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BIOSTIMULATORY ROLE OF *BACILLUS AMYLOLIQUEFACIENS* AUPPB02 IN BARLEY: FROM *IN-VITRO* CHARACTERIZATION TO POT TRIALS

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ABSTRACT

Plant growth promoting bacteria (PGPB) are an assemblage of specific kind of microbes which, reside in or around the plants in soil. These PGPB secrete specialized metabolites and influence to elevate the development of plants, through direct mobilization of unavailable mineral elements, including nitrogen, phosphorus and potassium. They are also involved in bioaugmentation of toxic metals, namely Zn, Co, Pb and Mn. *Bacillus* sp., *Pseudomonas* sp., *Rhizobium* sp. are some common examples of PGPB. While, investigating the plant growth promoting (PGP) activities, qualitatively and quantitatively, the *Bacillus amyloliquefaciens* AUPPB02 (OR187307.1) was revealed as a potent plant growth promoter. As per its qualitative evaluation, AUPPB02 showed the potentials to fix atmospheric N_2, solubilize P and K, produce IAA, siderophore and CMC by forming their characteristic halo zones of 20.5 mm, 7 mm, 17 mm, 16.5 mm, 22 mm and 21.75 mm, in respective manner. The production of HCN, ammonia and gibberellic acid (GA) was confirmed by change in color from yellow to brown, yellow to reddish brown and green to bluish green, respectively. It also presented significant results for bioaugmentation of toxic metals (Zn, Co, Mn and Pb). Quantification of PGP activities was done by preparing a standard curve, as per the established scientific protocols, implying absorbance verses concentrations of respective variables. It revealed N, P, IAA, GA, NH_3 and siderophore production to be 2.54 μgml^{-1}, 53.34 mgl^{-1}, 12.86 μgml^{-1} (in Trp^-) and 16.82 μgml^{-1} (in Trp^+), 577.41 μgml^{-1}, 46.40 μgml^{-1} and 77.69 SU%, respectively. Seeds of *Hordeum vulgare* L., when inoculated with AUPPB02, it greatly stimulated the seed's growth in terms of root (27.01%) and shoot (8.4%) length while, fresh weight, dry weight and biomass were escalated by 17.58%, 16.66% and 17.72%, respectively, as compared to the control. Therefore, PGPB can alleviate the consequences of chemical fertilizer usage, as their implementation in the form of microbial fertilizer can promote the development of soil microflora without causing any adverse effects.

***Keywords*:** PGPB, PGP activities, *Bacillus amyloliquefaciens* AUPPB02, bioaugmentation, *Hordeum vulgare* L.

Introduction

Approximately one out of ten individuals globally doesn't have sufficient food to maintain a healthy and active lifestyle (Kousar *et al*., 2021). Ensuring sufficient food for an expanding population has been a challenge since a long time. This issue remains unsolved as the global population continues to rise (Qaim, 2020). It is a big challenge to meet the food demands of the increasing population. Urbanisation is on the rise and people are switching to urban development rather than focusing towards agricultural production which, in turn raises the level of food insecurity (Kousar *et al*., 2021). To solve this problem, the Indian government launched Green Revolution in 1965, which marked a new era in the nation's agricultural growth. It was a time of momentous agricultural success in India (Stepha, 2022). It enhanced crop growth and yield by introducing high yielding variety (HYV) seeds, multiple cropping system, proper irrigation techniques and use of pesticides along with chemical fertilisers. Any ingredient either of synthetic or natural origin, which

when introduced to soil provides most of the essential elements like macronutrients - nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S) and micronutrients – iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), boron (B), nickel (Ni), chlorine (Cl) and molybdenum (Mo), which are vital for plant growth and development is known as a fertilizer (Das *et al*., 2023). As the pros of green revolution are many but, associated with it are some cons too. Chemical or synthetic fertilizers when applied to soil stimulate plant growth by providing all the essential nutrients to plants but excessive use of these fertilizers deteriorates soil fertility by increasing acidity of soil (Pundhir & Kumar, 2024). To overcome the harmful impacts of chemical fertilisers, biofertilizers were introduced. They are the formulations of live microorganisms, which assist in enhancing soil fertility by fixation of atmospheric nitrogen, solubilisation of phosphorus, decomposing organic wastes and synthesising plant growth hormones through their biochemical activities (Okur, 2018).

