

Original Research Article

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Assessment of the Plant Growth Promoting Potential of *Bacillus stercoris* AMC8 in *Vigna mungo* L. (Black Gram)

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ABSTRACT

Keywords

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Black gram (*Vigna mungo* L.) is an economically important pulse crop whose productivity is often constrained by environmental stresses and limited nutrient availability. The present study aimed to isolate and characterize indigenous plant growth promoting bacteria and evaluate their potential to enhance black gram growth. Bacterial isolates were obtained from compost enriched soils of municipal organic waste sites in Chennai, Tamil Nadu, India, and screened for phosphate solubilization and nitrogen fixing activity. Among 14 isolates, a potent isolate was identified through morphological, biochemical and 16S rRNA gene analysis as *Bacillus stercoris*, and strain name was designated as AMC8. Prepared seed coating bacterial formulations of liquid and powder forms were evaluated for seed germination and seedling growth of *Vigna mungo* plant. The powder formulation produced greater improvement in germination and early seedling growth than the liquid formulation. Besides, the plant growth promoting efficiency of *B. stercoris* AMC8 was evaluated by greenhouse cultivation of *Vigna mungo* plant. The plants were supplemented with bacterial culture in two ways as seed inoculation and soil inoculation methods. The plant growth promotion effect was found to be higher in soil supplemented with bacteria when compared to seed inoculation. These findings demonstrated the potential of *B. stercoris* AMC8 which could be utilized as a promising biofertilizer for sustainable *Vigna mungo* cultivation.

Introduction

Black gram (*Vigna mungo* L.) is an important pulse crop belonging to the family Fabaceae and is widely cultivated in South and Southeast Asia. It is valued for its high nutritional content, particularly proteins, carbohydrates, minerals, and essential amino acids, and contributes significantly to food and nutritional security. As a leguminous crop, black gram also has an important role

in sustainable agriculture through biological nitrogen fixation and improvement of soil fertility. However, its growth and productivity are frequently affected by environmental constraints such as drought, salinity, nutrient deficiency, and other abiotic and biotic stresses.

Therefore, the development of sustainable approaches for improving black gram productivity has become increasingly important.

Plant Growth Promoting Rhizobacteria (PGPR) are beneficial microorganisms that colonize the rhizosphere and enhance plant growth through a range of direct and indirect mechanisms. These bacteria can improve nutrient availability through nitrogen fixation and phosphate solubilization, produce phytohormones such as indole-3-acetic acid, and synthesize siderophores that facilitate iron acquisition. They can also contribute to plant protection through the production of antimicrobial compounds, hydrolytic enzymes, and induction of plant defence responses (Ehinmitan *et al.*, 2024). Recent reviews have emphasized that PGPR mediated improvement of nutrient acquisition, root development, phytohormonal regulation, and pathogen suppression makes these microorganisms promising candidates for sustainable biofertilizer development (Muneer *et al.*, 2024).

The use of indigenous soil bacteria is particularly valuable because naturally occurring microorganisms may be well adapted to the environmental and edaphic conditions of their native habitats. Screening such bacterial isolates for plant growth promoting characteristics can therefore help identify effective microbial inoculants. In black gram, recent studies have demonstrated significant benefits from bacterial inoculation under adverse environmental conditions. (Chandwani and Amaresan, 2024) reported that drought tolerant, ACC deaminase producing bacterial isolates belonging to genera including *Bacillus*, *Staphylococcus*, *Rhodococcus*, and *Delftia* promoted plant growth and biomass production of *V. mungo* under drought stress. The inoculated plants also showed improved relative water content, photosynthetic pigments, and nitrogen and phosphorus uptake (Chandwani and Amaresan, 2024).

Similarly, native salt tolerant bacterial isolates and compatible bacterial combinations have been reported to improve nodulation, nitrogen uptake, and growth of black gram under saline conditions (Tiwari *et al.*, 2024). More recently, a halophilic PGPR consortium containing *Halobacillus* species improved root development, biomass, nutrient uptake, antioxidant defence, and yield of *V. mungo* under salinity stress (Raphael and Parthasarathi, 2026). These findings demonstrate the potential of beneficial bacteria as environmentally sustainable tools for enhancing crop performance.

Therefore, the present study was undertaken to isolate bacteria from soil, evaluate their plant growth promoting properties, and investigate their effects on the growth and

development of *Vigna mungo* (black gram). The study may provide useful bacterial candidates for future development of microbial biofertilizers and sustainable crop production strategies.

