# Comparative Study of Plant Growth Promoters on Vegetative Soil and Slit Soil

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**Abstract:** Soil is the biologically active porous medium that has developed in the uppermost layer of Earth's crust. Free-living soil bacteria beneficial to plant growth, usually referred to as plant growth promoting rhizobacteria (PGPR), are capable of promoting the plant growth by colonizing the plant root. PGPR are also termed plant health promoting rhizobacteria (PHPR). Free-living nitrogen-fixing bacteria or associative nitrogen fixers example bacteria that belongs to the species Azospirillum, Enterobacter, Klebsiella and Pseudomonas, have been shown to attach to the root and efficiently colonize of root surfaces. The aim of this study is the potential of PGPB to enhance use efficiency of agro environmental resources.

**Key words:** PGPR, Pseudomonas, Azospirillum, Enterobacter.

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## 1. Introduction:

Soil is the biologically active porous medium that has developed in the uppermost layer of Earth's crust. Soil is one of the principal substrata of life on the world, serving as a reservoir of water and nutrients as a medium for the filtration and breakdown of injurious wastes and as a participant in cycling of carbon and other elements through the global ecosystem. It was evolved through the weathering processes driven by biological, climatic, geologic, and topographic influences (Garrison Sposito 2020). Soil bacteria are important in the biogeochemical cycles and have been used for crop production in decades. Plant–bacterial interactions in rhizosphere are determinants of plant health and its soil fertility. Free-living soil bacteria are beneficial to plant growth, usually referred to as plant growth promoting rhizobacteria (PGPR), are capable of promoting the plant growth by colonizing the plant root. PGPR are also termed plant health promoting rhizobacteria (PHPR). These are associated with the rhizosphere, which is the most important soil ecological environment for plant–microbe interactions (Hayat, R., *et al*.,2010).

The progression of life in all forms is not only dependent on the husbandry and food security but it also on the soil characteristics. The dynamic nature of soil is a direct manifestation of soil microbes and synergistic co-evolution with plants. With the rise in the number in world's population the demand for agriculture yield has increased tremendously and thereby leading to large scale production in the chemical fertilizers. Since the use of fertilizers and pesticides in the agricultural fields have cause degradation of soil quality and fertility, thus the expansion of agricultural land with fertile soil is near impossible, hence scientists have sifted their attention for a safer and productive means of agricultural practices. Plant an increase promoting rhizobacteria (PGPR) has been functioning as a co-evolution between plants and microbes showing antagonistic and

synergistic interactions with microorganisms in the soil (Sushanto Gouda, Rout George Kerry *et al*.,2018).

Free-living nitrogen-fixing bacteria or associative nitrogen fixers example bacteria that belongs to the species Azospirillum, Enterobacter, Klebsiella and Pseudomonas, have been shown to attach to the root and efficiently colonize of root surfaces. PGPR have the potential to contribute the sustainable growth promotion. Generally, PGPR functions in three different ways they are synthesizing particular compounds for the plants, facilitating the uptake of certain nutrients from the soil, and lessening or preventing the plants from diseases. Plant growth promotion and development can be facilitated by both directly and indirectly. Indirect plant growth promotion includes the prevention of the deleterious effects of phytopathogenic organisms. Direct plant growth promotion includes symbiotic and non-symbiotic PGPR which functions through the production of plant hormones such as auxins, cytokinins, gibberellins, ethylene and abscisic acid (Rifat Hayat *et al*.,2010).

The number and the type of the bacteria which are found in different soils are influenced by the soil conditions including temperature, moisture, and the presence of salt and other chemicals as well as the number and types of plants found in those soils (B. R. Glick, *et al*., 1999). In some time that the soil hosts a large number of bacteria (often around 108 to 109cells per gram of soil) and that the number of culturable bacterial cells in soil is generally only about 1% of the total number of cells present (L. Schoenborn *et al*., 2004). Bacteria are the most abundant microorganisms in the rhizosphere they influence the plants physiology to a greater extent, most considering their competitive in root colonization. Bacteria which colonize the rhizosphere are referred as plant growth-promoting rhizobacteria(cook *et al*.,2000). The search for microorganisms that improve soil fertility and that enhance plant nutrition has continued to attract the attention due to the increasing cost of the chemical fertilizers and some of their environmental impacts (Adesemoye *et al*., 2009).