Plant Growth Promoting Microorganism (PGPM) are an exclusive type of microbes, which nurture plant growth, suppress plant diseases and regulate abiotic stresses (Dhawi and Hess, 2017; Ma *et al*., 2020; Pratush *et al*., 2018). They include species of fungi, like *Aspergillus, Fusarium, Penicillium, Trichoderma* (Hossain *et al*., 2017) and many of cyanobacterial species, like *Anabaena, Aulosira, Nostoc, Tolypothrix* etc. are also prominent examples of PGPM (Chatterjee *et al*., 2017). Other than these microbes, Plant growth promoting bacteria (PGPB) are a diverse and advantageous group, which inhabit the rhizosphere, reside in the soil, having the ability to stimulate plant growth and development (Dimkpa *et al*., 2009; Grover *et al*., 2011; Glick, 2012). The PGPB may facilitate plant growth, through their own metabolism, which includes fixing nitrogen, solubilizing phosphates and synthesising hormones. They have immediate impact on plant metabolism by increasing water and mineral absorption. These organisms enhance root development, boosts plant's enzymatic activity and assist other favourable microorganisms to increase their action on the growth of plants and suppress the action of plant pathogens (Pérez-Montaño *et al*., 2014; Bhanse *et al*., 2022; Vocciante *et al*., 2022). Different PGPB species including, *Azotobacter, Azospirillum, Bacillus, Enterobacter, Klebsiella, Pseudomonas, Serratia* and *Variovorax* have been examined and marketed since a long time because of their positive impact on crop yield (Glick, 2012). Indirect methods by PGPB encompasses production of ACC deaminase, antibiotics, enzymes that degrade cell walls, competition, hydrogen cyanide, induced systemic resistance, quorum quenching and siderophores (Balogh *et al*., 2010; Frampton *et al*., 2012).

The aim of the present study is to solve the challenge of excessive use of chemical fertilisers and promote the use of bacteria as biofertilisers, to alleviate the consequences caused by synthetic fertilisers. Therefore, the objectives of the present investigation are (i) Qualitative screening for different plant growth promoting (PGP) activities (ii) Quantitative estimation for PGP activities (iii) Application of organism on barley (*Hordeum vulgare* L.) to assess the growth of seedlings.

Materials and Methods

Materials

All the chemicals used in the present investigation were of analytical grade and were purchased from Sigma, Merck, HiMedia and SRL India.

The bacterial strain i.e., *Bacillus amyloliquefaciens* AUPPB02 (OR187307.1) used in this piece of work was earlier isolated, characterised from fermented lentil seeds (Mishra *et al*., 2024), obtained from Microbial Biodiversity Laboratory, Department of Botany, Patna University, Patna, Bihar, India.

Screening For Different Plant Growth Promoting (PGP) Activities

The studied organism was tested for different PGP traits like nitrogen fixation, solubilization of phosphate and potassium, indole-3-acetic acid (IAA), gibberellin (GA), ammonia (NH_3), siderophore, hydrogen cyanide (HCN), carboxymethyl cellulase (CMC) production and solubilisation of heavy metals (zinc, manganese, cobalt and lead).

