

Role of Phosphate and Silicate Solubilizing Bacteria from Saline Soils in Promoting Seed Germination

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Abstract

Soil salinity lowers nutrient availability and has a detrimental influence on seed germination and crop growth. Phosphate-solubilizing bacteria (PSB) and silicate-solubilizing bacteria (SSB) play an essential role in turning insoluble phosphorus and silicon into bio-available forms, which improves plant development and stress tolerance. Soil samples were taken from salty and semi-arid parts of Sangli and Kolhapur districts in Maharashtra, India, to isolate and characterize PSB and SSB. A total of ten PSB and nine SSB isolates were biochemically analyzed. Under controlled laboratory conditions, selected isolates were evaluated for their ability to enhance germination and seedling vigor of *Sorghum bicolor*. Seeds were infected with individual isolates and a mixed inoculum, and germination parameters were monitored for up to six days. Microbial inoculation significantly increased the germination percentage, root and shoot elongation, and seedling vigor compared to uninoculated controls. PSSB8 consistently outperformed the other isolates, with the most significant germination percentage (96%), shoot length (9.2 cm), root length (11.5 cm), and seedling vigour index values. These data suggest that PSSB8 is a suitable bioinoculant for enhancing seed germination and early crop establishment in saline soils. The study emphasizes the potential use of PSB and SSB in sustainable agriculture to reduce reliance on chemical fertilizers while increasing crop output under abiotic stress conditions.

1. Introduction

Soil fertility and nutrient availability are essential indicators of plant health and agricultural productivity. Among vital nutrients, phosphorus (P) and silicon (Si) play critical roles in plant growth and stress tolerance (Schimel et al., 2007). However, both the elements frequently exist in insoluble forms in the soil, rendering them unavailable to plants. Microorganisms, such as phosphate-

solubilizing bacteria (PSB) and silicate-solubilizing bacteria (SSB), utilize natural biological mechanisms to circumvent this constraint. Phosphate-solubilizing bacteria (PSB) convert insoluble phosphorus into soluble orthophosphates for plant absorption. They sequester organic acids, lower soil pH, enhance root development, and produce growth-promoting substances (Qureshi et al., 2012). PSB is beneficial in

low-phosphorus soils and reduces reliance on chemical fertilizers, making it a sustainable agriculture tool. Phosphate-solubilizing bacteria (PSB) and Silicate-Solubilizing Bacteria (SSB) enhance soil fertility and plant growth by increasing nutrient bioavailability (Khan et al., 2019). Phosphorus, while abundant in many soils, is frequently bound in insoluble forms such as calcium, iron, and aluminium phosphates, which plants cannot readily absorb. PSBs are helpful microbes that release organic acids such as gluconic, oxalic, and citric acid, which decrease soil pH and chelate metal ions bound to phosphorus compounds, transforming them into soluble forms that plants can absorb. In addition to solubilising phosphorus, several PSB strains produce plant growth-promoting compounds such as indole-3-acetic acid (IAA) and siderophores, which promote root development and nutrient uptake. Silicate-solubilizing bacteria (SSB) release plant-available silicon from silicate minerals, enhancing plant resilience to stress (Shabbir et al., 2020). They produce organic acids, improve structural integrity, and strengthen resistance to drought, salinity, and pathogen attacks. Silicate Solubilising Bacteria (SSB) can transform insoluble silicate minerals in soil into plant-available silicon, particularly

monosilicic acid. Although silicon was not previously thought to be important, it is now recognized for its function in increasing plant tolerance to a variety of abiotic stresses, including drought, salt, and heavy metal toxicity, as well as biotic stresses such as pest and disease attacks. SSB accomplishes this by creating organic acids and releasing protons, which degrade complicated silicate structures. The soluble silicon produced reinforces plant cell walls, improves structural integrity, and increases resistance to lodging, particularly in cereal crops such as rice and wheat. SSB and PSB contribute to nutrient cycling, soil health, and sustainable crop production, aligning with eco-friendly practices and promoting resilient agro-ecosystems (Qureshi et al., 2012; Raturi et al., 2021).

Phosphate-solubilizing bacteria (PSB) and silicate-solubilizing bacteria (SSB) are crucial soil microbial groups that convert insoluble phosphorus into bioavailable forms, strengthen plant cell walls, and enhance resistance to abiotic stress. (Ahemad & Khan, 2010) investigated the phosphate-solubilizing and plant-growth-promoting properties of *Pseudomonas aeruginosa* PS1 in mustard soils. Their study suggests that reducing the phytotoxicity of herbicides to legumes could be achieved by applying the growth-

promoting herbicide-tolerant strain of *Pseudomonas aeruginosa* PS1. (Behera et al., 2017) studied a phosphate-solubilizing bacterium, PSB-26, isolated from a mangrove in the Mahanadi delta. The strain was identified as *Alcaligenes faecalis* and showed maximum phosphate solubilizing activity of 48 g/ml. The strain produced various organic acids during phosphate solubilization, and under optimized conditions, it showed maximum alkaline phosphatase activity of 93.7 U/ml. The bacterium's effect on the growth of *Arabidopsis thaliana* plants showed better growth compared to the control, suggesting potential biotechnological applications. (Naureen et al., 2015) worked on the Silicate Solubilizing Bacteria (SSB), according to their results, SSB can enhance soil fertility and plant defence mechanisms by solubilizing insoluble forms of silicates, potassium, and phosphates. The fundamental step of mineral phosphate solubilization is the formation of organic acids. Acid phosphatases play a vital role in the mineralization of organic phosphorus in soil. A few genes involved in mineral phosphate solubilization have been found, as well as numerous phosphatase-encoding genes that have been cloned and studied. Thus, cloning and expressing genes involved in mineral and organic phosphate

solubilization in specific rhizobacterial strains could be one means of genetically altering phosphate-solubilizing bacteria to increase their ability to promote plant growth.

2. Materials and Methods

2.1 Collection of Soil Sample:

Soil samples were collected from 15 sites in Maharashtra's Sangli and Kolhapur districts, including Jat, Dighanchi, Aatpadi, Kumbhari, Ravalgundewadi, Devnal, Birnal, and Sangali. The samples were divided into five homogeneous units, cleaned of surface debris, and placed in containers. After mixing, the sample amount was reduced to half a kilogram, and each was labeled with the collection number, date, site, and soil type. The samples were stored in polythene bags in the research laboratory at low temperatures until needed (Ghare & Kumbhar, 2021), (Raut & Kurhe, 2020), (Kekane et al., 2015). Based on climate and annual rainfall, semi-arid areas were selected for the isolation of drought-tolerant bacteria. At the same time, saline lands with poor crop growth were chosen for salt-tolerant bacteria in the western belt of Maharashtra.

2.2 Enrichment & isolation of Phosphate-Solubilizing Bacteria (PSB)

Multiple sub-samples were combined, each derived from individual soil samples. Serial dilutions were then performed on the mixed samples, reaching up to 10^{-6} . From the 10^{-4} , 10^{-5} , and 10^{-6} dilutions, a volume of 0.1 mL was taken. Cultures were grown on Pikovskaya medium, with the pH adjusted to 7.2. All plates were incubated at 30°C for 7 days. PSB isolates were selected and purified based on their distinct halos.

2.3 Characterization of Bacterial Isolates (PSB):

The isolates were examined for morphological, cultural, and biochemical characteristic tests such as IMViC, Oxferm test, Nitrate reduction test, and fermentation of glucose, fructose, sucrose, lactose, and mannitol. Enzymatic tests included amylase, caseinase, lipase, lecithinase, catalase, and oxidase, among others (Patel et al., 2016).