Chemical pesticides and their ill effects on environment to prove the majorsetbackto the unrestricted use of these chemicals; leaving us in a dilemma. Attempts to use chemicals and bio pesticide in combination and to gradually minimize use of the chemicals are necessary keeping in consideration environment protection for future. Bio controlling agents may take some time to establish in the soil before their significant effects are seen in disease control and crop yield. In certain cases, several applications schedules over a period of time may be required to obtain desired effects. This is limitation of biological methods but keeping in consideration human and animal health and ecology one has to give a thought to the problems that may rise from excessive use of chemical pesticides (Bowen G.D *et al*., 2000).

The soil organic matter provides a favorable habitat for the microorganisms to grow as compared to inorganic soil. The bacterial diversity present in the soil is greatly influenced by organic matter. It has been consistently reported that the soil organic matter will favors the growth of bacteria present in the soil. In most of the aerated or cultivated soils fungi share a major part of the major microbial biomass. Many important plant pathogens and plant growth promoting microorganisms are fungi. Proper soil management has and ameliorating effect on the biological effect on the biological effect in the soil. Difference in biological activity in these soils (Farzana Y *et al*., 2009).

PGPR have the potential to contribute in the development of sustainable agricultural systems (Schippers *et al.*,1995). PGPR are a group of functional bacteria that have the ability as a growth promoter (biostimulant). The process occurs by synthesizing and regulating the concentrations of various Growth Regulator (GR) or Plant Growth Regulator (PGR) such as Indole acetic acid or IAA (Bottini R *et al*., 2004). The functional bacteria (PGPR) produce IAA hormone and Acc-deaminase, and can provide nutrient on soil by fixating N2 from atmosphere with symbiosis and a symbiosis. They are equally able to dissolve P nutrient bound with Al, Fe, and Ca on soil by synthesizing PMEase enzyme (Phosphomonoesterase). PGPR can control the activity of pathogens of plant-disturber organisms (biocontrol) and decomposers of agrochemical compounds (Gunalan 1996), and may control pathogens derived from soil (bioprotectant) by multiple compounds or anti-pathogenic metabolites such as siderophore, chitinase, antibiotics, and cyanide (Glick BR 1995).

The group of PGPR will instantly occupy and colonize the plant roots (rhizosphere) aggressively and fast after inoculation in seeds, germs, and soil. This PGPR will immediately provide nutritional inputs needed for plant growth. Some of the bacteria belonging to PGPR are Flavobacterium, Herbaspirillum, Acetobacter, Acinetobacter, Alcaligenes, Arthrobacter, Bacillus, Beijerinckia, Burkholderia, Serratia, Enterobacter, Erwinia, Pseudomonas, Az spirillum, Azotobacter, and Rhizobium (Sudhakar P *et al*.,2000).

The fertility of soil is determined by several components including soil structure, type of soils, microbe communities, type of microbes, and type of plants. If one of the components is damaged due to the continuous use of chemical fertilizer, the environment of soil will become infertile (marginal). This condition disturbs a germination process of seed in soil. The germination process started from imbibition and terminated on the process of elongated radicle (Salisbury FB *et al*., 1995). Velocity and diversity of radicle growth on germination are early indications of seed quality and crop production for optimization (Parera CA *et al*., 1994). Germination process from dormant seed to radicle release until growth phase of seed depends on seed viability, suitability of environment

condition, and breaking effort of seed dormant (Har Jadi SS 1993).The aim of the experiment was to check whether it is possible to increase the growth stimulants (growth activators) alone or together with a preparation containing PGPR (such as *Bacillus subtilis, Pseudomonas and Bacillus subtilis+Pseudomonas*).

## 2. Materials And Methods:

### 2.1 Collection of the soil:

The soil samples were collected from various places of Coimbatore district, Tamil Nadu, India.The collected samples brought to the laboratory in sterile polythene bag.

### 2.2 Isolation of soil microbes:

Soil particles adhering to the rhizosphere soil of Eleusine coracana were collected and microorganisms was isolated by serial dilution method. Colonies were collected from Nutrient agar media after24hrs growth at 25 C microbes was isolated by using Nutrient agar medium.

### 2.3 Serial dilution method:

A serial dilution method was used for each plate of soil sample on the nutrient media.

### 2.4 Isolation of pure culture:

Colonies were transferred to agar media from dilution plates on the media. The streak plate method was used to isolate individual colonies.

