor of ethylene.

Reduced Ethylene Levels: High ethylene levels can inhibit root growth under stress. By lowering ethylene levels, PGPB allow roots to continue growing and exploring the soil for water, even under drought conditions.

Enhanced Root Growth and Stress Resilience: Lower ethylene levels lead to better root elongation, allowing plants to access water more effectively in drought-prone soils.

3. Modulation of Soil Microbiome and Rhizosphere Effects

Rhizosphere Colonization and Biofilm Formation: PGPB form biofilms on the root surface, creating a stable environment that enhances root colonization and drought tolerance.

Improved Soil Structure and Water Retention: Through biofilm formation and EPS production, PGPB enhance the water-holding capacity of the soil around roots, reducing water stress.

Positive Impact on Rhizosphere Microbiome: PGPB can promote a more diverse and resilient microbial community in the rhizosphere. This microbial diversity helps improve soil structure, nutrient availability, and pathogen resistance, contributing to drought resilience.

4. Examples of PGPB with Drought-Resistance Abilities

1) *Rhizobium and Sinorhizobium:*

2) Pseudomonas spp

3) Bacillus spp

5)Azospirillum

6) Paenibacillus polymyxa:

5. Agricultural Applications and Future Prospects

PGPB as Bio-inoculants: The application of PGPB as bio-inoculants in arid and semi-arid regions can enhance crop productivity and sustainability under drought conditions. Formulating PGPB-based biofertilizers tailored for specific crops and soil types is an active area of research.

Use in Integrated Drought Management: Combining PGPB with other drought management practices, such as improved irrigation techniques, organic amendments, and crop selection, provides a comprehensive approach to drought resilience.

Genomic and Metabolic Engineering of PGPB: Advances in genomics and synthetic biology allow scientists to enhance the drought tolerance mechanisms of PGPB. Genetic engineering may allow for the development of PGPB strains with optimized traits like increased ACC deaminase activity, enhanced phytohormone production, or specific VOC profiles for drought resilience.

6 Environmental Impact and Sustainability of PGPB in Drought Management

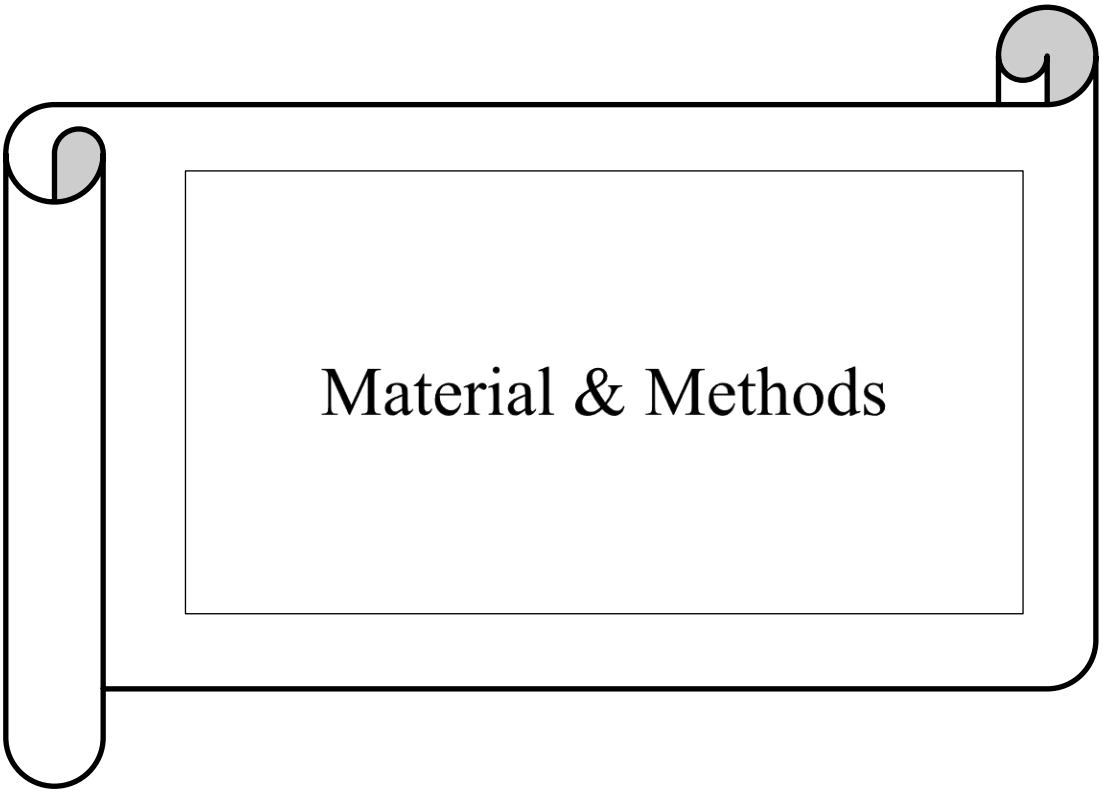
PGPB offer a sustainable and eco-friendly approach to enhancing drought tolerance in soils, contrasting with conventional methods that often involve intensive irrigation and fertilizer use. Several studies emphasize that PGPB application can reduce the reliance on chemical inputs, lowering costs for farmers and minimizing environmental pollution (Adesemoye et al., 2009). Additionally, PGPB improve soil microbial diversity and function, which is critical for long-term soil health, particularly in ecosystems prone to drought (Beneduzi et al., 2012).

❖ Challenges and Limitations

Despite the benefits of PGPB, several challenges remain in implementing them on a large scale. Soil variability, environmental conditions, and the complex interactions between PGPB and native microbial communities can affect PGPB efficacy. For instance, strains that perform well under controlled conditions may not show the same effectiveness in diverse field environments (Compant et al., 2010). Additionally, more research is needed to optimize formulations and delivery methods for PGPB to ensure consistent results in drought-stressed soils (Sarkar et al., 2020).

❖ Future Directions in PGPB Research

The potential of PGPB to mitigate drought effects in soils has led researchers to explore various avenues for enhancing their effectiveness. Genomic studies, for example, are helping identify specific traits in PGPB strains that contribute to drought tolerance, paving the way for the development of bioengineered strains with improved efficacy (Ali et al., 2014). Research is also focusing on formulating consortia of PGPB to harness the synergistic effects of multiple beneficial strains, which could lead to more resilient and adaptive drought solutions (Singh et al., 2020). Finally, integrating PGPB into sustainable agricultural practices and policies will be essential for broad-scale adoption in drought-prone regions.


Material & Methods

Material & Methods

❖ Collection of Soil Sample

- The study Focused on soil sample collected from various Location Like Bambavade, Sangli, Shivaji University and Shenda Park.
- A systematic approach was employed. Sample was collected from drought area

Table 1 :- Soil Sample & Their Location

Sr. No	Sample	Location
1	A	Bambavade
2	B	Shenda Park
3	C	Shivaji University
4	D	Sangli

❖ Physical Analysis :-

Collected Soil sample needs to analysed for further study.