2.4 Enrichment & isolation of Silicate-Solubilizing Bacteria (SSB)

To get fine-sized particles, the soil samples were pulverized, air-dried, and then sieved through a 0.5 mm sieve. Silicate-solubilizing bacteria are enriched by combining Minimal broth with 0.25 %

of magnesium trisilicate in 100 mL sterile broth. One gram of soil sample was added to each flask, and the samples were maintained on a Rotary shaker at room temperature for 6 to 8 days to allow bacteria to develop. The components were thoroughly combined by vortexing to achieve a 10:1 dilution. For the isolation of silicate-solubilizing bacteria, we proceeded with two experiments: first, using the serial dilution and plating method, utilizing nutrient agar (glucose 5.0g; peptone 5.0g; beef extract 3.0g; NaCl 5.0g; agar 20.0g; tap water 1000 ml; pH 7.0) (Fatharani & Rahayu, 2018). After the proper incubation period, the isolated bacteria were incubated for four days at 30°C to undergo a silica-solubilizing test on Bunt and Rovira Agar with 0.25% magnesium trisilicate. After air-drying, the silica source (quartz) was run through a 325-mesh filter. The medium has the following contents (g/750 mL): 20 grams of glucose, 20 grams of agar, 1 gram of peptone, 1 gram of yeast extract, 0.5 grams of $(\text{NH}_4)_2\text{SO}_4$, 0.4 grams of K_2HPO_4 , 0.1 grams of MgCl_2 , 0.01% of FeCl_3 , 250 milliliters of soil extract, 750 milliliters of aquades, pH 6,6-7,0 (Engineering, 2013) (SANTI & GOENADI, 2017). In Bunt and Rovira Agar, isolates that form a clear zone can solubilize 0.25 percent quartz. The

Solubilizing Index was used to measure each isolate's clear zone (Chopra et al., 2021).

$$SI = \frac{\text{Diameter of colony} + \text{Diameter of halozone}}{\text{Diameter of colony}}$$

2.5 Characterization of bacterial isolates (SSB):

The isolates were examined for morphological characteristics & biochemical tests such as IMViC, Oxferm test, Nitrate reduction test, and fermentation of glucose, fructose, sucrose, lactose, and mannitol. Enzymatic tests such as amylase, caseinase, lipase, lecithinase, catalase, and oxidase (Fatharani & Rahayu, 2018).

2.6 Experimental Design for Seed Germination Test

- Seeds of *Sorghum bicolor* (L.) Moench, cultivar Maldandi (M-35-1), a medium-duration, drought-resistant variety suitable for rainfed dryland farming, was used in this study.
- To ensure the elimination of surface contaminants, seeds were surface sterilized by immersing them in a 1.5% sodium hypochlorite solution for 15 minutes, followed by multiple rinses with sterile distilled water to remove any residual disinfectant.

- Post-sterilization, seeds were soaked in microbial suspensions of individual isolates (PSSB4, PSSB6, PSSB7, PSSB8, PSSG11, and PSSA1) for 30 minutes to allow microbial adherence.
- Control seeds were soaked in sterile distilled water.
- The treated seeds were then air-dried under sterile conditions using filter paper. Twenty-five seeds per treatment were placed in sterile petri plates containing two layers of Whatman No. 2 filter paper.
- Experimental treatments included a negative control (no microbial inoculation), a positive control (seeds treated with a known bioinoculant), individual microbial isolates, and a mixed-inoculum treatment.
- Every treatment was performed in triplicate.
- All petri plates were incubated in a growth chamber maintained at a constant temperature of $28 \pm 2^\circ\text{C}$ and 50% relative humidity.
- To maintain uniform moisture, 5 ml of sterile distilled water was added to each dish every two days.
- Seed germination was monitored daily, and seeds were considered germinated once the radicle length

reached at least 2 mm. Observations were recorded explicitly on the 3rd, 5th, and 6th days after sowing.

- From each treatment, ten seedlings were randomly selected to measure shoot and root lengths using a standard ruler.
- Germination percentage was calculated, and seedling vigour was assessed using three indices: SVI-I (Germination % $\times$ Shoot length), SVI-II (Germination % $\times$ Root length), and SVI-III (Germination % $\times$ Total seedling length).
- These parameters were used to evaluate the influence of microbial inoculation on seed germination and early seedling development ; .

3. Results & Discussion:

3.1 Collection of Soil Sample:

A total fifteen soil samples were collected from agricultural fields of Sangli and Kolhapur districts. The sample codes, source, place and description of each sample are presented in the table 1

Sangli is a region in India with a semiarid climate, characterized by dry soil and low water retention. The region is

prone to aridification, which affects the persistence of specialized microbial populations. The semiarid zone features high temperatures, low rainfall, and 60% humidity. Kumbhari, the area with the most rainfall, has the highest rainfall, indicating a semiarid climate. Ravalgundewadi, Devnal, Birnal, Sangli, Miraj, Akiwat, Majarewadi, Narsobawadi, Arjunwad, Kurundwad, and Rajapur are all located in Sangli district. Akiwat in Kolhapur has a mild, humid climate with saline terrain, supporting halophilic microbial species. Majarewadi experiences temperatures of 32°C, 60% humidity, and 700 mm of rainfall. Narsobawadi has moderate temperatures and higher humidity but receives less rainfall. Arjunwad records the lowest average temperature at 25.2°C and a moderately humid environment. Kurundwad has a warm climate with 700 mm of rainfall and 60% humidity, which influences soil structure and microbial diversity. Rajapur, in western Kolhapur, has an unusually high humidity and a balanced climate. These locations contribute to the microbial ecosystems in Sangli.

Table 1: Sample code, source, place and description of samples

SR. NO	SAMPLE CODE	SOURCE	PLACE	DESCRIPTION
1	SJ	Agricultural field	Sangli	Semiarid region
2	SD1	Agricultural field	Sangli	Semiarid region
3	SA	Agricultural field	Sangli	Semiarid region
4	SK	Agricultural field	Sangli	Semiarid region
5	SR	Agricultural field	Sangli	Semiarid region
6	SD2	Agricultural field	Sangli	Semiarid region
7	SB	Agricultural field	Sangli	Semiarid region
8	SS	Agricultural field	Sangli	Medium saline soil
9	SM	Agricultural field	Sangli	Medium saline soil
10	KA1	Agricultural field	Kolhapur	Saline land
11	KM	Agricultural field	Kolhapur	Saline land
12	KN	Agricultural field	Kolhapur	Medium saline soil
13	KA2	Agricultural field	Kolhapur	Medium saline soil
14	KK	Agricultural field	Kolhapur	Saline land
15	KR	Agricultural field	Kolhapur	Medium saline soil

***Note: SJ -Jath, SD1- Dighanchi, SA- Aatpadi, SK- Kumbhari, SR - Ravalgundewadi, SD2 - Devnal, SB - Birnal, SS – Sangli, SM - Miraj from Sangli District
KA1 - Akiwat, KM - Majarewadi, KN - Narsobawadi, KA2 - Arjunwad, KK - Kurundwad, KR – Rajapur from Kolhapur District**

3.2 Isolation and Screening of PSB

A total of ten morphologically distinct bacterial isolates (PSSB1-PSSB8, PSSG11 & PSSA1) with a phosphate solubilization zone were isolated from semiarid soil samples.

3.3 Characterisation of phosphate solubilizing bacteria

Based on their growth on solid media, ten microbial isolates (PSSB1 to

PSSB8, PSSG11, and PSSA1) were examined for colony characteristics. The size, shape, elevation, margin, opacity, color, and consistency varied among all isolates. Most isolates (PSSB1, PSSB3, PSSB4, PSSB6, PSSB7, PSSB8, and PSSA1) formed round colonies with smooth, complete edges, mainly convex or elevated, and a translucent to transparent

appearance. In contrast, PSSB5 and PSSG11 showed irregular margins and opaque colonies, while PSSB2 exhibited a regular shape with mucoid consistency. Additionally, PSSG11 had a moist texture and a level elevation. White and creamy colors predominated, and colony sizes ranged from 1 to 2 mm. The consistency varied from mucoid to smooth, indicating potential differences in extracellular polymer synthesis. These colony traits may reflect the isolates' physiological or functional characteristics.

The morphological, physiological, and biochemical characteristics of ten phosphate-solubilizing bacterial isolates (PSSB1 to PSSA1) showed significant variation (Table 2). Most isolates were gram-negative rods, except for PSSG11 (a gram-positive rod), and two isolates (PSSB4 and PSSB7) classified as gram-negative coccobacilli. All strains, except PSSG11 and PSSA1, were motile, suggesting they could effectively colonize the rhizosphere. All isolates except PSSA1 tested positive for oxidative-fermentative (Oxfer) activity, and all showed positive nitrate reduction, indicating aerobic metabolism and denitrification capacity. Most strains were excellent fermenters of glucose, fructose, sucrose, and mannitol, with some producing acid and gas (a⁺⁺), reflecting high carbohydrate metabolism.