## 3. Identification Of Soil Microbes:

### 3.1 Gram staining:

The Gram Stain on any sample requires four basic steps that include applying a primary stain (crystal violet) to a heat-fixed smear, followed by the addition of a mordant (Gram's Iodine), rapid decolorization with alcohol, acetone, or a mixture of alcohol and acetone and lastly, counterstaining with safranin.

## 4. BIOCHEMICAL TEST:

### 4.1 Indole test:

- Inoculate the tryptophan broth with broth culture or emulsify isolated colony of the test organism in tryptophan broth.
- Incubate at 37°C for 24-28 hours in ambient air.
- Add 0.5 ml of Kovac's reagent to the broth culture.

### 4.2 **Methyl red test**:

- Inoculate Methyl Red-Voges Proskauer broth with a pure culture of the organism.
- Incubate at 35°-37°C for a minimum of 48 hours in ambient air.
- Add 5 or 6 drops of methyl red reagent per 5 mL of broth.

### 4.3 Voges-Proskauer test:

The test is performed by adding alpha-naphthol and potassium hydroxide to the Voges-Proskauer broth which has been inoculated with bacteria.

### 4.4 Citrate utilization test:

Slants of Simon's citrate agar were prepared and streaked with bacterial culture. The tubes were incubated at 28°C for 48 hrs. The change in color of Slants was observed.

### 4.5 Oxidase test:

The isolates were streaked on yeast extract mannitol agar plates and incubate for 3 days 28°C.After incubation, isolates were placed over oxidase disc. Developed of blue or purple color.

### 4.6 Urea's test:

The isolates were streaked on Christensen urea agar Slants and incubate for 3 days at 28°C.Observed the Slants for a color change at 6hrs every day up to 6 days.

### 4.7 Catalase test:

A drop of 3% hydrogen peroxide was added to a bacterial colony on a sterile glass slide and mixed well. Production of air bubble was observed for a minute.

### 4.8 Pot culture:

Seed of Pennisetum glaucum were soaked in water over night and surface sterilized with 70% ethanol for 15 minutes and washed with sterile distilled water. After 35 days the plants were uprooted and measured root and shoot length.

## 5. Results:

### 5.1 Identification of soil microbes:

Microbes were isolated from rhizosphere soil sample of Eleusine coracana and cultured were maintained.

### 5.2 Gram staining:

Gram-positive bacteria have a thick mesh-like cell wall made up of peptidoglycan (50%- 90% of cell envelope), and rod shaped bacteria as a result are stained purple by crystal violet.

### 5.3 Biochemical test:

The soils were isolated from rhizosphere soils viz, Coimbatore and the culture were maintained. Bacillus isolated from rhizosphere soil sample of Eleusine coracana and the culture were maintained. staining and growth characteristics. The results on biochemical and growth characteristics of the isolates are presented in table 1. All the isolates(Bacillus sp.,) were gram positive, maximum growth at 280C,370C and 450

indicated that isolates grow well on nutrient agar medium. Extra cellular enzymes of all the isolates were positive of the catalyses, oxidases and negative of the urea's production.

**5.4 Indole test:**

Results of an indole test are indicated by a change in color following a reaction with an added reagent. A negative result is shown by the presence of a colorless or light yellow.

**5.5 Methyl red test:**

A negative methyl red was shown by the appearance of yellow or orange color.

**5.6 Voges Proskauer test:**

A positive test is represented by the development of a red color 15 minutes or more after the addition of the reagents indicating the presence of diacetyl, the oxidation product of acetoin.

**5.7 Citrate utilization test:**

Inoculating the microorganisms into an organic synthetic medium Simon's citrate agar medium where sodium citrate is the only source of carbon and energy. Bromo methyl blue where is used as an indicator from green to blue and this constitutes a positive test. Slants of Simon's citrate agar were prepared and streaked with the bacterial culture. The tubes were incubated at 280C for 48 hours. The change in color of the slants were observed.

**5.8 Oxidase test:**

The oxidase negative result just means that these organisms do not have the cytochrome c oxidase that oxidase the test reagent. When the enzymes not present, the reagent remains reduced and is colorless.

**5.9 Urea's test:**

The isolates were streaked on Christensen urea agar slants and incubated for 3 days at 280C.Observed the slant for a color at 6 hours ,24 hours, and every day for up to 6 days. Urea's production is indicated by a negative organisms produced no color change or yellow as a result of acid production.