For analysis we determine various characteristic of Soil

- **Texture :-**

Collected soil sample number 1 was found to be moist, Sample number 2 was found to be loam, sample number 3 was found to be sandy & sample number 4 was found to be loam

- **Colour**

Soil colour is often and indicator of its composition and organic matter contain

Dark soil are typically rich in organic matter

Red soil are typically rich in iron oxide

Light colour are often Sandy and low in organic matter

- **Temperature**

Soil temperature impact seed germination root growths and microbial activity

Dark side often warm up faster than light soil impacting the growing season for plants

❖ Isolation

The Study aimed to isolate and characterise drought tolerant bacteria from the collecting soil sample.

The isolation process involves following steps

- Preparation of soil dilutions
  - 1 gm of soil sample from each samples was majored and added in 1st sterile distilled water (10ml) tubes and labelled as 10^{-1}.
  - Then serially add 1ml sample from 10^{-1} test tube to next sterile distilled water tubes having D/W 9 ml
  - This process carried out up to 10^{10} Labelled Tubes

❖ Inoculation :-

- To isolate bacteria from sample sterile nutrient agar plate were prepared
- On each plate respective dilutions of respective samples [dilution 10^{-3},10^{-4},10^{-5}] were spread by using spread plate technique

❖ Incubation :-

- For isolation of PGPB we need to incubated the plates
- Incubation done at room temperature for 24 hrs
- After incubation individual bacterial colony was observed on plate
- We selected colonies from respective nutrient agar plate

	Dilution	Selected Colonies
Sample 1:-	10^{-3}	6
	10^{-4}	5
	10^{-5}	3

	Dilution	Selected Colonies
Sample 2:-	10^{-3}	5
	10^{-4}	5
	10^{-5}	5

	Dilution	Selected Colonies
Sample 3:-	10^{-3}	6
	10^{-4}	3
	10^{-5}	5

	Dilution	Selected Colonies
Sample 4:-	10^{-4}	4
	10^{-5}	4
	10^{-6}	2

For our convenient purpose we labelled that sample & as well as selected bacterial colonies

a) For sample

Sample	label
1	A
2	B
3	C
4	D

b) For dilutions

Sample	Dilution	label
1	10^{-3}	a
	10^{-4}	b
	10^{-5}	c
2	10^{-3}	a
	10^{-4}	b
	10^{-5}	c
3	10^{-3}	a
	10^{-4}	b
	10^{-5}	c
4	10^{-4}	a
	10^{-5}	b
	10^{-6}	c

Isolated bacteria were then maintained on nutrient agar slant at suitable storage condition

Morphological characters [garam staining] was observed

❖ Screening

To study characterization of PGPB we need to perform some test for example.

- Phosphate solubilizing ability
- Nitrogen fixing ability
- Siderophore.
- IAA production ability
- Exopolysaccharide production ability

❖ Phosphate solubilizing ability

- For determining the phosphate solubilizing ability we performed the following steps

- Phosphate solubilizing ability was studied using the method of Fiske & Subbarow (1925)
- We have prepared sterile Pikovskaya agar plats.
- Then each selected colony was inoculated by spot inoculation method.
- plates were incubated at room temperature for 3-4 days
- After incubation we have observed clear zone around some Colonies

Table 2 :- Composition of Pikovskaya agar

Sr. No	Media	Quantity
1	Peptone	10 g
2	Dextrose	10 g
3	Potassium Chloride	0.5 g
4	Agar	15 g
5	Distilled Water	1 Lit

❖ Nitrogen fixation Ability

- For determining the phosphate solubilizing ability we performed the following steps
- Nitrogen fixation ability was studied using the method of sergei winogradsky (1888)
- We have prepared sterile Ashby's mannitol agar plates and add bromothymol blue
- Selected organism were inoculated on each separate plates. by using spot inoculation method.
- Each Plates was incubated at room temperature (25-28 ºC) for 4-5 days &noted down the Observation

Table 3 :- Composition of Ashbey's mannitol agar

Sr. No	Media	Quantity
1	Mannitol	20.0 g
2	K_2HPO_4	0.2 g
3	$MgSo_4\ 7H_2O$	0.2 g
4	NaCl	0.2 g
5	K_2So_4	0.1 g
6	$CaCO_3$	5.0 g
7	Agar	20.0 g
8	D/W	1000 ml
9	pH	7.2

❖ IAA production ability.

➢ For determination of IAA production ability of we performed following steps

- IAA production ability was studied using the method of Gordan & weber (1951)
- We prepared 50ml nutrient broth for each isolates.
- Loopful of isolates were inoculated into the broth.
- Flask were incubated at room temperature for 5-6 days.
- After incubation 1ml Sample was collected for centrifugation.
- After centrifugation 1ml supernatant from each tubes was Collected
- Then we added 2ml Salkowaski reagent & 2 drops of Orthophosphoric acid in each tube then we optioned pink colour
- Optical density was checked using colorimeter at 530 nm filter.

❖ Siderophore Production Ability :-

➢ For Determination of siderophore production ability of we performed following steps

- siderophore production ability was studied using the method of Schwyn & Neiland (1987)
- We prepared sterile CAS agar plate
- Selected organism were inoculated on plates. by using spot inoculation method.
- Incubation was done at room temperature for 4-5 days & noted down the observation

➢ Preparation of CAS agar

1) Blue dye-

solution 1: [CAS = Chrome Azorol S]

dissolve 0.06 gm of CAS in 50ml d/w

Solution 2

dissolve 0.0027. gm of $Fecl_3$ in 10 ml of 10mM HCI

Solution 3 :-

dissolve 0.073 gm of HDTMA in 40 ml of d/w

-Mix solution 1 with 9ml of solution 2 then mix with solution 3.

solution should be blue in colour.

2) Mixture solution =

A) Minimal media -

dissolve 15 gm of KH_2PO_4, 25 gm NaCl, 50gm NH_4Cl in 500ml d/w

(B) 20% glucose stock-

20 gm glucose +100 ml d/w

C) NaOH stock

dissolve 25 gm NaOH in 150 ml d/w PH=12.

3) CAS agar preparation.

A) Add 100ml of mm-9 solution to 750 ml d/w

B) Dissolve 32.24gm PIPES (Piperzine NN base 2-ethene sulphuric acid)

PIPES do not dissolve below pH 5 bring pH up to 6 and slowly add PIPES while stirrings the pH will draw as PIPES dissolve while stirring slowly bring pH. up to 6.8.

C) Add 15 gm agar powder and autoclave it

Add 10 ml of sterile 20% glucose to MM 9 PIPES Mixture.

slowly add 100ml. of blue dye solution along the glass wall with enough agitation to mix thourghly aseptically pour plates.