Test for nitrogen fixation

Nitrogen (N_2) fixation ability was determined by dot inoculation of the bacterial inoculum on nitrogen free malate agar media, added with bromothymol blue (BTB), as an indicator. The plate was incubated for 3-4 days at 30 ± 2°C. Appearance of blue color zone on agar plate, indicated N_2 fixation (Gothwal *et al*., 2008). The method described by Cordova-Rodriguez *et al*. (2022) was utilized for the quantitative assessment of N_2 fixation. Bacterial strain was cultured in NFb medium with bromothymol blue (BTB) serving as the indicator and was incubated at 30 ± 2ºC in a shaker cum incubator (Rivotek) at 150 rpm for 48 h. The culture broth was centrifuged for 10 min at 5,000 rpm after incubation. Subsequently, the supernatant was

collected and absorbance was measured at 610 nm, using UV-VIS spectrophotometer (Systronics, 119). The uninoculated NFb medium served as reference. The amount of N_2 produced was determined by plotting the standard curve of ammonium hydroxide (NH_4OH).

Test for phosphate solubilization

Evaluation of the bacterial isolate for phosphate (P) solubilization was done by using Pikovskaya's agar media with tricalcium phosphate. The studied organism was dot inoculated on agar plate and incubated at 30 ± 2°C for 5 days. Presence of clear halo zone around the growing colony illustrated P solubilization (Nautiyal, 1999). Quantitative estimation of P solubilization was done by culturing 10 µl of 48 h old bacterial culture (10^{-7} CFU ml^{-1}) in nutrient broth. The solution was transferred into a 250 ml Erlenmeyer flask containing 50 ml of PVK broth and incubated at 30 ± 2°C in a shaker cum incubator at 150 rpm for 5-6 days. After incubation, the bacterial suspension was centrifuged at 10,000 rpm for 10 minutes. The pellet was discarded, and the amount of phosphorus solubilized in the supernatant was quantified using the chlorostanous reduced molybdophosphoric acid blue method (Jackson, 1967). 1 ml of supernatant was mixed with 10 ml of chloromolybdic acid. Subsequently, 0.25 ml chlorostannous acid was added and the final volume was made to 50 ml with DDW. The supernatant containing phosphate, develops a blue color and absorbance was recorded at 610 nm using a UV-VIS spectrophotometer. The quantity was estimated by referring the blank and using the standard curve of potassium dihydrogen orthophosphate.

Test for potassium solubilization

To assess potassium (K) solubilization, the bacterial inoculum was line and dot inoculated on Aleksandrow's agar plate along with 1% bromothymol blue (BTB) as indicator (Parmar and Sindhu, 2013). Plate was incubated at 30 ± 2°C for 8 days. Appearance of yellow coloured zone around the bacterial colony indicated K solubilization.

Test for indole acetic acid production

This assessment is conducted to check the transformation of trypytophan into indole (IAA) by the bacterial isolate. Following the method of Gordon and Weber (1951), the qualitative test for IAA was conducted, by preparing tryptone agar plate with 100 µg ml^{-1} of tryptophan. After solidification, a sterile cork borer was used to create a well of approximately 1-2 cm in diameter and 0.5 cm in depth. Then 0.1 ml of overnight grown culture was added to the well and the plate was incubated at 30 ± 2°C. Following an overnight growth period, 0.2 ml of Salkowaski reagent was added into the well, resulting in the formation of a pink zone surrounding the bacterial colony. According to the Salkowaski method (Gordon and Weber, 1951), the isolate was cultivated in tryptone broth, with 1 gl^{-1} tryptophan (Trp^+) and without tryptophan (Trp^-), maintaining the pH at 5.8. The broth solution was incubated at 30 ± 2°C in a shaker cum incubator (150 rpm) for 3-4 days. Following incubation, the culture was centrifuged at 10,000 rpm for 30 minutes. After that, 1 ml of the supernatant was mixed with 2 ml of Salkowaski reagent. The resulting mixture was left at room temperature for 25 min and absorbance was measured using a UV-VIS spectrophotometer at a wavelength of 540 nm. The quantity was estimated by using the standard curve of IAA, referring the blank.