Lactose fermentation varied, with only PSSB1, PSSB4, PSSB6, PSSB7, and PSSB8 exhibiting strong results. PSSB1, PSSB2, PSSB3, and PSSB5 all produced indole; however, only a few strains tested positive for methyl red (MR) and Voges-Proskauer (VP), indicating differences in glucose metabolism pathways. Citrate utilization was generally positive across all strains, suggesting they can use citrate as their sole carbon source. Hydrolytic enzyme production was inconsistent: only PSSG11 and PSSA1 produced amylase; caseinase was found in five isolates; and lipase and lecithinase production varied among strains, indicating differing capacities for protein and lipid degradation. Catalase activity was high across all isolates, while oxidase activity was detected in only four. PSSB4, PSSB7, PSSB8, PSSG11, and PSSA1 produced high levels of ammonia, an important trait for nitrogen supply to plants. Several isolates tested positive for urease activity, including PSSB4, PSSB6, PSSB7, PSSB8, and PSSG11. The activity of decarboxylase and deaminase enzymes varied considerably. Some strains possessed arginine dihydrolase, ornithine decarboxylase, lysine decarboxylase, and deaminase, demonstrating differences in amino acid metabolism and stress adaptation. Overall, this biochemical

profile highlights the functional diversity among PSB isolates from semiarid soils, identifying several promising strains—particularly PSSG11 and PSSA1—with enhanced enzyme production, ammonia

generation, and multiple stress-related biochemical capabilities, making them strong candidates for biofertilizer development in saline and drought-prone agricultural environments.

Table 2 : Characterization of phosphate solubilizing bacteria

SR. NO.	CHARACTERISTICS	PSSB1	PSSB2	PSSB3	PSSB4	PSSB5	PSSB6	PSSB7	PSSB8	PSSG11	PSSA1
1	Gram Nature	Gram negative rods	Gram negative rods	Gram negative rods	Gram negative cocobacilli	Gram negative rods	Gram negative rods	Gram negative cocobacilli	Gram negative rods	Gram-positive rods	Gram-negative rods
2	Motility	Motile	Motile	Motile	Motile	Motile	Motile	Motile	Motile	Non motile	Non motile
3	Oxferm test	++	++	++	++	++	++	++	++	++	-
4	Nitrate reduction test	++	++	++	++	++	++	++	++	++	+
5	Glucose fermentation	a++	++	a++	a++	++	++	a++	++	a++	-
6	Fructose fermentation	a++	++	++	+	a++	++	+	+	-	-
7	Sucrose fermentation	a++	++	++	+	++	++	+	+	++	-
8	Lactose fermentation	++	--	--	+	--	++	+	+	-	-
9	Mannitol fermentation	a++	++	++	+	++	++	+	+	+	-
10	Indole	+	+	+	-	+	+	-	-	+	-
11	MR	-	-	-	-	-	-	-	-	+	-
12	VP	-	-	-	+	-	-	+	+	+	-
13	Citrate Utilization	+	+	+	+	+	+	+	+	+	+
14	Amylase	-	-	-	-	-	-	-	-	+	+
15	Caseinase	+	+	+	-	+	-	-	-	+	-
16	Lipase	+	-	+	+	-	-	+	+	+	+
17	Lecithinase	-	-	-	+	-	+	+	-	+	-

18	Catalase	+	+	+	+	+	++	+	+	+	+
19	Oxidase	-	+	-	-	-	+	-	-	+	+
20	Ammonia production	+	-	+	+++	-	+	+	+++	+++	+++
21	Urease	-	+	-	+	+	+	+	+	+	-
22	Arginine dihydrolase	+	-	-	-	-	-	-	-	+	-
23	Ornithin decarboxylase	-	-	+	+	+	+	+	-	+	+
24	Lysine decarboxylase	+	-	+	-	+	-	+	+	-	+
25	Phenyl alanine Deaminase	+	+	-	-	+	-	-	-	-	-

*Note: ^a represents acid and gas formation

3.4 Isolation and screening of SSB

A total of nine morphologically distinct bacterial isolates (PSSB1-PSSB8, PSSG11) with a silicate solubilization zone were isolated from semiarid soil samples.

3.5 Characterization of silicate solubilizing bacteria

Table 3 describes the size, shape, margin, elevation, opacity, color & consistency characteristics of these silicate-solubilizing bacterial colonies. It summarizes the colony shape of the bacterial isolates that solubilize silicate. Shape, margin, elevation, opacity, color, and consistency were all different among the isolates. Except for PSSB2 and PSSB5, which showed irregular morphologies, the majority of isolates formed circular

colonies. The elevation ranged from flat to elevated and convex, and the colony borders were either complete or uneven.

The isolates were either transparent (PSSB4, PSSB6, PSSB7, and PSSB8) or opaque (PSSB1, PSSB2, PSSB3, PSSB5, and PSSG11) in terms of opacity. Except for PSSB4, PSSB6, PSSB7, and PSSB8, which were all white, and PSSG11, which was off-white, the majority of the colonies were creamy in color. The colony's consistency varied; the majority were smooth, but PSSB4 and PSSB6 had mucoid textures, and PSSG11 appeared moist. The variety of silicate-solubilizing bacteria that were separated from the semiarid soil samples is shown by these morphological traits.

Table 3. Colony morphology of silicate solubilizing bacteria

Sr. No.	Isolate	Size (mm)	Shape	Margin	Elevation	Opacity	Colour	Consistency
1	PSSB1	2	ROUND	ENTIRE	RAISED	OPAQUE	CREAMY	SMOOTH
2	PSSB2	2	IRREGULAR	IRREGULAR	FLAT	OPAQUE	CREAMY	SMOOTH
3	PSSB3	2	ROUND	IRREGULAR	RAISED	OPAQUE	CREAMY	SMOOTH
4	PSSB4	2	ROUND	ENTIRE	CONVEX	TRANSLUCENT	WHITE	MUCOID
5	PSSB5	2	IRREGULAR	IRREGULAR	RAISED	OPAQUE	CREAMY	SMOOTH
6	PSSB6	2	ROUND	ENTIRE	CONVEX	TRANSLUCENT	WHITE	MUCOID
7	PSSB7	2	ROUND	ENTIRE	CONVEX	TRANSLUCENT	WHITE	SMOOTH
8	PSSB8	2	ROUND	ENTIRE	FLAT	TRANSLUCENT	WHITE	SMOOTH
9	PSSG11	2	ROUND	IRREGULAR	FLAT	OPAQUE	OFF WHITE	MOIST

Table 4 provides a summary of the physiological and biochemical tests of the bacterial isolates that solubilize silicate. In Gram Nature and morphology, except for PSSB8 and PSSG11, which were Gram-positive, most of the isolates were Gram-negative. Interestingly, PSSB8 and PSSG11 showed out as Gram-positive cocci and rods, respectively, whereas PSSB4 and PSSB7 were found to be Gram-negative coccobacilli. The results of motility showed that PSSB4, PSSB7, PSSB8, and PSSG11 were non-motile, while PSSB1, PSSB2, PSSB3, PSSB5, and PSSB6 were motile. The oxidative-

fermentative (Oxferm) and nitrate reduction assays yielded positive results for every isolate. The isolate's fermentation processes for glucose, fructose, sucrose, lactose, and mannitol differed, and some of them produced gas and acid (PSSB1, PSSB5, and PSSB7 in certain sugars). With concern for enzymatic activities, except for PSSB6, PSSB7, and PSSB8, the majority of isolates had positive caseinase tests. All isolates tested positive in the catalase test, with PSSB6 exhibiting a positive response. Lecithinase activity was only seen in PSSB4, PSSB6, and PSSB7, whereas

amylase production was seen in PSSB7, PSSB8, and PSSG11. PSSB1, PSSB3, PSSB7, PSSB8, and PSSG11 all produced lipase. The results of IMViC and other biochemical tests showed that all isolates except PSSG11 had positive indole production, and all isolates except PSSB8 and PSSG11 had negative Methyl Red (MR) test results. Only PSSG11 passed the Voges-Proskauer (VP) test. Except for PSSB4 and PSSB6, the majority of

isolates showed positive citrate utilization.

The oxidase and catalase test results are PSSB2, PSSB4, PSSB6, and PSSB8; all had positive reactions in the oxidase test; however, isolate PSSG11 results were inconsistent.

The diversity of the silicate-solubilizing bacterial isolates is highlighted by this biochemical test study, which also points to possible functions in mineral solubilization and plant growth promotion.