❖ Exopolysaccharide production ability

➢ For Determination of siderophore production ability of we performed following steps

- Eps production ability was studied using the method of Nicolous (1999)
- we prepared 50 ml. Nicolous broth for each isolate
- loopful of isolates were inoculated into the broth
- Flasks were incubated at room temperature for 5-6 days
- After incubation sample was collected for centrifugation
- After centrifugation the 1 ml supernatant was collected and added with chilled ethanol
- The white precipitate formed shows positive result


Result & Discussion

Result & Discussion

❖ Result

❖ Collection of Soil

After Collection of Soil Sample it is necessary to analyse collected soil for further studied. We have determined various characteristics of Soil for Example Texture, Colour& Temp

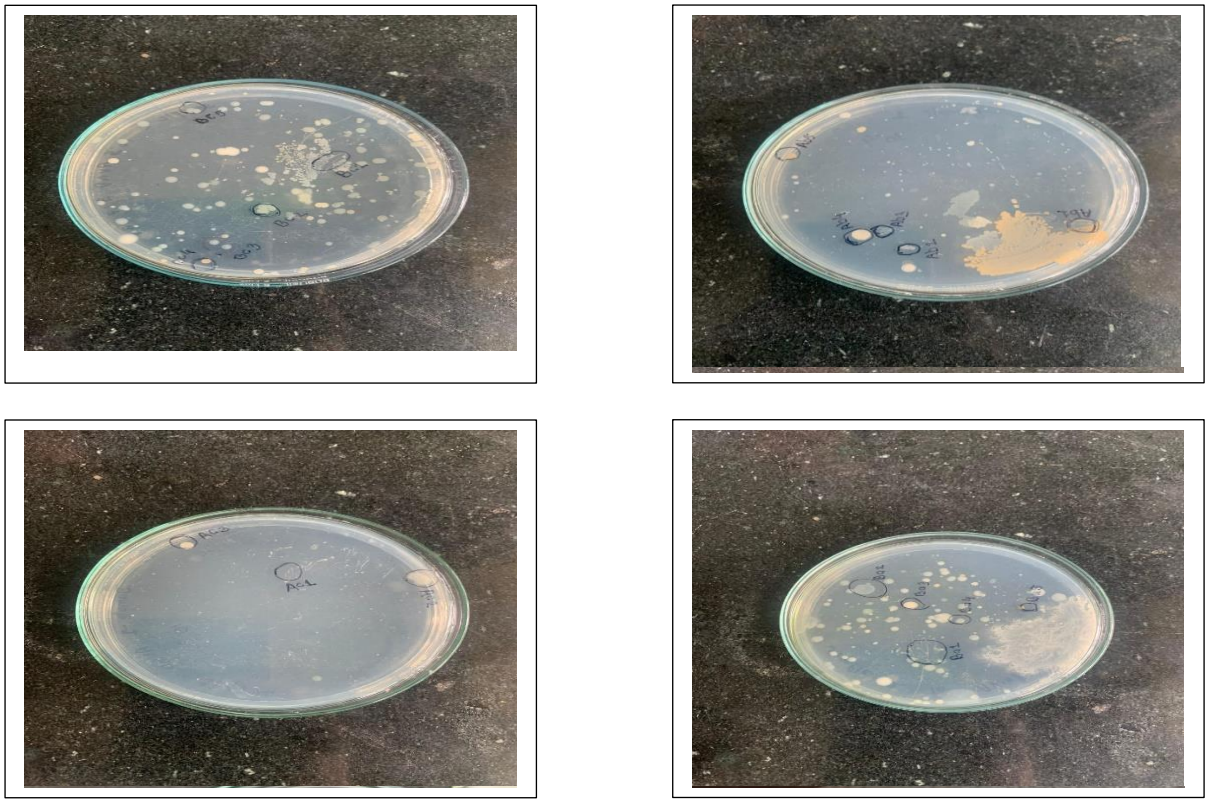
Table 4 :- Properties of collected soil samples

Sr. No	Location	Texture	Colour
1	Bambavade	Moist	Dark brown
2	Shenda Park	Loam	Brown
3	Shivaji University	Sandy	Light
4	Sangli	Loam	Red

❖ Isolation

After incubation at room temperature for 24 hrs. On Nutrient agar various types of colonies was observed. There are 48 organism we selected for further Study.

Fig No 1 : Colonies of organism on nutrients agar



For study aimed we were focused on to get potent organism where character of selected organism were noted down

➢ Table No. 5. Colony Characters of isolated organisms

Organism	Size (mm)	Shape	Margin	Colour	Elevation	Opacity	Surface	Consistency
Aa 1	1	Irregular	Entire	Peal Yellow	Flat	Opaque	Smooth	Buttery
Aa 2	1	Irregular	Wavy	White	Flat	Opaque	Smooth	Buttery
Aa 3	8	Irregular	Entire	White	Flat	Opaque	Smooth	Sticky
Aa 4	2	Circular	Entire	Yellow	Raised	Translucent	Smooth	Buttery
Aa 5	3	Circular	Entire	Peal Yellow	Raised	Opaque	Smooth	Sticky
Aa 6	2	Circular	Entire	White	Flat	Translucent	Smooth	Sticky
Ab 1	1	Circular	Entire	Yellow	Raised	Opaque	Smooth	Buttery
Ab 2	1	Circular	Entire	White	Flat	Translucent	Smooth	Buttery
Ab 3	1	Irregular	Wavy	White	Raised	Transparent	Slimy	Sticky
Ab 4	5	Circular	Entire	White	Convex	Opaque	Smooth	Dry
Ab 5	3	Circular	Entire	Yellow	Raised	Opaque	Slimy	Sticky
Ac 1	1	Circular	Entire	White	Flat	Opaque	Smooth	Buttery
Ac 2	6	Irregular	Entire	White	Raised	Transparent	Mucoid	Sticky
Ac 3	4	Circular	Entire	Off White	Falt	Opaque	Smooth	Buttery
Ba 1	1	Circular	Entire	White	Raised	Opaque	Smooth	Sticky
Ba 2	7	Irregular	Entire	White	Flat	Translucent	Slimy	Buttery
Ba 3	5	Circular	Entire	White	Convex	Opaque	Smooth	Buttery
Ba 4	3	Circular	Entire	White	Raised	Translucent	Smooth	Sticky
Ba 5	3	Circular	Entire	Yellow	Convex	Opaque	Slimy	Sticky
Bb 1	4	Irregular	Entire	White	Convex	Translucent	Smooth	Buttery
Bb 2	3	Irregular	Wavy	White	Raised	Opaque	Slimy	Buttery
Bb 3	2	Circular	Entire	White	Raised	Opaque	Smooth	Sticky
Bb 4	8	Circular	Wavy	White	Raised	Opaque	Smooth	Sticky
Bb 5	4	Circular	Entire	White	Raised	Transparent	Smooth	Buttery
Bc 1	1	Irregular	Wavy	White	Flat	Opaque	Slimy	Sticky
Bc 2	4	Irregular	Wavy	White	Raised	Opaque	Smooth	Sticky
Bc 3	4	Circular	Entire	White	Convex	Translucent	Smooth	Sticky
Bc 4	3	Circular	Entire	Pale Yellow	Raised	Opaque	Slimy	Buttery
Bc 5	2	Circular	Entire	White	Convex	Translucent	Smooth	Sticky
Ca 1	1	Irregular	Wavy	White	Raised	Opaque	Slimy	Sticky
Ca 2	2	Circular	Entire	White	Flat	Translucent	Smooth	Buttery
Ca 3	1	Circular	Entire	Yellow	Raised	Opaque	Smooth	Sticky
Ca 4	2	Circular	Entire	Off White	Convex	Opaque	Smooth	Buttery
Ca 5	6	Irregular	Wavy	White	Raised	Opaque	Slimy	Sticky
Ca 6	4	Circular	Wavy	White	Flat	Translucent	Smooth	Sticky
Cb 1	1	Circular	Entire	White	Convex	Transparent	Smooth	Sticky
Cb 2	2	Irregular	Wavy	White	Raised	Opaque	Smooth	Buttery
Cb 3	4	Irregular	Entire	Off White	Raised	Translucent	Slimy	Sticky
Da 1	2	Circular	Wavy	Off White	Raised	Opaque	Smooth	Buttery