Materials and Methods

Collection of Soil Samples and Isolation of Bacterial Strains

Soil samples were collected from different municipal organic waste dumping and compost preparation sites in and around Chennai, Tamil Nadu, India. The soil samples were collected in the depth of approximately 5 cm using a sterile spatula and collected aseptically in sterile polythene bags. The samples were brought to the laboratory within 6 hours and processed immediately. The collected samples were subjected to serial dilution up to 10^{-7} using sterile physiological saline as the diluent and plated onto sterile nutrient agar plates. The plates were incubated at 37°C for 24 hours. The obtained bacterial isolates were purified by repeated streaking on nutrient agar plates and maintained for further studies.

Screening of Bacterial Isolates for Plant Growth Promoting Activities

The purified bacterial isolates were screened for selected plant growth promoting (PGP) traits like phosphate solubilization and nitrogen fixation.

Phosphate solubilizing activity was evaluated using Pikovskaya's agar medium. Sterile Pikovskaya's agar plates were prepared and each bacterial isolate was streaked onto the medium. The plates were incubated at 37°C for 24 hours and observation was made for the development of a clear zone surrounding the bacterial growth was considered as indicative of phosphate solubilizing activity.

The nitrogen fixing ability of the bacterial isolates were assessed using nitrogen free Malate Agar medium supplemented with bromothymol blue as an indicator. The sterile medium was prepared and bacterial isolates were streaked onto the medium. The streaked plates were incubated at 37°C for 24 hours and observation was made for the development of a blue colour around the bacterial growth was considered as positive indication of nitrogen fixing activity. Based on the intensity of phosphate solubilization and nitrogen fixation responses,

the isolate exhibiting prominent activity in both assays was selected for subsequent plant growth promotion studies.

Identification of the Selected Bacterial Isolate

The selected bacterial isolate was characterized by conventional Microbiological and Biochemical methods. Molecular identification was performed by sequencing the 16S rRNA gene. The resulting sequence was compared with sequences available in the National Centre for Biotechnology Information (NCBI) database using the BLAST (Basic Local Alignment Search Tool) program.

Preparation of Bacterial Cell Biomass and Concentrate

Bacterial biomass was prepared for subsequent seed treatment experiments. One litre of sterile nutrient broth was inoculated with 3 ml of bacterial culture. The inoculated broth was incubated at 37°C for 24 hours on a rotary shaker at 75 rpm. The bacterial culture was concentrated by centrifuged at 10,000 rpm for 5 min. The supernatant was discarded and the cell pellet was collected in a sterile container. The pellet was resuspended in 100ml of sterile nutrient broth. The bacterial cell concentration was estimated using a Neubauer counting chamber. The concentrated bacterial suspension was subsequently used for seed treatment formulations.

Preparation of Bacterial Formulations for Seed Treatment

Two formulations, namely liquid and powder formulations, were prepared for seed coating. For the liquid formulation, 20 ml of bacterial cell concentrate at a concentration of 5×10^5 cells/ml was mixed with 0.5 ml of glycerol. Subsequently, 2 ml of polyethylene glycol and 0.1 g of xanthan gum were added to the mixture. The components were mixed thoroughly using a magnetic stirrer to obtain homogeneous formulation.

The powder formulation was prepared using a basal powder mixture containing 10 g of magnesium silicate and 2 g of calcium carbonate. Xanthan gum (0.1 g) was added to the basal powder mixture and mixed thoroughly. Twenty ml of bacterial cell concentrate at a concentration of 5×10^5 cells/ml was then incorporated

into the mixture and homogenized to obtain the bacterial powder formulation.

Seed Treatment of *Vigna mungo*

Healthy seeds of *Vigna mungo* were selected for the seed treatment. A total number of 100 seeds were washed twice with sterile distilled water and dried using sterile blotting paper. Fifty seeds were immersed in the liquid bacterial formulation for 10 min. The treated seeds were then placed on clean filter paper to remove excess content. The remaining 50 seeds were coated with the bacterial powder formulation by rolling the seeds in the powder and allowed to dry for 10 min under aseptic conditions.