Test for gibberellin production

To check the GA production, 10 ml tryptone broth (TB) was taken in a test tube/ McCartney bottle, inoculated with 24 h old bacterial culture and incubated at 30 ± 2°C for 2 days. Following the incubation period, 1 ml of Folin- Ciocalteu reagent was added and left for 5 minutes. The appearance of greenish-blue color indicated the presence of GA (Graham and Henderson, 1961). The bacterial strain was introduced in TB media and incubated in a shaker cum incubator at 30 ± 2°C for 24 h at 150 rpm, in order to quantify its ability to produce GA. Following incubation, the culture was centrifuged at 10,000 rpm for 5 min. The supernatant was collected in a test tube and 1 ml Folin- Ciocalteu reagent and 1 ml concentrated HCl was added to it. After 5 min of boiling in a water bath, it was left to cool at room temp. (Graham and Henderson, 1961). Absorbance of greenish blue color was measured at 760 nm using a UV-VIS spectrophotometer. The quantity was estimated by referring the standard curve of GA_3 in the range of 10-100 µg ml^{-1}, along with blank.

Test for ammonia production

Ammonia production ability was illustrated by inoculating the studied organism in 10 ml of 4% peptone broth, followed by incubating it for 48-72 h at 30 ± 2°C. Then 0.5 ml of Nessler's reagent was added. Appearance of yellow to brown color signified ammonia production, whereas no color indicated negative test for ammonia production (Cappuccino and Sherman, 1992). The amount of NH_3 produced by each strain was examined quantitatively. For this, 10 ml peptone broth was used to inoculate the 24 h old bacterial cultures, which was then incubated for 48 h at 30 ± 2°C. The culture was then centrifuged for 10 min at 10,000 rpm. Test tubes were filled with 0.5 ml of the supernatant, 1 ml of freshly prepared Nessler's reagent

and left at room temp. for 10 min. The liquid was diluted 6 times to attain final volume of 9 ml (Cappuccino and Sherman, 1992). Change in color from yellow to reddish brown indicated formation of NH_3. Absorbance was measured at 450 nm using a UV-VIS spectrophotometer. The reference was the uninoculated combination. In the range of 10 and 100 µg ml^{-1}, a standard curve was plotted.

Test for siderophore production

For siderophore production, 24 h old bacterial isolate was dot inoculated on modified chrome auzoral sulphonate agar plate following the method of Schwyn and Neilands (1987). Agar plate was incubated at 30 ± 2°C for 24 to 72 h. Development of orange zone on the media indicates siderophore production. Siderophore production was quantitatively assessed using the CAS shuttle assay (Schwyn and Neilands, 1987). The isolates were cultured in succinate medium and incubated at 30 ± 2°C in a shaker cum incubator at 150 rpm for 24 hours. The cultured broth was then centrifuged at 10,000 rpm for 10 minutes, while maintaining a temperature of 4°C. An aliquot of 0.5 ml of the supernatant (cell-free extract) was mixed with 0.5 ml of CAS solution. After 20 min of incubation period, the resulting color was analysed using a UV-VIS spectrophotometer at a wavelength of 630 nm, with the uninoculated CAS solution serving as the reference. The alteration in the CAS color was used to calculate the percentage of siderophore units (SU%) using the given formula:

$$SU\% = [(Ar - As)/ Ar] \times 100$$

where,

SU%: percentage of Siderophore Units; Ar: absorbance of reference (CAS assay solution + uninoculated media) and As: absorbance of the sample (CAS assay solution + cell-free supernatant).

Test for hydrogen cyanide production

HCN production was determined by the method of Bakker and Schippers (1987). For this, nutrient agar plate was prepared along with 4.4 gl^{-1} glycine. Bacterial isolate was dot inoculated on the media plate. A Whatman no. 1 filter paper, dipped in picric acid solution (0.5%) was placed on the underside of petri dish lid. After that, it was sealed with parafilm and incubated at 30 ± 2°C for 6 days. Picric acid, in the presence of sodium carbonate (Na_2CO_3) reacts with HCN, resulting in color change of the filter paper from deep yellow to orange brown, light brown and finally dark brown, indicating weak, moderate or strong cyanogenic potential, respectively. If the filter paper maintains its deep yellow color, even after the growth of bacteria, the test was declared negative.