Da 2	3	Circular	Entire	White	Convex	Opaque	Slimy	Sticky
Da 3	1	Irregular	Entire	Yellow	Flat	Opaque	Slimy	Sticky
Da 4	1	Circular	Entire	Yellow	Convex	Opaque	Smooth	Sticky
Db 1	3	Irregular	Entire	Off White	Convex	Transparent	Smooth	Sticky
Db 2	2	Circular	Entire	Orange	Flat	Opaque	Smooth	Sticky
Db 3	1	Irregular	Entire	Yellow	Flat	Opaque	Smooth	Sticky
Db 4	3	Irregular	Entire	Orange	Raised	Opaque	Smooth	Sticky
Dc 1	3	Irregular	Entire	Off White	Convex	Opaque	Smooth	Sticky
Dc 2	1	Circular	Entire	White	Flat	Opaque	Smooth	Sticky

- **Table No. 6. : Gram nature & shape of organism**

Organism	Gram Nature	Shape
Aa 1	Gram Positive	Short Rod
Aa 2	Gram Positive	Cocci
Aa 3	Gram Positive	Short rod
Aa 4	Gram Positive	Cocci
Aa 5	Gram Positive	Rod
Aa 6	Gram Positive	Short rod
Ab 1	Gram Positive	Short rod
Ab 2	Gram Positive	Rod
Ab 3	Gram Positive	Short rod
Ab 4	Gram Positive	Bacilli
Ab 5	Gram Positive	Cocci
Ac 1	Gram Positive	Cocci
Ac 2	Gram Positive	Short rod
Ac 3	Gram Positive	Rod
Ba 1	Gram Positive	Cocci
Ba 2	Gram Positive	Short rod
Ba 3	Gram Positive	Short rod
Ba 4	Gram Positive	Cocci
Ba 5	Gram Positive	Bacili
Bb 1	Gram Positive	Short rod
Bb 2	Gram Positive	Short Rod
Bb 3	Gram Positive	Cocci
Bb 4	Gram Positive	Short rod
Bb 5	Gram Positive	Cocci

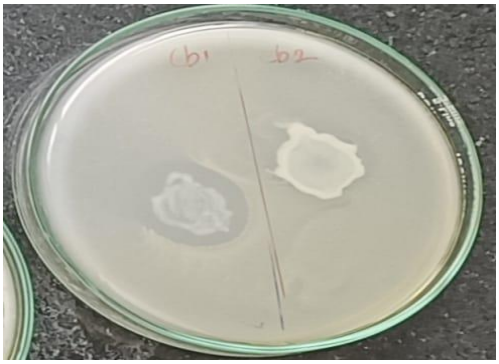
Organism	Gram Nature	Shape
Bc 1	Gram Positive	Cocci
Bc 2	Gram Positive	Cocci
Bc 3	Gram Positive	Cocci
Bc 4	Gram Positive	Rod (Arranged in Chain)
Bc 5	Gram Positive	Short rod
Ca 1	Gram Positive	Cocci
Ca 2	Gram Positive	Cocci
Ca 3	Gram Positive	Cocci
Ca 4	Gram Positive	Short rod
Ca 5	Gram Positive	Cocci
Ca 6	Gram Positive	Cocci
Cb 1	Gram Positive	Cocci
Cb 2	Gram Positive	Rod
Cb 3	Gram Positive	Cocci
Da 1	Gram Positive	Short rod
Da 2	Gram Positive	Cocci
Da 3	Gram Positive	Rod
Da 4	Gram Positive	Short rod
Db 1	Gram Positive	Short rod
Db 2	Gram Positive	Short rod
Db 3	Gram Positive	Cocci
Db 4	Gram Positive	Rod
Dc 1	Gram Positive	Short rod
Dc 2	Gram Positive	Short rod

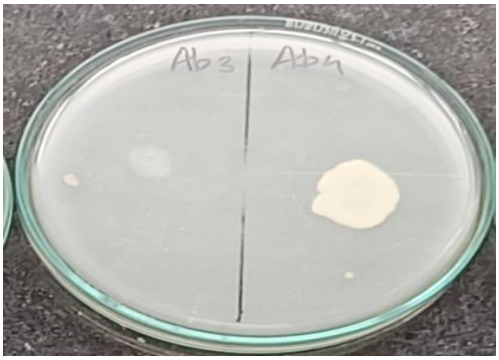
❖ Screening of isolated organisms for their different abilities

➢ Phosphate Solubilising Bacteria

On Pikovskaya (Pk) agar medium the spot inoculated 4 isolates showed the Clear zone around colony after 48 hours. The spot inoculated some organisms shown the positive results