Seed Germination Studies

Seed germination was evaluated using the Rolled Towel method. Whatman No. 1 filter paper was used to prepare the germination towels. The filter papers were folded into rectangular sections and 10 numbers of seeds from each treatment were uniformly placed on the surface of the folded paper. The paper was then rolled and the outer surface was covered with polythene to minimize moisture loss. The rolls were moistened periodically with sterile distilled water and maintained under suitable conditions for 3 days. Germination was monitored at 24 hours intervals. After 3 days of incubation, various parameters like number of germinated seeds, number of leaves produced and measurement of root and shoot length were observed. The roots and shoots were separated by trimming and wet weights of the roots and shoots were determined using pre weighed containers. For determination of dry weight, the plant materials were dried in a hot air oven and the final dry weights were recorded.

Greenhouse Growth Promotion Experiment

A greenhouse experiment was conducted to evaluate the effect of the selected bacterial isolate on the growth of *Vigna mungo*. Garden soil was collected and sterilized in a hot air oven at 160°C for 1 hour to eliminate indigenous microorganisms and spores. One kg of sterilized soil was transferred into each of three similar sized pots to establish three experimental treatments. The first pot served as the control in which untreated seeds were sown. In the second pot, the soil was amended with 100 ml of a 48 hours old bacterial culture and subsequently sown with untreated seeds. The second pot

was representing the soil application of the bacteria as biofertilizer. In the third pot, pretreated seeds with the bacterial binder formulation were sown to represent the bacterial seed coating treatment. All pots were maintained under greenhouse conditions with adequate sunlight and aeration and growth was monitored periodically for 10 days.

At the end of the 10 day experimental period, the plants were carefully harvested to minimize damage to the shoot and roots. Water was added to the pots to loosen the soil and inner peripheral soil was gently disturbed. The entire soil mass containing the plants was transferred into a larger container containing water. The soil was carefully dispersed in water and the plants were removed without damaging the root system. The following growth parameters like total plant length, shoot length, root length, shoot weight, root weight, number of leaves, number of primary roots, number of secondary roots and total fresh weight of the *Vigna mungo* plants were measured.

Results and Discussion

Isolation and Screening of Plant Growth Promoting Bacteria

A total number of 14 morphologically distinct bacterial isolates were obtained from compost enriched soil samples collected from different municipal dumping sites. The isolates were screened for plant growth promoting (PGP) traits specifically for phosphate solubilization and nitrogen fixation potential.

Among the 14 isolates, several isolates showed positive both characters but one isolate exhibited comparatively higher activity for both PGP traits (Figure 1 & 2). Therefore, the potent isolate was selected for subsequent studies.

The presence of multiple PGP characteristics in a single isolate is agriculturally significant because plant growth promotion is generally mediated through several complementary mechanisms rather than through a single bacterial trait. (Bakki *et al.*, 2024). PGP bacteria may improve plant nutrition through nitrogen fixation and phosphorus solubilization while also influencing root development through phytohormone production, siderophore synthesis, and other plant-microbe interactions (Díaz Rodríguez *et al.*, 2024).

Identification of the Potent Bacterial Isolate

Based on the conventional identification methods, the selected bacterial isolate found to be *Bacillus* sp. The molecular identification results showed that obtained 16S rRNA gene sequence consisted of 944 nucleotides in size (Genbank Accession number QB034211). BLAST analysis revealed 97% sequence similarity with *Bacillus stercoris* with 100% query coverage (Table.1). Based on the morphological, biochemical, and molecular characteristics, the isolate was identified as *Bacillus stercoris* and designated strain as AMC8. The identification of an isolate closely related to *B. stercoris* is noteworthy because recent studies have reported phosphate solubilizing and other plant growth promoting characteristics among *B. stercoris* isolates (Tripathi *et al.*, 2026).

Members of the genus *Bacillus* are widely investigated as PGP microorganisms because they can express several beneficial traits, including phosphate solubilization, phytohormone production, siderophore synthesis, nutrient mobilization, and suppression of phytopathogens (Díaz Rodríguez *et al.*, 2024). Previous studies have similarly demonstrated that bacteria belonging to the genera *Bacillus* and *Pseudomonas* can combine nutrient solubilizing properties with other PGP activities and consequently improve plant growth (Vitorino *et al.*, 2024).

Preparation and formulation of bacterial biomass for seed coating

The selected *B. stercoris* AMC8 strain was cultivated on a larger scale for biomass production and total cell density was determined as 18×10^{12} cells. The concentrated bacterial biomass was incorporated into two forms as liquid and powder formulations.