Table 4. Characterization of silicate solubilizing bacteria

SR. NO.	CHARACTERISTICS	PSSB1	PSSB2	PSSB3	PSSB4	PSSB5	PSSB6	PSSB7	PSSB8	PSSG11
1	Gram Nature	Gram negative	Gram negative	Gram negative	Gram negative cocobacilli	Gram negative	Gram negative	Gram negative cocobacilli	Gram-positive cocci	Gram-positive rods
2	Motility	Motile	Motile	Motile	Non motile	Motile	Motile	Non motile	Non motile	Non motile
3	Oxferm test	++	++	++	++	++	++	++	++	++
4	Nitrate reduction test	++	++	++	++	++	++	++	++	++
5	Glucose fermentation	a++	++	a++	++	++	++	++	++	a++
6	Fructose fermentation	a++	++	++	++	a++	++	a++	++	++
7	Sucrose fermentation	a++	++	++	a++	++	++	++	++	++
8	Lactose fermentation	++	--	--	--	--	++	++	--	++
9	Mannitol fermentation	a++	++	++	++	++	++	++	++	++
10	Indole	+	+	+	+	+	+	+	+	-
11	MR	-	-	-	-	-	-	-	+	+
12	VP	-	-	-	-	-	-	-	-	+
13	Citrate Utilization	+	+	+	-	+	-	+	+	+
14	Amylase	-	-	-	-	-	-	+	+	+
15	Caseinase	+	+	+	+	+	-	-	-	+
16	Lipase	+	-	+	-	-	-	+	+	+

17	Lecithinase	-	-	-	+	-	+	+	--	-
18	Catalase	+	+	+	+	+	++	+	+	+
19	Oxidase	-	+	-	+	-	+	-	+	variable

*Note: ^a represents acid and gas formation

3.6 Seed Germination Study using Individual Isolates

Day 3 Study:

The table 5a, Figure 1a, b & c presents the early effects of microbial inoculation on seed germination and seedling growth of *Sorghum bicolor* on day 3 after sowing. Germination percentages across treatments were relatively high, ranging from 68% in the uninoculated control to 96% in the mixed and PSSB8 treatments. However, shoot elongation was minimal or absent in most treatments, with only PSSB8 showing a slight increase (0.1 cm). Root length was marginally improved in a few treatments, with the highest (0.4 cm) recorded in PSSB8 and the known control. In terms of

seedling vigor indices, only PSSB8 demonstrated a notable improvement, with SVI-I (9.6), SVI-II (38.4) and SVI-III (48) indicating the beginning of a positive growth response. All other treatments, including the known bioinoculant, exhibited zero shoot growth and lower vigor values, reflecting limited seedling development at this early stage. Overall, these results suggest that while germination had initiated, substantial seedling elongation had not yet occurred by Day 3 with PSSB8 emerging as the most promising isolate for early root and shoot stimulation.

Table 5a: Seed Germination study using individual isolates (Observation Day 3)

TREATMENT	GERMINATION %	MEAN SHOOT LENGTH (CM)	MEAN ROOT LENGTH (CM)	SVI-I (GERMINATION % X SHOOT LENGTH)	SVI-II (GERMINATION % X ROOT LENGTH)	SVI-III (Germination % x Seedling length)
CONTROL (NO CULTURE)	68	0	0.2	0	13.6	13.6
CONTROL (KNOWN)	92	0	0.4	0	36.8	36.8
MIXED	96	0	0.3	0	28.8	28.8
PSSB4	88	0	0.2	0	17.6	17.6
PSSB6	88	0	0.3	0	26.4	26.4

PSSB7	88	0	0.2	0	17.6	17.6
PSSB8	96	0.1	0.4	9.6	38.4	48
PSSG11	92	0	0.2	0	18.4	18.4
PSSA1	92	0	0.2	0	18.4	18.4

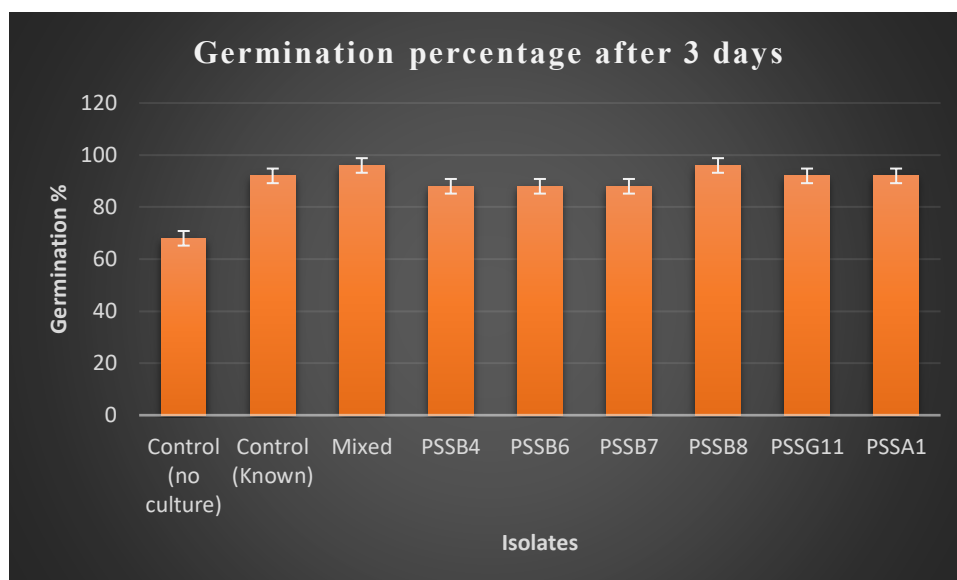


Figure 1a: Germination percentage after 3 Days

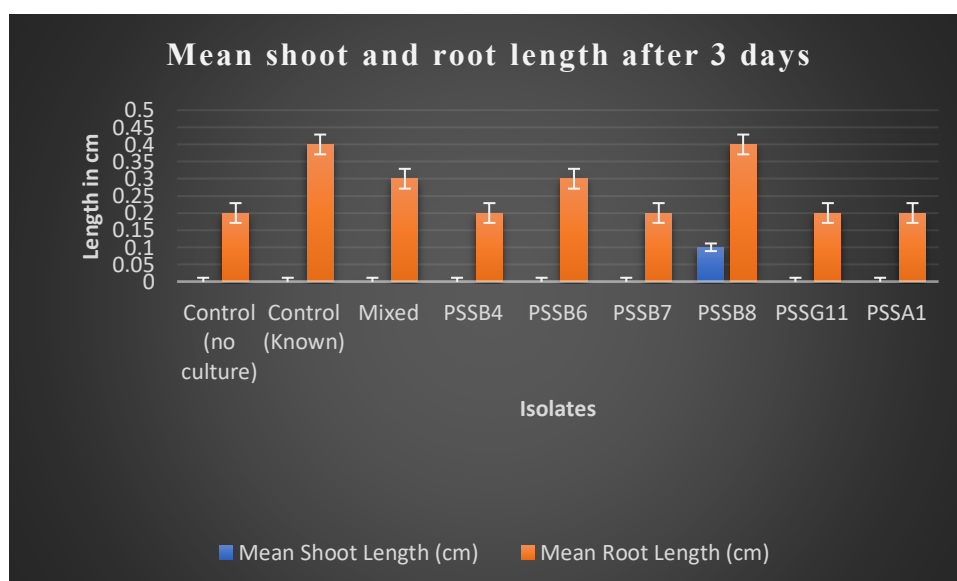


Figure 1b: Mean shoot and root length after 3 days

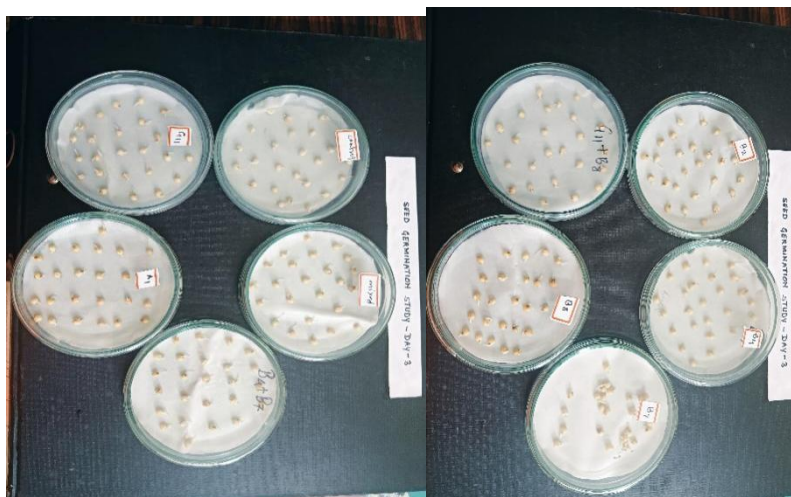


Figure 1c: Seed germination using individual isolates after 3 days

The statistical analysis of seed germination parameters on day 3 provides insights into early-stage variability among treatments (Table 5b). The mean germination percentage was high at 88.89%, with a standard deviation of 8.43, indicating moderately uniform germination across treatments, though the range spanned from 68% to 96%. Mean shoot length was extremely low (0.011 cm), with a maximum of only 0.1 cm, suggesting minimal shoot development at this stage. The root length showed slightly more growth, with a mean of 0.27 cm and a narrow range (0.2 to 0.4 cm), highlighting