Fig No 2: phosphate solubilizing





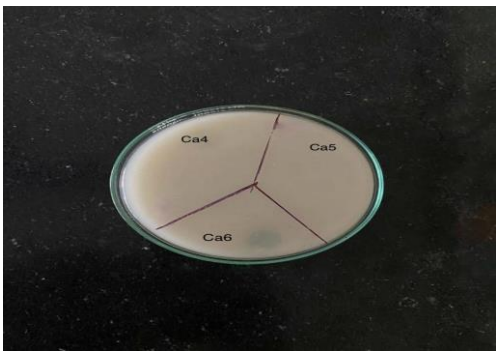


Table No. 7 :- Result of phosphate solubilizing organisms

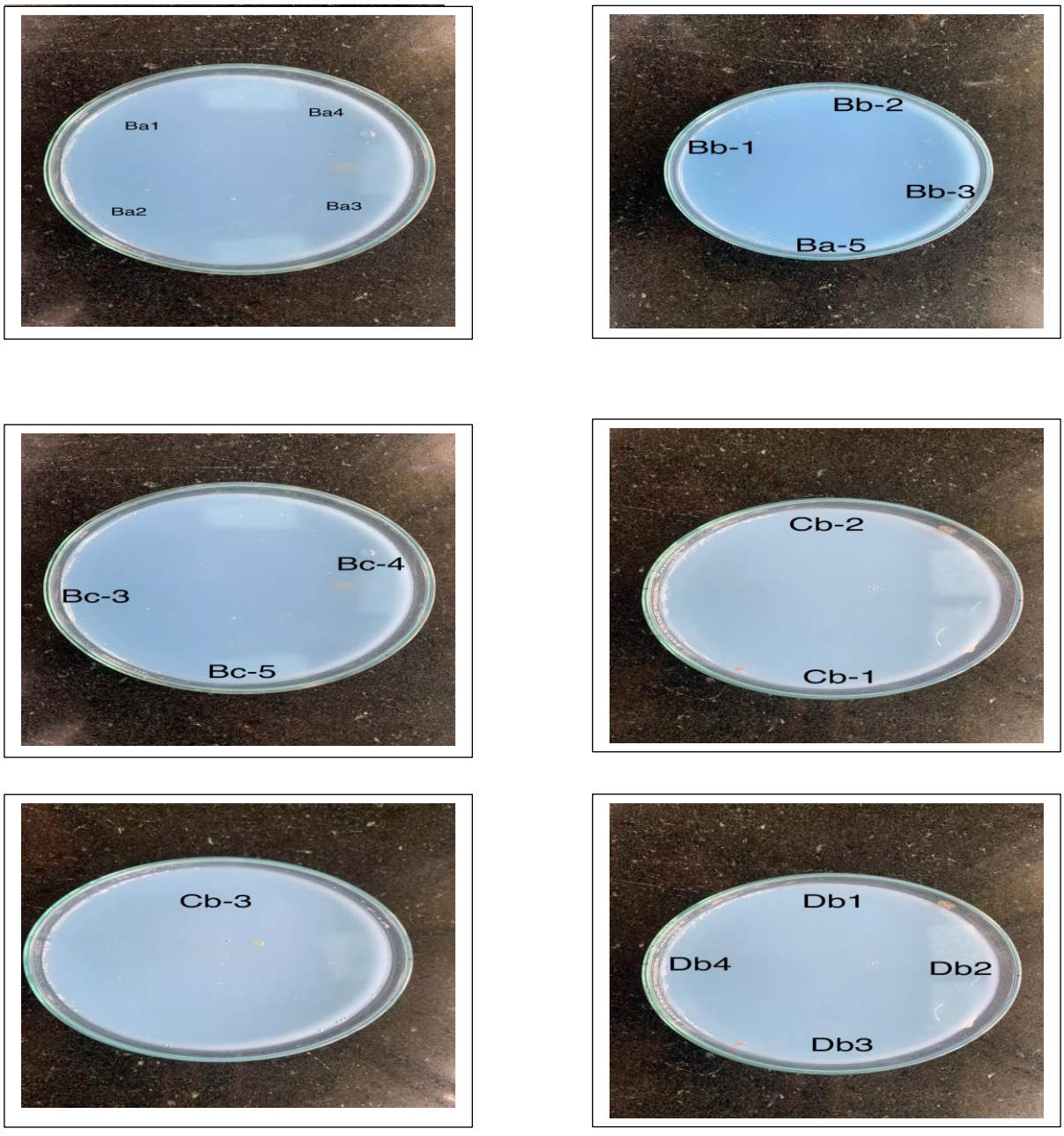
Organism	Result
Aa 1	Negative
Aa 2	Negative
Aa 3	Negative
Aa 4	Negative
Aa 5	Negative
Aa 6	Negative
Ab 1	Negative
Ab 2	Negative
Ab 3	Negative
Ab 4	Negative
Ab 5	Negative
Ac 1	Negative
Ac 2	Negative
Ac 3	Negative
Ba 1	Negative
Ba 2	Negative
Ba 3	Negative
Ba 4	Negative
Ba 5	Negative
Bb 1	Negative
Bb 2	Negative
Bb 3	Negative
Bb 4	Negative
Bb 5	Negative

Organism	Result
Bc 1	Negative
Bc 2	Negative
Bc 3	Negative
Bc 4	Negative
Bc 5	Negative
Ca 1	Negative
Ca 2	Negative
Ca 3	Negative
Ca 4	Negative
Ca 5	Negative
Ca 6	Negative
Cb 1	Positive
Cb 2	Positive
Cb 3	Negative
Da 1	Negative
Da 2	Negative
Da 3	Negative
Da 4	Negative
Db 1	Negative
Db 2	Negative
Db 3	Negative
Db 4	Negative
Dc 1	Negative
Dc 2	Negative

➢ Nitrogen fixation Ability-

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

Fig No 3 : Organism showing nitrogen fixation



❖ Table No. 8 :- Result of nitrogen fixation organism

Organism	Result
Aa 1	Negative
Aa 2	Negative
Aa 3	Negative
Aa 4	Positive
Aa 5	Negative
Aa 6	Negative
Ab 1	Negative
Ab 2	Negative
Ab 3	Negative
Ab 4	Positive
Ab 5	Negative
Ac 1	Positive
Ac 2	Negative
Ac 3	Negative
Ba 1	Negative
Ba 2	Positive
Ba 3	Negative
Ba 4	Negative
Ba 5	Negative
Bb 1	Negative
Bb 2	Negative
Bb 3	Negative
Bb 4	Negative
Bb 5	Positive

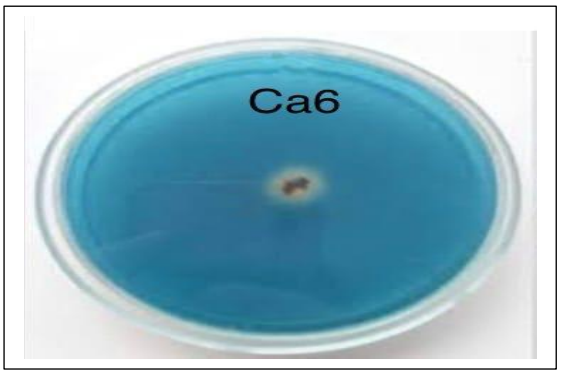
Organism	Result
Bc 1	Negative
Bc 2	Positive
Bc 3	Negative
Bc 4	Negative
Bc 5	Negative
Ca 1	Negative
Ca 2	Negative
Ca 3	Positive
Ca 4	Negative
Ca 5	Positive
Ca 6	Negative
Cb 1	Negative
Cb 2	Negative
Cb 3	Negative
Da 1	Negative
Da 2	Negative
Da 3	Positive
Da 4	Negative
Db 1	Negative
Db 2	Negative
Db 3	Positive
Db 4	Negative
Dc 1	Negative
Dc 2	Negative

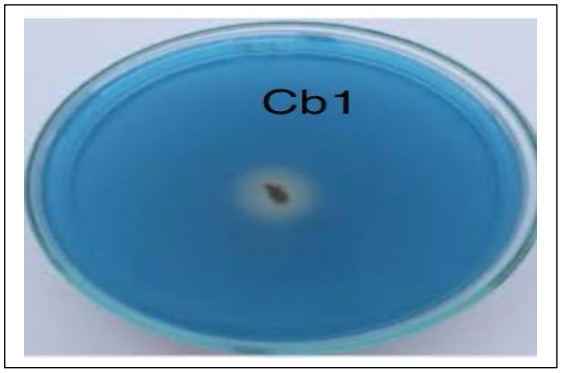
❖ Siderophore :-

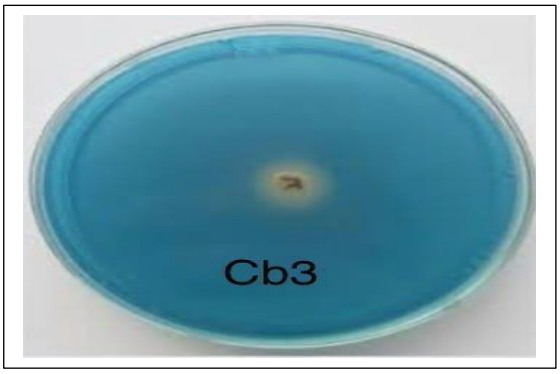
Siderophore :-

The isolates were spot inoculated on CAS medium. After 3-4 Days , Yellow colour zone around colonies were observed.