Test for CMC production

Cellulase production ability was determined by dot inoculation of isolate on Czapek agar media and incubating the plate at 30 ± 2°C for 2-5 days. The plate was then flooded with 0.1% of Congo red, followed by destaining it with 1M NaCl for 10-15 mins. Appearance of clear zone around the bacterial colony confirms degradation of CMC by synthesis of extracellular enzyme, i.e., cellulase (Hankin and Anagnostakis, 1977).

Test for heavy metals

The ability of the bacterial isolate to solubilize heavy metals (zinc, manganese, cobalt, lead) was tested on nutrient agar plates amended with 0.01% salt of the respective heavy metal. Plates were incubated at 30 ± 2°C for 8 days. Appearance of clear halo zone around bacterial colony indicated solubilization of heavy metals (Aleem *et al*., 2003).

Assessment of Growth Promoting Characteristics of Bacteria on Barley

To evaluate the impact of *Bacillus amyloliquefaciens* AUPPB02 (OR187307.1) on plant development, *Hordeum vulgare* L. (barley) was chosen as the experimental crop. Seeds of *Hordeum vulgare* were procured from a local market of Boring Road, Patna, Bihar.

Preparation of bacterial suspension

The bacterial strain was cultured in broth medium in a shaker cum incubator at 150 rpm for 4-5 days at a temperature of 30 ± 2°C, then centrifuged for 10 minutes, after which, the pellet was re-suspended in normal saline solution. Cell density of the bacterial suspension used for seed inoculation was determined through the cell count method, using a haemocytometer and dilution plate technique. Subsequently, the bacterial suspension was diluted to achieve a final concentration of 10^8 CFU ml^{-1}.

Seed sterilization and seed germination bioassay

Sterilization of seeds was done by immersing them in 95% ethanol for 3-4 seconds and then in 0.2% solution of $HgCl_2$ for 3 minutes, followed by multiple rinses with sterile DDW, to eliminate the traces of remaining sterilant, if any (Russel *et al*., 1982).

Seed germination experiment was performed using paper towel method (Raj *et al*., 2004). Appropriate number of thoroughly sterilized seeds were inoculated with bacterial strain by soaking them in bacterial suspension for 30 minutes and were left to

dry at room temperature for 1 h. The control seeds were soaked in sterile DDW. Seeds treated with bacterial culture were placed in separate petri dish with three replicates for each. A sterilized filter paper was placed over all the seeds and moisture level was maintained, by adding 10 ml sterile DDW to each petri dish. Then, at an interval of 2 days, bacterial broth culture was re-applied to seeds. Seeds were incubated for 7 days at 30 ± 2°C. Average lengths of radicle and plumule were calculated and through that vigor index was calculated, referring the formula (Abdul - Baki and Anderson, 1973), as follows:

Vigor index = (Mean plumule length + Mean radicle length) x germination %

where

Germination % = (Total no. of seed germinated/total no. of seeds) x 100

Pot experiment

Pot experiments were performed in the glass house of Department of Botany, Patna University. For this, farm soil was collected, allowed to air dry, sieved (2 mm) and tyndallised before filling the pots. Pots (diameter of 9 cm) were first sterilised with 0.2% $HgCl_2$.

Appropriate number of pre sterilized and pre inoculated seeds (05) were sown, in each pot, keeping control pot as uninoculated. To reduce air contamination, a thin layer of sterile sand and soil was spread over the pot and it was regularly watered with 10 ml sterile DDW. Similarly, as a supplemental dose, 2 ml of bacterial inoculum was added to the inoculated pots after every 7-8 days. After that, the pots were exposed to 16/8 h of light/dark phase for 15 days. The plant seedlings were then carefully removed and a number of variables, including root length, shoot length and % increase in biomass were measured, calculated and statistically examined.