**5.10 Catalase test:**

Catalase positive bacteria include strict aerobes as well as facultative anaerobes. They all have the ability to respire using oxygen as terminal electron acceptor.

## 6. Plant Growth Promotor (Pgpr) :

In this study we are using Pennisetum glaucum. Microbes (Bacillus subtilis, Pseudomonas sp.,) were cultured with different broth culture in conical flask. Each culture medium was also used in the Liquid form of well shake with shakers apparatus at 24 hours. After 24 hours, the broth cultures of microbes were added in treated pots of

inoculated plants. The microbes were not added and selected one pot as a control (non-treated plants).

## 7. Growth Characteristics Of Pennisetum Glaucum Shoot, Root, Fresh Weight, Dry Weight At 35 Day (Cm/Plant)

In the present study, shoot and root length Pennisetum glaucum were analyzed at 35 days of isolates treatment compared to control. All isolates were highly promoting the plant growth of shoot and root length compared to control (uninoculated pot) then inoculation of Bacillus subtilis, Pseudomonas sp., and Dual inoculated study of inoculation of Bacillus subtilis, Pseudomonas sp., and Dual inoculated study of Pseudomonas +Bacillus subtilis was increased in the shoot and root length when compared to control.

Fresh weight of shoot and root, were scientifically isolated in Bacillus subtilis, Pseudomonas sp., and combination of Bacillus subtilis + Pseudomonas sp., increased when compared to control.

Dry weight of shoot and root were scientifically isolated in Bacillus subtilis, Pseudomonas sp., and combination of Bacillus subtilis + Pseudomonas sp., increased when compared to control.

## Identification Of Soil Microbes:

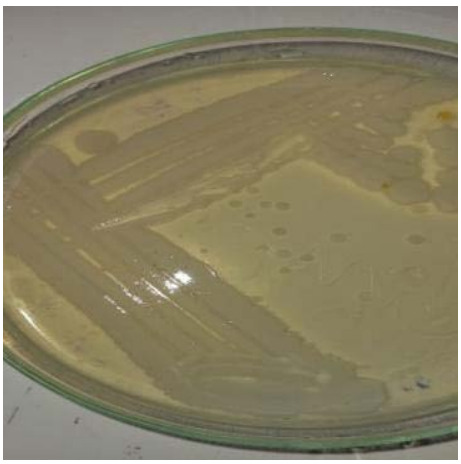


**Fig :1. a. Isolation of Bacillus sp.,**

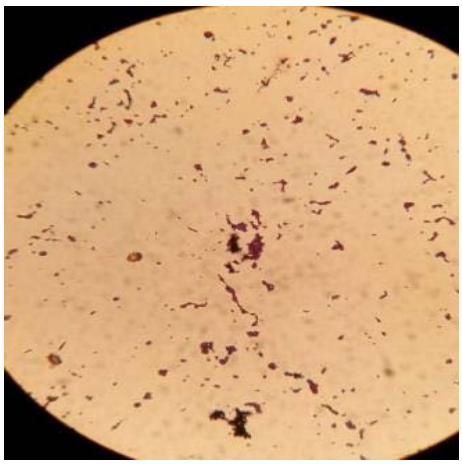
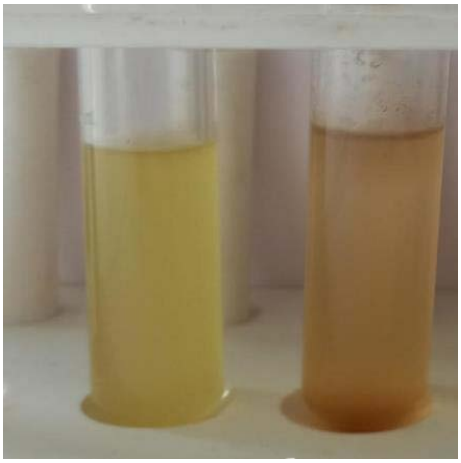