limited variation among treatments. The seedling vigor indices reflect these trends. SVI-I (based on shoot length) had a low mean of 1.07 and a standard deviation of 3.2, driven largely by the contribution of isolate PSSB8. SVI-II, which reflects root vigor, showed more variation (mean 24, SD 9.03, range 13.6–38.4), indicating that root elongation contributed more to early seedling vigor than shoots. Similarly, SVI-III (total seedling length) showed a broader range (13.6 to 48) with a mean of 25.07, again primarily due to the improved performance of PSSB8.

Table 5b: Statistical analysis seed germination study using individual isolates (Observation Day 3)

	COUN T	SUM	MEA N	STANDAR D DEVIATIO N	MIN	MAX	RANG E
GERMINATIO N %	9	800	88.888 9	8.43274	68	96	28

MEAN SHOOT LENGTH(CM)	9	0.1	0.0111 1	0.03333	0	0.1	0.1
MEAN ROOT LENGTH(CM)	9	2.4	0.2666 7	0.0866	0.2	0.4	0.2
SVI-I (GERMINATIO N %X SHOOT LENGTH)	9	9.6	1.0666 7	3.2	0	9.6	9.6
SVI-II (GERMINATIO N %X ROOT LENGTH)	9	216	24	9.03327	13.6	38.4	24.8
SVI-III (GERMINATIO N %X SEEDLING LENGTH)	9	225.6	25.066 7	11.2428	13.6	48	34.4

Overall, the data suggest that while germination was generally good, actual seedling growth was minimal at day 3, with isolate PSSB8 being the only treatment showing early and measurable growth response, particularly in root elongation and seedling vigour.

Day 5 Study:

The seed germination observations recorded on day 5 highlight the significant impact of microbial inoculation on early seedling development of *Sorghum bicolor*. Across treatments, germination rates remained high, with an average of 88.89%, showing consistent seed viability. However, seedling vigor varied notably based on the microbial treatment applied (Table 6a & Figure 2a, b & c). The uninoculated control exhibited minimal

growth, with low shoot (1.0 cm) and root (0.2 cm) lengths, resulting in a low SVI-III of 81.6. In contrast, the known bioinoculant showed a marked improvement in seedling parameters, recording shoot and root lengths of 7.1 cm and 6.5 cm, and a high SVI-III of 1251.2, validating its effectiveness as a growth enhancer. Among the test isolates, PSSB8 was the most effective, achieving the highest values in all parameters: 8.3 cm shoot length, 8.2 cm root length, and a maximum SVI-III of 1675. This indicates excellent seedling vigor and a strong potential for early plant establishment. PSSB6 and PSSB7 also performed well, with SVI-III values of 1002.8 and 911.1, respectively, signifying good root and shoot development. The mixed inoculum

treatment also showed substantial improvement over the control, although it was less effective than the best-performing individual isolates.

Table 6a: Seed Germination study using individual isolates (Observation Day 5)

TREATMENT	GERMINATION %	MEAN SHOOT LENGTH (CM)	MEAN ROOT LENGTH (CM)	SVI-I (GERMINATION % X SHOOT LENGTH)	SVI-II (GERMINATION % X ROOT LENGTH)	SVI-III (Germination % x Seedling length)
CONTROL (NO CULTURE)	68	1	0.2	68	13.6	81.6
CONTROL (KNOWN)	92	7.1	6.5	653.2	598	1251.2
MIXED	96	5.5	2.9	528	278.4	806.4
PSSB4	88	2.2	4.3	193.6	378.4	572
PSSB6	88	4.7	6.2	432.4	570.4	1002.8
PSSB7	88	3.1	6.8	285.5	625.6	911.1
PSSB8	96	8.3	8.2	796.8	878.2	1675
PSSG11	92	5.2	4.6	478.8	423.2	902
PSSA1	92	2.1	4	193.2	368	561.2