The liquid formulation exhibited a slightly oily consistency and dried rapidly on application to the seed surface. In contrast, the powder formulation exhibited a comparatively semisolid consistency with appreciable moisture content.

Microbial seed coating and biopriming have received increasing attention because these approaches allow beneficial microorganisms to be positioned close to the germinating seed and developing root system (Sasani *et al.*, 2026).

Figure.1 Phosphate solubilizing activity of Bacterial isolate in Pikovskaya's agar plates.



Figure.2 Nitrogen fixation activity of Bacterial isolate in nitrogen free Malate agar plates.

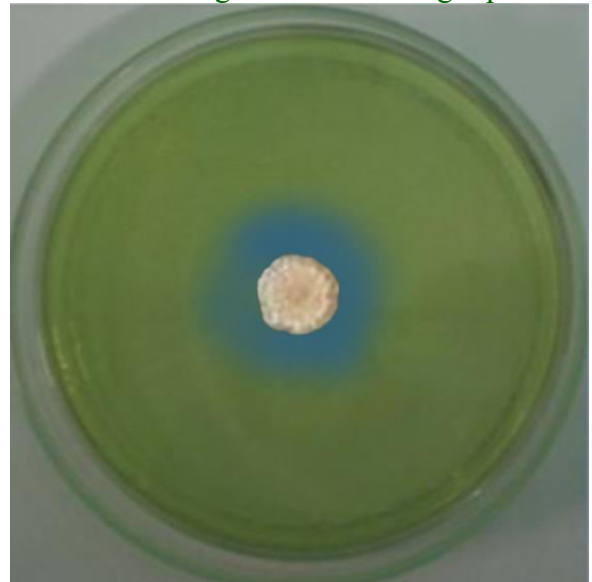


Figure.3 Seed Germination Study of *Vigna mungo*



1. Seed treated with powder formulation
2. Untreated seeds (Control)
3. Seed treated with liquid formulation

Figure.4 Green House Growth study of *Vigna mungo*



1. Plain soil (Control) 2. Seed inoculated with bacteria 3. Soil amended with bacteria

Figure.5 Shoot and root of *Vigna mungo* grown in green house



1. Growth in plain soil (Control) 2. Growth from seed inoculated with bacteria
3. Growth from soil inoculated with bacteria

Table.1 BLAST analysis of 16S rRNA gene of bacterial isolate.

Description	Scientific Name	Query Cover	Per. Ident	Accession
<i>Bacillus stercoris</i> strain HOAI_2 16S ribosomal RNA gene, partial sequence	<i>Bacillus stercoris</i>	100%	99.15%	PX363063.1
<i>Bacillus subtilis</i> strain M3C4 16S ribosomal RNA gene, partial sequence	<i>Bacillus subtilis</i>	100%	97.67%	OQ346200.1
<i>Bacillus</i> sp. (in: firmicutes) strain P39 16S ribosomal RNA gene, partial sequence	<i>Bacillus</i> sp. (in: firmicutes)	100%	97.35%	OQ381162.1
<i>Bacillus subtilis</i> strain BCRS_107 16S ribosomal RNA gene, partial sequence	<i>Bacillus subtilis</i>	100%	97.35%	ON680843.1
<i>Bacillus velezensis</i> strain AH3 16S ribosomal RNA gene, partial sequence	<i>Bacillus velezensis</i>	100%	97.35%	PZ626852.1
<i>Bacillus subtilis</i> strain JSS01 16S ribosomal RNA gene, partial sequence	<i>Bacillus subtilis</i>	100%	97.35%	PX471274.1
<i>Bacillus subtilis</i> strain CPM6 16S ribosomal RNA gene, partial sequence	<i>Bacillus subtilis</i>	100%	97.35%	PV715728.1
<i>Bacillus halotolerans</i> strain S1-group2_SFR-group2 16S ribosomal RNA gene, partial sequence	<i>Bacillus halotolerans</i>	100%	97.35%	PP990534.1
<i>Actinomycetes bacterium</i> strain RSA35 16S ribosomal RNA gene, partial sequence	<i>Actinomycetes bacterium</i>	100%	97.35%	PP956894.1
<i>Bacillus tequilensis</i> strain 59_3a 16S ribosomal RNA gene, partial sequence	<i>Bacillus tequilensis</i>	100%	97.35%	OP811573.1

Table.2 Seed germination studies of *Vigna mungo*.