Fig No 4 : Organism showing siderophore production abilit







❖ IAA (Indole Acetic acid) :-

Result:- Qualitative and Quantitative Estimation of IAA

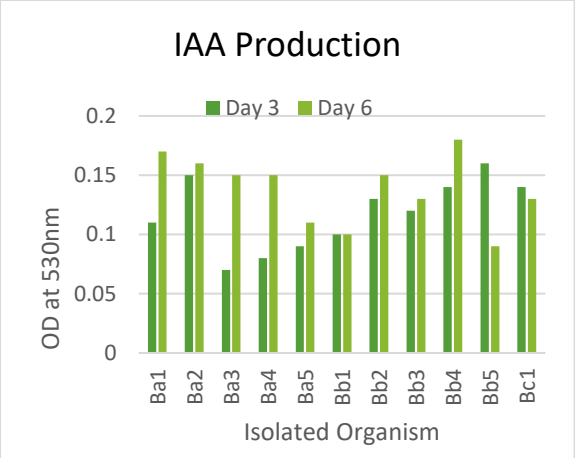
Tested IAA producing activity All of them are positive & show slightly pink colour.

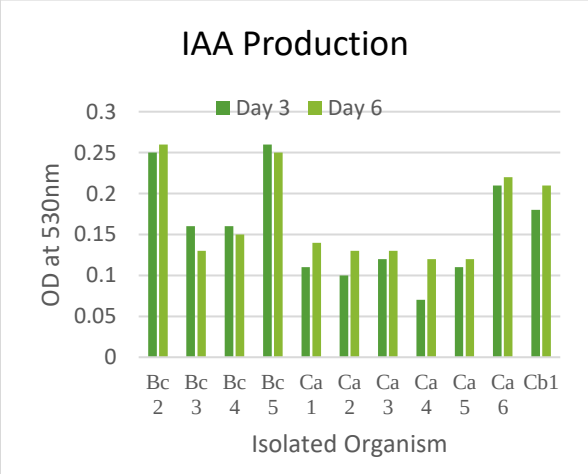
Fig No 5: IAA produced by

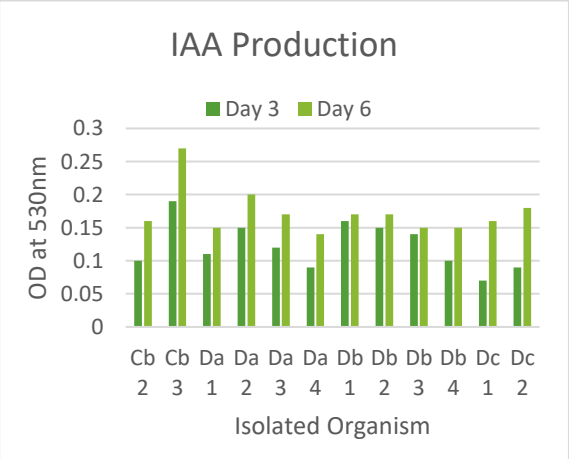
Table No 9:- Quantitative estimation of IAA

Organism	Day 3	Day 6
Ba 1	0.11	0.17
Ba 2	0.15	0.16
Ba 3	0.07	0.15
Ba 4	0.08	0.15
Ba 5	0.09	0.11
Bb 1	0.10	0.10
Bb 2	0.13	0.15
Bb 3	0.12	0.13
Bb 4	0.14	0.18
Bb 5	0.16	0.09
Bc 1	0.14	0.13
Bc 2	0.25	0.26
Bc 3	0.16	0.13
Bc 4	0.16	0.15
Bc 5	0.26	0.25
Ca 1	0.11	0.14
Ca 2	0.10	0.13

Organism	Day 3	Day 6
Ca 3	**0.12**	**0.13**
Ca 4	**0.07**	**0.12**
Ca 5	**0.11**	**0.12**
Ca 6	**0.21**	**0.22**
Cb 1	**0.18**	**0.21**
Cb 2	0.10	0.16
Cb 3	0.19	0.27
Da 1	0.11	0.15
Da 2	0.15	0.20
Da 3	0.12	0.17
Da 4	0.09	0.14
Db 1	0.16	0.17
Db 2	0.15	0.17
Db 3	0.14	0.15
Db 4	0.10	0.15
Dc 1	0.07	0.16
Dc 2	0.09	0.18





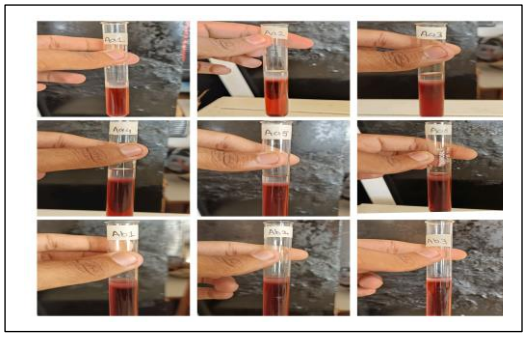


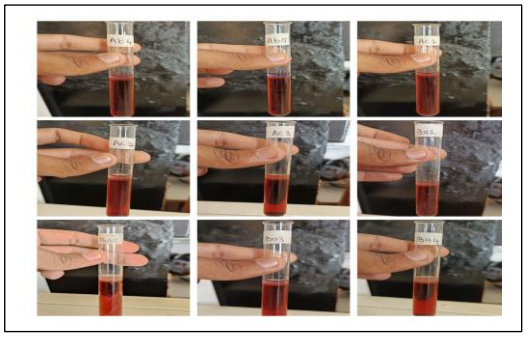
❖ Exopolysaccharides :-

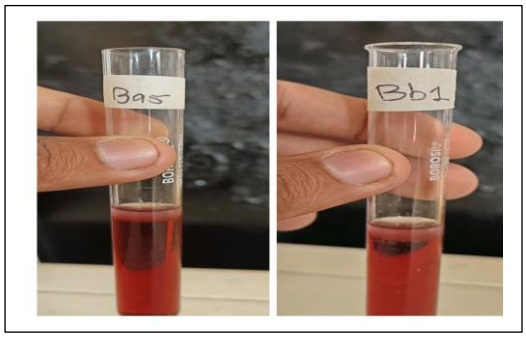
Result :- Exopolysaccharide Activity

exopolysaccharides activity was studied alll of them are positive & they show white colour precipitated when chilled ethanol is added