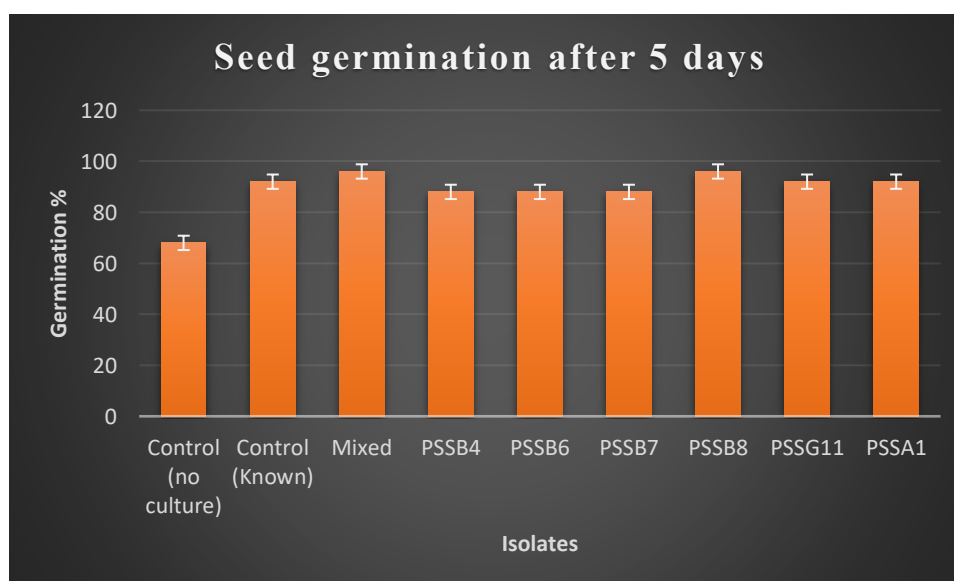


Figure 2a: Seed germination after 5 days

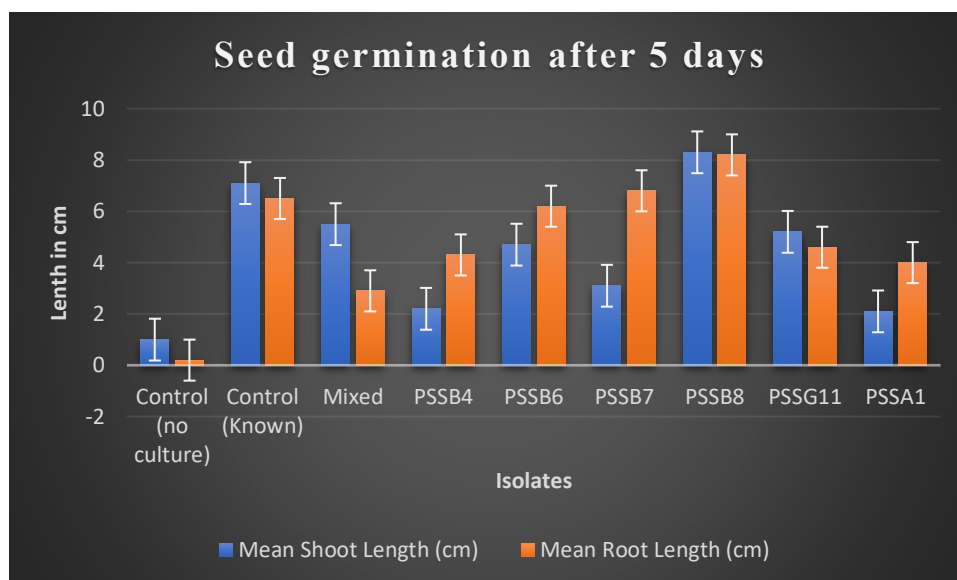


Figure 2b: Mean shoot & root length after 5 days

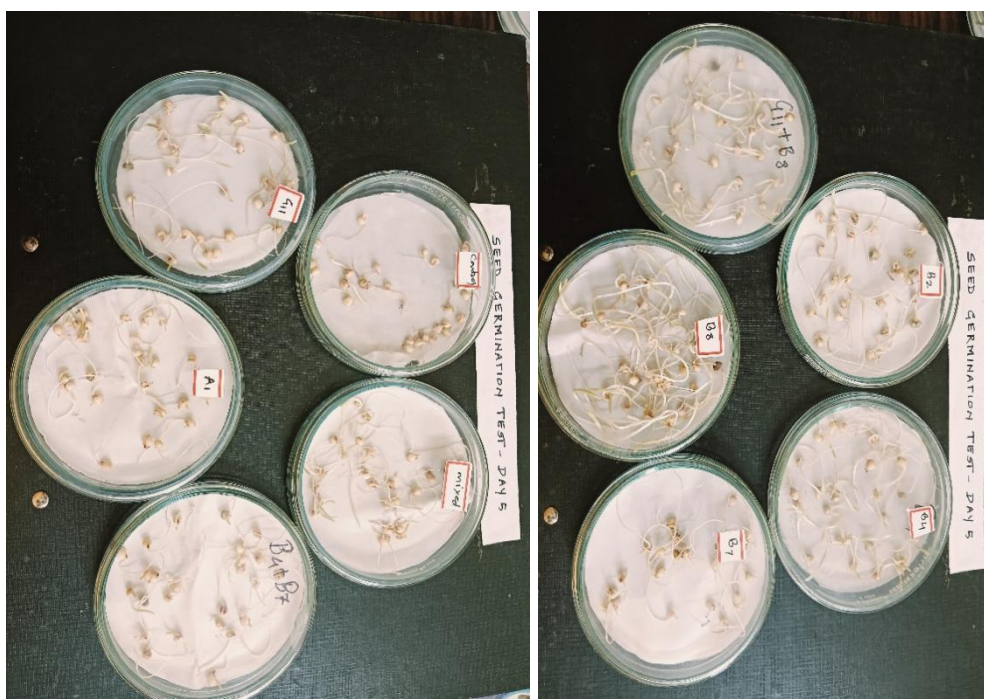


Figure 2c: Seed germination using individual isolates after 5 days

**Table 6b: Statistical analysis seed germination study using individual isolates
(Observation Day 5)**

	COUNT	SUM	MEAN	STANDARD DEVIATION	MIN	MAX	RANGE
GERMINATION %	9	800	88.8889	8.43274	68	96	28

MEAN SHOOT LENGTH(CM)	9	39.2	4.35556	2.44444	1	8.3	7.3
MEAN ROOT LENGTH(CM)	9	43.7	4.85556	2.40006	0.2	8.2	8
SVI -I (GERMINATION %X SHOOT LENGTH)	9	3629.5	403.278	238.172	68	796.8	728.8
SVI -II (GERMINATION %X ROOT LENGTH)	9	4133.8	459.311	245.528	13.6	878.2	864.6
SVI -III (GERMINATION %X SEEDLING LENGTH)	9	7763.3	862.589	450.413	81.6	1675	1593.4

The statistical analysis supports these observations. The mean shoot and root lengths across treatments were 4.36 cm and 4.86 cm respectively, indicating considerable overall growth (Table 6b). The highest variability was observed in seedling vigor indices, particularly SVI-III, with a range from 81.6 to 1675 and a standard deviation of 450.41, highlighting the distinct influence of different microbial treatments. In summary, day 5 data confirm that microbial inoculation significantly improves seedling vigor, with PSSB8 emerging as the most effective isolate, followed by PSSB6 and PSSB7. These results suggest their strong potential for use as bioinoculants to promote early plant growth in sustainable agriculture.

Day 6 Study:

By day 6, the effects of microbial inoculation on seed germination and

seedling development became even more pronounced. Germination rates remained consistently high across treatments (mean: 88.89%), while shoot and root elongation showed significant enhancement compared to earlier days (Table 7a, Figure 3a, b & c). The uninoculated control recorded limited seedling growth with shoot and root lengths of 3.0 cm and 4.0 cm, respectively, resulting in a modest SVI-III of 476. In contrast, the known bioinoculant demonstrated a marked improvement with 10.0 cm shoot and 9.6 cm root lengths, achieving a SVI-III of 1803. Among the tested isolates, PSSB8 stood out as the most effective, recording the highest seedling vigor index (SVI-III of 1987.2), along with shoot and root lengths of 9.2 cm and 11.5 cm, respectively. This indicates its superior ability to enhance early seedling development. Isolates

PSSB6 and PSSB7 also performed well, with SVI-III values of 1531.2 and 1469.6, reflecting strong root and shoot growth.

Other isolates such as PSSB4, PSSG11

and PSSA1 showed moderate to high improvements in seedling vigour compared to the control.

Table 7a: Seed Germination study using individual isolates (Observation Day 6)

Treatment	Germination %	Mean Shoot Length (cm)	Mean Root Length (cm)	SVI-I (Germination % x Shoot Length)	SVI-II (Germination % x Root Length)	SVI-III (Germination % x Seedling length)
Control (no culture)	68	3	4	204	272	476
Control (Known)	92	10	9.6	920	883	1803
Mixed	96	5.8	7	556.8	672	1228.8
PSSB4	88	5	9.8	440	862.4	1302.4
PSSB6	88	6.4	11	563.2	968	1531.2
PSSB7	88	6.5	10.2	572	897.6	1469.6
PSSB8	96	9.2	11.5	883.2	1104	1987.2
PSSG11	92	5.8	7	533.6	644	1177.6
PSSA1	92	3.5	9.7	322	892.4	1214.4