**Fig:1. b. Gram staining**



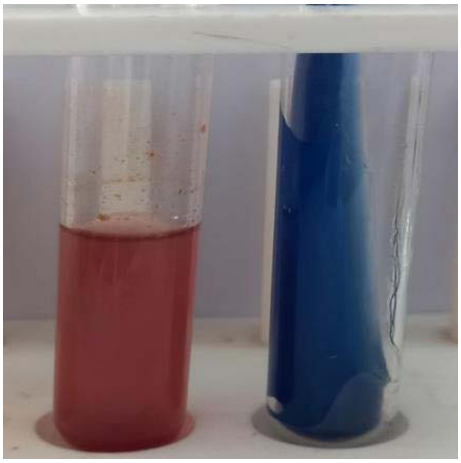


**Fig: 1.c Indole test and Methyl red test**

**Fig: 1.d VP and Citrate test**

**Fig: 1.e Catalase test**

**Fig: 2. Soils treated with microbes**

## Table: 1 Identification Of Soil Microbes

| S.NO | CHARACTERISTICS | BACILLUS SP., |
|---|---|---|
| 1. | Gram staining | + |
| 2. | Indole test | - |
| 3. | Methyl red test | - |
| 4. | Voges Proskauer test | + |
| 5. | Citrate test | + |
| 6. | Oxidase test | - |
| 7. | Urease test | - |
| 8. | Catalase test | + |

("+" Present, "-" Absent)

**Table:2 Effect of Isolated Growth Promoter Organism on Pennistum Glaucum at 35 days**

| Treatment | Shoot length (cm/ plt) | Root length(cm/plt) | Fresh weight (g/plt) | Dry weight (g/plt) |
|---|---|---|---|---|
| Control | 6.34 ± 0.57 | 1.00 ± 0.03 | 0.73 ± 0.16 | 0.03 ± 0.02 |
| *Bacillus subtilis* | 8.67 ± 0.58 | 1.67 ± 0.58 | 3.98 ± 0.07 | 4.18 ± 0.17 |
| *Pseudomonas Sp.,* | 11.34 ± 0.58 | 3.00 ± 0.03 | 3.29 ± 0.25 | 2.19 ± 0.16 |

| | | | | |
|---|---|---|---|---|
| *Bacillus subtilis* + *Pseudomonas Sp.,* | 12.34 ± 0.58 | 4.00 ± 0.03 | 5.15 ± 0.13 | 4.36 ± 0.20 |

Values are expressed as Mean ± Standard deviation

## 8. Discussion:

The present study, the samples were collected from vegetative soil, Coimbatore district, Tamil Nadu. Biochemical characterization of bacterial strains isolated from soil; after gram staining technique biochemical test were carried out for characterization purpose.

The results on biochemical and growth characteristics of the soil isolates are presented in (table1). All the isolates (*Bacillus subtilis)* were gram positive, cocci motile, maximum growth at 28 C ,37 C and 45 C indicated that isolates grow well on nutrient agar medium. Extra cellular enzymes of all the isolates were positive and catalase, oxidase and negative of the urea's production.

After 35 days of inoculation of isolates were compared to *Bacillus* subtilis. All the isolates were highly promoting the plant growth of shoot, root, fresh weight dry weight compared to control. Then inoculation of *Bacillus subtilis, Pseudomonas and Bacillus subtilis+ Pseudomonas* are inoculated to the pot in Pennisetum glaucum After 35 days the results were observed for fresh weight, dry weight, root and shoot growth of the plant. Similarly the toxicity caused by the species is a concern because it inhibits plant growth thus leading to a lower yield; it also causes a deterioration in solanaceous vegetable crop quality (Guo *et al.*,2004).High level of Bacillus subtilis., in the soil had a deleterious effect on the plant growth (Table2).

After inoculation, the effect was significantly higher in soil samples inoculated with bio inoculant for the soil sample studied as compared with inoculated control. The present study a Pennisetum glaucum plant shoot, root length, fresh weight and dry weight increased in the *Bacillus subtilis+ Pseudomonas sp.,* plant when compared to control in decreased.

Due to increase in both human population growth and environmental pressure, it is necessary to raise agricultural productivity without enhancing environmental footprint.With in this context, soil inoculation with PGPB may be considered a promising tool of integrated management systems. PGPB comprise different functional and taxonomic groups of bacteria like Pseudomonas, *Bacillus, Rhizobium* and others. In particular PGPB may improve plant growth either directly, by facilitating resource use or

modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogenic agents. In particular, the main topic of this project is the potential of PGPB to enhance use efficiency of agro environmental resources.

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