Fig No 6 : Organism showing exopolysaccharides activity







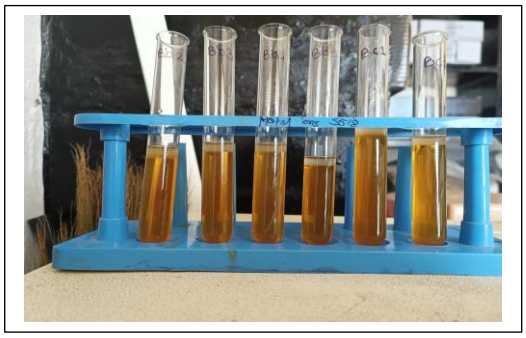


Table No 10:- Result of exopolysaccharide activity

Organism	Gram Nature
Aa 1	Positive
Aa 2	Positive
Aa 3	Positive
Aa 4	Positive
Aa 5	Positive
Aa 6	Positive
Ab 1	Positive
Ab 2	Positive
Ab 3	Positive
Ab 4	Positive
Ab 5	Positive
Ac 1	Positive
Ac 2	Positive
Ac 3	Positive
Ba 1	Positive
Ba 2	Positive
Ba 3	Positive
Ba 4	Positive
Ba 5	Positive
Bb 1	Positive
Bb 2	Positive
Bb 3	Positive
Bb 4	Positive
Bb 5	Positive

Organism	Gram Nature
Bc 1	Positive
Bc 2	Positive
Bc 3	Positive
Bc 4	Positive
Bc 5	Positive
Ca 1	Positive
Ca 2	Positive
Ca 3	Positive
Ca 4	Positive
Ca 5	Positive
Ca 6	Positive
Cb 1	Positive
Cb 2	Positive
Cb 3	Positive
Da 1	Positive
Da 2	Positive
Da 3	Positive
Da 4	Positive
Db 1	Positive
Db 2	Positive
Db 3	Positive
Db 4	Positive
Dc 1	Positive
Dc 2	Positive

DISCUSSION

Discussion

Plant growth promoting bacteria (PGPB) exhibit a variety of mechanism that enhance plant growth and resilience under draught stress. In this study, the abilities of selected PGPB, including nitrogen fixation phosphate solubilization, siderophore production. IAA production and exopolysaccharide (Eps) Synthesis were investigated

Nitrogen fixation by PGPB is a key trait that support plant growth in nitrogen limited soils The isolates in this study demonstrated nitrogenase activity foaming nitrogen Fixation forming zones. These results are consistent with (Singh et al., 2022), where *Rhizobium leguminosarum* and *Azospirillum brasilense* recorded nitrogen fixation zones This similarity highlights the effectiveness of the studied isolates in providing bioavailable nitrogen to plants under drought condition. The isolate Aa4,Ab4,Ac1,Ba2,Bb5,Bc2,Ca3,Ca5,Da3,&Db3 have Show positive result for nitrogen fixation ability which was observed in the form of clear zone around the colonies. Nitrogen is essential for plants as it is a key component of proteins, nucleic acids, and chlorophyll. Without nitrogen, plants show stunted growth, yellowing of older leaves (chlorosis) due to reduced chlorophyll, weak structures, and poor flowering and fruiting. However, when plant growth-promoting bacteria (PGPB) fix atmospheric nitrogen (N_2) into a usable form like ammonia, it significantly benefits plant growth by providing nitrogen to plants, leading to greener leaves, stronger stems, and higher yields. This process also enhances soil fertility, reduces the need for synthetic fertilizers, and increases plant resilience to environmental stress, contributing to sustainable agriculture.

In term of phosphate solubilization, isolates in this study formed zones on Pikovskaya's agar. These results align closely with those reported by [Khan et al., 2020), where *pseudomonas Fluorescens* and *Bacillus Subtilis* produced zone The high solubilisation ability of those bacteria enhances phosphorus availability to plants especially in nutrient-deprived, water-scarce soil. The Isolates Cb1&Cb2 from our Research Shown positive result

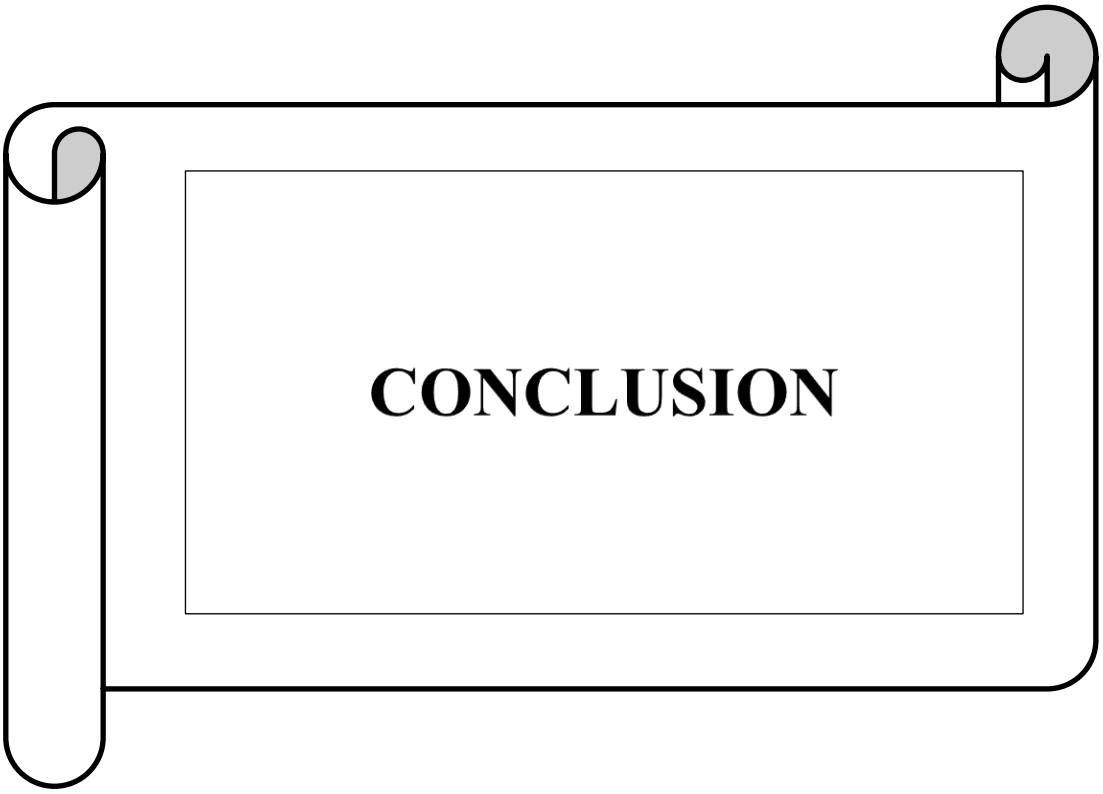
For phosphate solubilization, Which was observed in the form of Clear zone around the colonies. When plants lack phosphate, they exhibit stunted growth, dark green or purple discoloration of leaves (especially older ones), poor root development, delayed flowering, and reduced yield. However, plant growth-promoting bacteria (PGPB) capable of phosphate solubilization can convert insoluble phosphates in the soil into soluble forms that plants can absorb. This process improves phosphorus availability, promoting healthy root and shoot growth, enhanced flowering, and higher productivity. Additionally, phosphate-solubilizing PGPB improve soil fertility, support nutrient uptake, and reduce the dependence on chemical