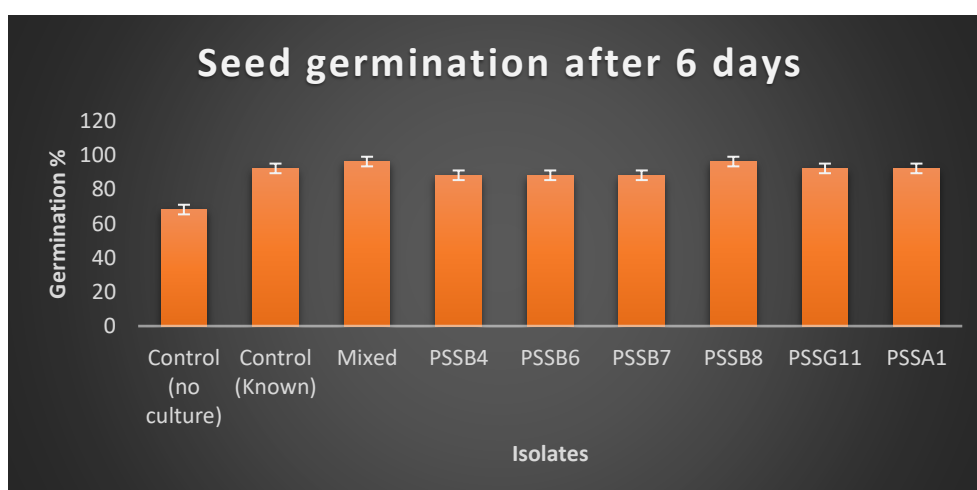


Figure 3a: Germination percentage after 6 days

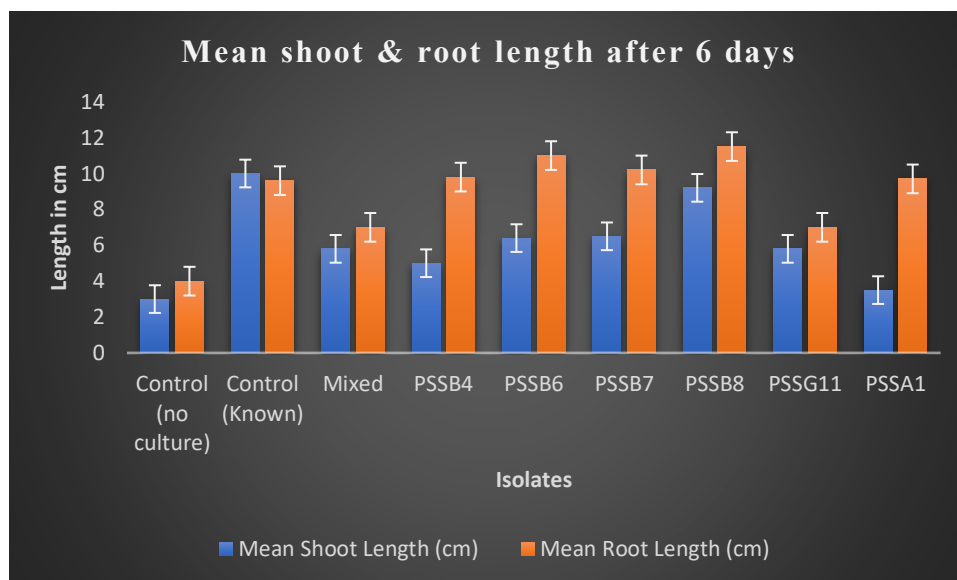


Figure 3b: Mean shoot & root length after 6 days

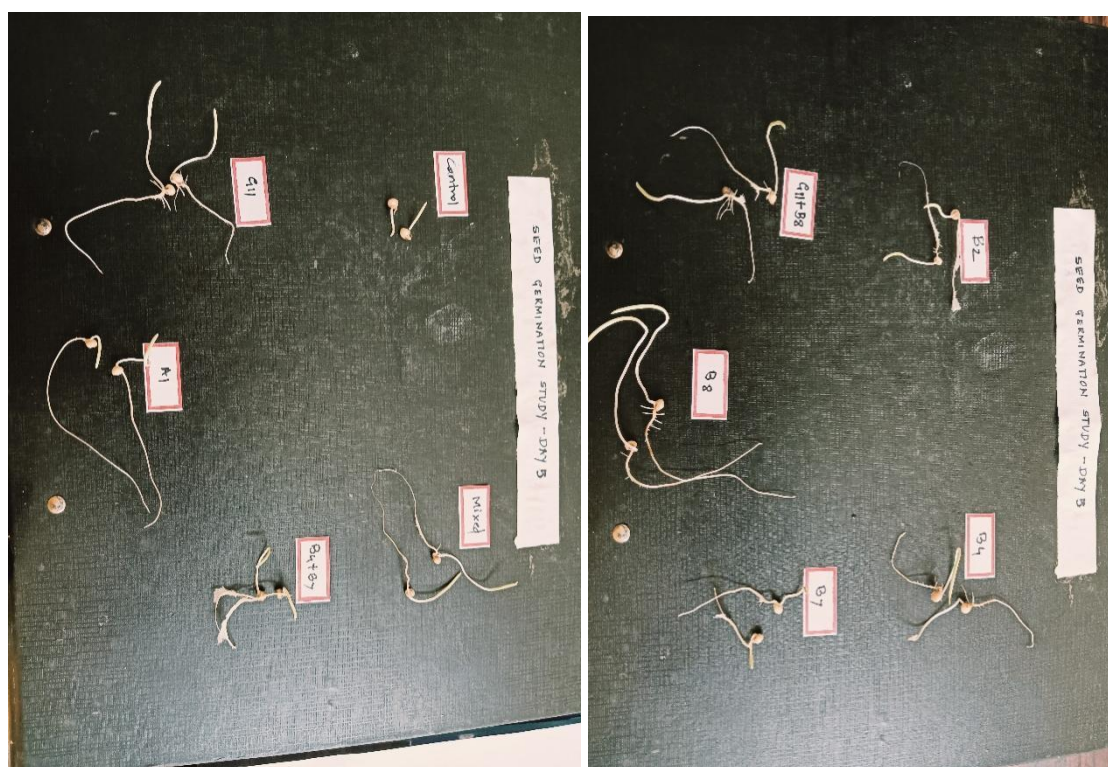


Figure 3c: Seed Germination study using individual isolates after 6 days

**Table 7b: Statistical analysis seed germination study using individual isolates
(Observation Day 6)**

	COUNT	SUM	MEAN	STANDARD DEVIATION	MIN	MAX	RANGE
GERMINATION %	9	800	88.8889	8.43274	68	96	28
MEAN SHOOT LENGTH(CM)	9	55.2	6.13333	2.31355	3	10	7
MEAN ROOT LENGTH(CM)	9	79.8	8.86667	2.39844	4	11.5	7.5
SVI -I (GERMINATION %X SHOOT LENGTH)	9	4994.8	554.978	232.506	204	920	716
SVI -II (GERMINATION %X ROOT LENGTH)	9	7195.4	799.489	242.123	272	1104	832
SVI -III (GERMINATION %X SEEDLING LENGTH)	9	12190.2	1354.47	431.151	476	1987.2	1511.2

The statistical analysis supports these findings. The mean shoot length was 6.13 cm and mean root length was 8.87 cm indicating robust growth overall (Table 7b). The seedling vigor indices, especially SVI-III, showed a wide range (476 to 1987.2) and a high standard deviation (431.15), reflecting the differential effects of various treatments. In conclusion, day 6 observations confirm the continued growth-promoting effects of microbial inoculation, with PSSB8 consistently delivering the best performance. These results strongly suggest its potential as a high-value bioinoculant for improving

seedling establishment in sustainable crop management.

4. Discussion:

This study isolated and characterized phosphate-solubilizing bacteria from soil samples in the Kolhapur and Sangli districts of Maharashtra under conditions of salt and drought stress. The soil analysis revealed moderate fertility, alkaline pH, and sufficient levels of nitrogen, phosphorus, potassium, calcium, and magnesium for crop growth. Similar to our study, (Karpagam & Nagalakshmi, 2014) investigated the isolation of PSBs from agricultural soil samples and examined the

soil's physicochemical properties. The current study analyzed ten microbial isolates (PSSB1 to PSSB8, PSSG11, and PSSA1) for colony characteristics. Most displayed round, translucent colonies, while others had irregular margins. The majority were gram-negative rods, motile, and efficient fermenters of glucose, fructose, sucrose, and mannitol. Lactose fermentation varied among isolates, and hydrolytic enzyme production was inconsistent. Catalase activity was high across all isolates, suggesting they are promising candidates for biofertilizer development in saline and drought-affected agricultural conditions. Similarly, (Patel et al., 2016) collected samples from rhizospheric soil and studied the isolation and characterization of PSB. (Karpagam & Nagalakshmi, 2014) isolated 37 bacteria showing halo zone phosphate solubilization from agricultural soils. (López-Gutiérrez et al., 2004) examined the morphological and biochemical characteristics of PSBs isolated from soil. Bacterial isolates PSSB6, PSSB7, PSSB4, PSSB8, and PSSG11 demonstrated high phosphate solubilization indices, indicating potential for biofertilizer formulations to enhance phosphorus availability in agricultural settings. Studies by (A. Kumar et al., 2010), (Mohamed et al., 2019), and (López-Gutiérrez et al.,

2004) also focused on the phosphate solubilization activity of these isolates. Additionally, (Patel et al., 2016) investigated the effects of salt and drought stress on microbial growth, aligning with our research. *Enterobacter* spp., *Bacillus* spp., *Pseudomonas* spp., and *Azotobacter* spp. are effective phosphate solubilizers across various pH, salt, and temperature ranges, as demonstrated by their high efficiency. These isolates thrive in environments with high salt, pH, and temperature, making them ideal bio-inoculants for soils containing insoluble phosphorus. However, field trials are necessary to fully evaluate their potential in agricultural ecosystems.