Type	Parameters Analysed									
	Total no. of seeds planted	No. of seeds germinated	Percentage of germination	Shoot (Mean value of 5 plantlets)				Root (Mean value of 5 plantlets)		
				No. of leaves	Total Length (cm)	Wet weight (mg)	Dry weight (mg)	Length (cm)	Wet weight (mg)	Dry weight (mg)
Control	10	5	50	1.4	8	49.7	13.2	4.7	30.1	15.9
Powder formulation	10	9	90	2.6	10.8	58.4	31.7	7.4	46.3	25.5
Liquid formulation	10	9	90	2.0	10.2	55.5	28.6	6.8	45.2	24.7

Table.3 Greenhouse growth studies of *Vigna mungo*.

Pot types	Total weight of the plants (Mean of 5 plants) (gm)	Parameters of Shoot System of Plants (Mean value of 5 plants)			Parameters of Root System of Plants (Mean value of 5 plants)			
		Shoot Length (cm)	Shoot Weight (gm)	No. of leaves	Root length (cm)	Root Weight (gm)	Primary root (Nos.)	Secondary root (Nos.)
Control (Growth on Plain soil)	5.4	12.5	38.3	4.6	4.1	15.1	4	8
Growth on soil amended with <i>Bacteria</i>.	9.6	17.7	44.4	5.8	9.4	10.7	4	26
Growth from the seed inoculated with bacteria	8.7	16.2	42.3	5.0	7.7	9.8	4	22

Effect of Bacterial Seed Coating on Seed Germination and Seedling Growth

Seeds of *Vigna mungo* were coated with the liquid and powder formulations of *B. stercoris* AMC8 and subjected to germination under controlled environment. During germination, seed imbibition and swelling of the seed coat were observed during the first two days. Plumule emergence was observed on the third day followed by progressive shoot and root development up to the fifth day (Figure3). Germination percentage, number of germinated seeds, shoot length, root length, number of leaves and dry weight of shoots and roots were recorded (Table.2). The bacterial seed treatments produced an overall positive effect on germination and seedling development compared with the untreated control.

Among the two formulations, the powder formulation showed comparatively greater improvement in germination and seedling growth characteristics than the liquid formulation. The positive response observed in the present study may be associated with the combined PGP properties of the isolate. Phosphate solubilization can increase the availability of phosphorus required for energy metabolism, nucleic acid synthesis, membrane formation, and root development, whereas nitrogen fixing activity may contribute to nitrogen availability under suitable conditions. In addition, PGP bacteria can influence plant development through the production or modulation of plant hormones and other signaling compounds (Díaz Rodríguez *et al.*, 2024).

Greenhouse evaluation of plant growth promotion

The plant growth promoting ability of *B. stercoris* AMC8 was further evaluated under greenhouse conditions using *Vigna mungo*. Plants were maintained under three different treatments involving bacterial inoculation. Seed germination was observed within 48 hours after sowing and complete leaf development was observed by the third day. The plants were maintained for 10 days and growth related parameters were recorded (Figure. 4). Plants receiving bacterial treatment exhibited greater growth than the control plants (Figure.5). Furthermore, the soil inoculation treatment produced greater growth promotion than the seed inoculation treatment (Table.3). This indicated that application of bacterial isolate directly to the soil was comparatively more effective under the conditions tested.

The response of plants to bacterial inoculation under greenhouse conditions provides important evidence of the functional potential of the isolate. However, in vitro PGP characteristics do not necessarily guarantee equivalent performance under soil or field conditions because bacterial activity can be affected by soil properties, indigenous microbial populations, temperature, moisture, plant genotype, and nutrient availability. Therefore, greenhouse evaluation represents an important intermediate step between laboratory screening and field level validation (Raphael and Parthasarathi, 2026).

Overall, the study results demonstrated that the *Bacillus stercoris* AMC8 possessed Plant Growth Promoting potential and its application through either seed inoculation or soil inoculation enhanced the early growth and further development of *Vigna mungo*.

Author Contributions

Arulpandi I: Work designing and ideology behind the work, Scientific preparations. Asha. M: Lab work of experimentation part. Kanimozhi. S: Work designing and ideology behind the work, Scientific preparations.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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