fertilizers, leading to sustainable plant growth and development

The production of Indole acetic acid (IAA) is another critical trait. In this Study. IAA production was checked Formation of pink colour after addition of Salkowski Reagent Showes positive test When plants lack IAA, they experience stunted growth, poor root and shoot development, reduced branching, and an inability to form lateral roots or root hairs, which limits nutrient and water uptake. However, plant growth-promoting bacteria (PGPB) can produce IAA and supply it to plants, stimulating root elongation, lateral root formation, and overall root biomass. This improved root architecture enhances nutrient and water absorption, leading to better plant growth, increased resistance to environmental stress, and higher yields. By promoting root development and nutrient efficiency, PGPB producing IAA significantly contribute to healthy plant growth and sustainable agriculture

siderophore production, the studied isolates exhibited yellow zone on chrome azurol S (CAS) agar These values are comparable to the finding of (Patel. et al., 2019), where *pseudomonas putida* and *Enterobacter cloacae* showed siderophore production The ion-chelating ability of siderophores contributes to mitigating Oxidative stress in plants under drought condition The isolate Ca6,Cb1&Cb3 from our research Shows Positive result by forming yellow zone around the colonies They enhance iron availability by binding to insoluble iron in the soil and transporting it to plant roots, ensuring proper uptake for chlorophyll synthesis, enzyme activity, and overall growth. This prevents iron deficiency symptoms such as chlorosis, stunted growth, and weak stems. They also help plants tolerate abiotic stresses like drought, salinity, and heavy metal toxicity by improving nutrient availability and reducing oxidative damage.

Finally Exopolysaccharide Production was studied, organism show positive result when chilled ethanol added into Supernatant of isolate. Forming white colour precepted When plants lack access to EPS-producing microorganisms, they struggle with poor soil aggregation, reduced water retention, and limited nutrient availability, which can lead to restricted root growth and reduced tolerance to environmental stress. However, plant growth-promoting bacteria (PGPB) that produce EPS enhance soil structure by binding soil particles, improving porosity, and increasing water and nutrient retention around plant roots. EPS also protect plants by forming a protective biofilm around roots, reducing the impact of pathogens and environmental stresses such as drought and salinity. This leads to improved root growth, better nutrient uptake, and increased plant resilience, ultimately supporting healthier and more robust plant development


CONCLUSION

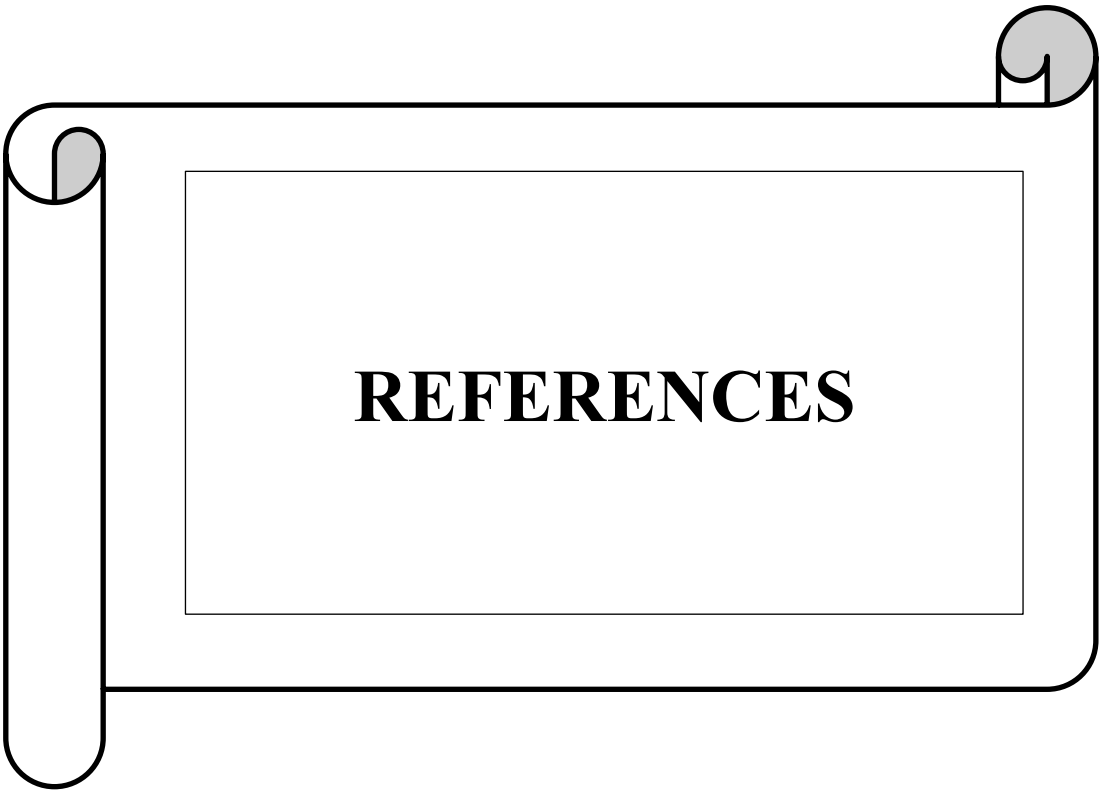
Conclusion:

Drought stress is one of the primary abiotic factor limiting agricultural productivity globally. Reduced water, availability in soil leads to stunted plant growth. nutrient deficiencies and diminished crop yields Drought condition not only affect plant physiology but also degrade soil Structure and diminish microbial biodiversity, further aggravating the challenge of achieving sustainable crop yield under water Scarcity

Plant growth promoting bacteria (PGPB) play a vital role in mitigating drought stress by enhancing plant water uptake. Improving Root architecture and promoting osmotic balance through the production of phytohormones and osmolytes Their ability to produce exopolysaccharides, reduce oxidative stress and modulate stress hormones like ethylene and abscisic and further support plant resilience under water- deficit condition Additionally, PGPB contribute to nutrient availability through Nitrogen fixation and improved nutrient uptake while their ACC deaminase activity helps plants maintain growth during stress

We collected soil sample from different location like shenda park, Sangli, bambavade, and isolated organism from the soil. We have screened the isolated organisms on the basis of different plant growth promoting activities like phosphate Solubilisation, Nitrogen fixation, siderophore, IAA and EPS production. Among the isolated organisms we have selected potent organism which shows phosphate Solubilisation, Nitrogen fixation, siderophore, IAA and EPS production effectively. The presence of these activities will help to mitigate drought stress in plants and increase agricultural yield.

Further in next phase of this project will focus on the preparation of bio inoculum ensuring its effectiveness and Sustainability Additionally. The formulated bioinoculum will be tested for its ability to ameliorate drought condition in plants. These efforts aim to validate the findings under real-world Conditions and contribute to the development of on eco friendly and cost effective solution for plants growth under drought condition.



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