The second most prevalent mineral in the crust of the Earth is silica. Even though silica has several benefits, plants are unable to directly use the complex silicates. In this case, bacteria were essential to the bioweathering process, which transformed the insoluble silica into a form that plants could use (Santi et al., 2018). Plants that accumulate silica will be able to withstand both biotic and abiotic stress. Some biochemical mechanisms were important in the mechanism of bioweathering, even though bacteria dissolve silica through several physical interactions (LIAN et al., 2008). Microbe-induced bioweathering is thought to be a

key mechanism that liberates vital nutrients from soil and sediments (Lee et al., 2019), (Vasanthi et al., 2018). A silicate-solubilizing bacterium was isolated from the semiarid soil samples used in the current study. It was common practice to isolate silicate-solubilizing bacteria using the traditional Bunt and Rovira selective medium. It can be difficult to observe silicate solubilising zones in traditional selective media since they will be off-white and translucent. Despite possible inhibitions by salt stress, some bacterial strains can continue to exhibit significant silicate solubilisation activity even in the presence of salt stress, suggesting their potential as bio-inoculants in saline soils. Because some SSB strains can withstand high salinity and retain silicate solubilisation, they may be used as bio-inoculants in salty and semiarid soils to improve crop development and soil fertility. In a similar manner, (Lee et al., 2019) isolated and characterised the novel strain of *Enterobacter ludwigii* GAK2 that improves rice plant growth by solubilising phosphate and silicate. Numerous crops could benefit from the employment of the promising silicate-solubilizing bacteria as silicon biofertilizers, according to research by (Chopra et al., 2021). Since silicate-solubilizing bacteria use glucose to secrete organic acids, silicate-solubilization is also

an acidolysis process. After four days of incubation, the PSSB7, PSSG11 isolates out of the nine collected displayed a higher silicate solubilisation index. A thorough biochemical characterisation was conducted in this investigation to assess the potential of the screened isolates. A significant barrier to crop quality and productivity is soil salinity. Using phytostimulation and phytoremediation, plant growth-promoting rhizobacteria (PGPR) are essential for recycling nutrients (Sharma et al., 2021). Although strain-specific responses and variations in silicate solubilisation efficiency point to the need for strain-specific research to gain a better understanding of stress adaptation processes, the work validates the importance of salt-tolerant SSB in nutrient cycling.

Phosphate-solubilizing bacteria (PSB) are typically isolated from soil samples, especially from the rhizospheres of plants with active microbial populations. This study collected soil samples from the Kolhapur and Sangli districts in Maharashtra to isolate and analyze PSB under salt and drought stress, focusing on saline barren lands and drought-affected farms. Soil analysis indicates moderate fertility, with an alkaline pH of 8.57, suggesting potential nutrient availability issues. The soil

contains sufficient nitrogen, phosphorus, and potassium for crop growth, along with adequate calcium and magnesium levels. However, low levels of iron, manganese, zinc, and copper may limit plant development. The soil could benefit from vitamin enrichment and pH adjustment to optimize crop production. Similar to our study, (Karpagam & Nagalakshmi, 2014) isolated PSBs from agricultural soils and studied their physicochemical characteristics. The current study examined ten microbial isolates (PSSB1 to PSSB8, PSSG11, and PSSA1) for colony features. Most colonies were round and translucent to transparent, while others had irregular margins and appeared opaque. PSSB2 formed a regularly shaped, mucoid colony, whereas PSSG11 exhibited a moist texture. These colony traits may relate to the isolates' physiological or functional features. The study assessed the morphological, physiological, and biochemical traits of ten phosphate-solubilizing bacterial isolates (PSSB1 to PSSA1). Most were gram-negative rods, with a few being gram-positive. All but two were motile, indicating strong colonization potential. They efficiently fermented glucose, fructose, sucrose, and mannitol. Lactose fermentation varied, with PSSB1, PSSB4, PSSB6, PSSB7, PSSB8, and PSSA1 showing positive

results. Hydrolytic enzyme production was inconsistent, with only PSSG11 and PSSA1 producing amylase and lipase. Catalase activity was high across all isolates, and several displayed differing decarboxylase and deaminase activities. These strains are promising candidates for biofertilizer development in saline and drought-prone agricultural settings. Similarly, (Patel et al., 2016) also collected rhizospheric soil samples for PSB isolation and characterization. (Karpagam & Nagalakshmi, 2014) isolated 37 bacteria demonstrating halo zone phosphate solubilization from agricultural soils. (López-Gutiérrez et al., 2004) studied the morphological and biochemical features of PSBs isolated from soil. PSSB6, PSSB7, PSSB4, PSSB8, and PSSG11 showed high phosphate solubilization indices, indicating their potential for biofertilizer formulations to improve phosphorus availability. Research by (A. Kumar et al., 2010), (Mohamed et al., 2019), and (López-Gutiérrez et al., 2004) examined the phosphate solubilization activity of these isolates. The findings show that bacteria's adaptability to different pH levels varies. Isolates like PSSG11 and PSSB8 showed the highest optical density (OD) at neutral pH 7, while PSSB2 and PSSB5 thrived more at alkaline pH 9. Similar results were reported by (A.

Kumar et al., 2010) and (Mohamed et al., 2019). The study further revealed that salt levels influence PSB growth; some strains tolerate salinity, with high OD values at 1% NaCl, but a significant decline at 5% NaCl. A study on salt stress showed growth declines with increasing salinity, though some isolates showed notable tolerance. (Jiang et al., 2020) analyzed stress tolerance by varying NaCl and Na₂CO₃ levels and pH. (Johri et al., 1999) presented evidence that LCR3 increases phosphate solubilization at low salt levels. Under stress, organisms grow more slowly, reducing phosphorus solubilization. Despite this, *Enterobacter* species from agricultural farms solubilized 1.6-2.5 times more phosphate under stress than previous studies (Malboobi et al., 2009; Nautiyal et al., 2000). (Nautiyal et al., 2000) showed similar trends. This study examined drought effects on bacterial growth and phosphate activity, finding all isolates tolerated moderate drought stress at 20% PEG but none grew at 25% or 30%. (Patel et al., 2016) also investigated salt and drought impacts on microbial growth. *Enterobacter*, *Bacillus*, *Pseudomonas*, and *Azotobacter* species are effective phosphate solubilizers across various pH, salt, and temperature ranges, indicated by their high efficiency. These isolates thrive in harsh conditions, making

them ideal bio-inoculants, although field trials are necessary to fully assess their potential in agricultural ecosystems.

The study on *Sorghum bicolor* revealed that microbial isolates significantly influence early seedling growth. On day 3, germination percentage was high, but shoot and root elongation were minimal. By day 5, all microbial treatments had a stimulatory effect on seedling development, with PSSB8 showing the highest vigor. On day 6, PSSB8 showed the most robust growth, with shoot and root lengths of 9.2 cm and 11.5 cm respectively. Isolate PSSB8 consistently outperformed others, showing potential for improving nutrient uptake efficiency during early growth stages. The results suggest that microbial isolates can enhance seed germination and early vigor of sorghum, with PSSB8 showing promise as a potent plant growth-promoting rhizobacteria candidate for bioformulation development. The study done by (Chaithra V., 2017) did seed germination with the field experiment evaluated sorghum genotypes for cold tolerance under different sowing dates. According to their results, quality parameters like seed germination, root length, vigour index, and dry weight differed with dates and genotypes. The investigation by (Rajendran et al., 2011) used seed

germination experiment to study drought tolerance in Sorghum genotype. Their study examined ten-day plant growth, revealing significant differences in germination percentage, root length, shoot length, seed vigour index, coleoptile length, root to shoot length ratio, and membrane stability, identifying poor genotypes like ICS213A and CO26 at low water supply. Similarly, (Chiranjeevi et al., 2024) used the paper towel method to assess and classify rhizobacteria from sorghum (*Sorghum bicolor* L.) for promoting seed germination during the research work. Their research work analyzed 197 isolates, one reference, and one control for seed bio-priming. According to their results, *Pseudomonas fluorescence* treatment improved seed germination by 3.5% compared to PG-152 and PG-145 test isolates, with a median of 70.50%. Root length and shoot length were also significantly shorter in PG-152 plants compared to the reference strain. The water treatment had little impact on seed germination. The report by (Rashid et al., 2022) found that drought-tolerant PGPR, specifically MU2 and MU8, improved wheat seed germination percentage and index under well-watered and water-deficit conditions, with MU2 showing the highest germination percentage and promptness index.

5. Conclusion:

This study found that phosphate- and silicate-solubilizing bacteria isolated from saline soils greatly improve seed germination and seedling vigour in Sorghum bicolor. PSSB8 significantly outperformed all other studied isolates in terms of shoot and root elongation and vigour indices, indicating a high potential as a biofertilizer strain. The findings demonstrate that these microbes can effectively mobilise critical nutrients, boosting early plant growth even under saline stress conditions. The findings highlight the importance of microbial inoculants in sustainable agriculture by lowering the need for artificial fertilisers and promoting crop establishment in stress-prone soils. Further field trials are recommended to evaluate the efficacy of PSSB8 and other promising isolates in real-world agricultural situations, as well as to produce bioformulations for large-scale use.

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