Sampling and processing

For sample processing, the plant samples were collected after 15 days. To preserve the roots, soil stuck to it was carefully rinsed away. An electronic balance was used to record the fresh weight of the entire plant. Then, it was air dried for 48 h at 72ºC in an oven and dry weight was determined (Vázquez *et al*., 2000). By positioning a thread and ruler, next to the entire plant and taking 5 arbitrary readings (in cm), shoot and root length, were determined. Total biomass was calculated after subtracting dry weight from fresh weight.

Results

Qualitative assessment

The qualitative assessments of the bacterial strain, *Bacillus amyloliquefaciens* AUPPB02 (OR187307.1) showed positive results for different plant growth promoting activities, with zones measuring for each, as 20.5 mm for N_2 fixation, 7 mm and 17 mm for P and K solubilization, respectively. For the production of IAA, siderophore and CMC, the zones were as 16.5 mm, 22 mm and 21.75 mm, respectively. Also, appearance of yellow to dark brown color and greenish blue color was observed, which demonstrated NH_3 and GA production, respectively. HCN production was confirmed by color change of filter paper from yellow to brown. The strain also proved to be a resilient heavy metal solubilizer, as affirmative results were observed for Zn, Co, Mn and Pb solubilization.

Quantitative estimation

N_2 concentration was determined quantitatively, using standard curve of nitrogen (R^2 = 0.984). To calculate the concentration of phosphate, standard curve of phosphate was prepared (R^2 = 0.9869). IAA concentration was evaluated by standard curve of indole-3-acetic acid (R^2 = 0.9988), whereas GA concentration was assessed by plotting standard curve of gibberellic acid (R^2 = 0.9903). Quantification of ammonia was done by plotting standard curve of ammonia (R^2 = 0.9934) whereas, amount of siderophore present was estimated by calculating SU%. N_2 concentration was found to be 0.82 µg ml^{-1}, P concentration was 53.34 mgl^{-1}, that of IAA was 12.86 in Trp^- and 16.82 µg ml^{-1} in Trp^+ (it was higher in Trp^+ due to presence of L-tryptophan), GA as 577.41 µg ml^{-1} and ammonia as 46.40 µg ml^{-1}. For siderophore, the SU% was observed to be 77.69 SU%.

Seed germination assay

Following qualitative and quantitative screening, the bacterial strain was further evaluated for its ability to promote plant growth in seeds of *Hordeum vulgare* L. Significant increase in germination percentage and vigor index was observed after watering the seeds with bacterial inoculum. Radicle and plumule lengths were found to be 0.8 cm and 3.8 cm, respectively. Germination percentage was 48% and vigor index was calculated to be 220.8. This was more in comparison to control where, radicle and plumule length were 0.5 cm and 1.5 cm, germination percentage and vigor index were 30% and 60, respectively.

Pot experiment

Pot experiment is economical and flexible method for assessment of various applications. This experiment

helps to demonstrate how the establishment of plant growth promoting bacteria can enhance the biomass and yield of any plant. The findings of this study show that inoculating seedlings of *Hordeum vulgare* L. with bacterial strain has a beneficial impact on all evaluated growth parameters including root length, shoot length, fresh weight and dry weight, after 15 days of treatment (Fig 1). Significant enhancement was noted in root length (12.32 cm), shoot length (16.50 cm), fresh weight (1.07 gm), dry weight (0.14 gm) and biomass (0.93 gm) whereas, in control the results, obtained were, as 9.7 cm (root length), 15.22 cm (shoot length), 0.91 gm (fresh weight), 0.12 gm (dry weight) and 0.79 gm (biomass).

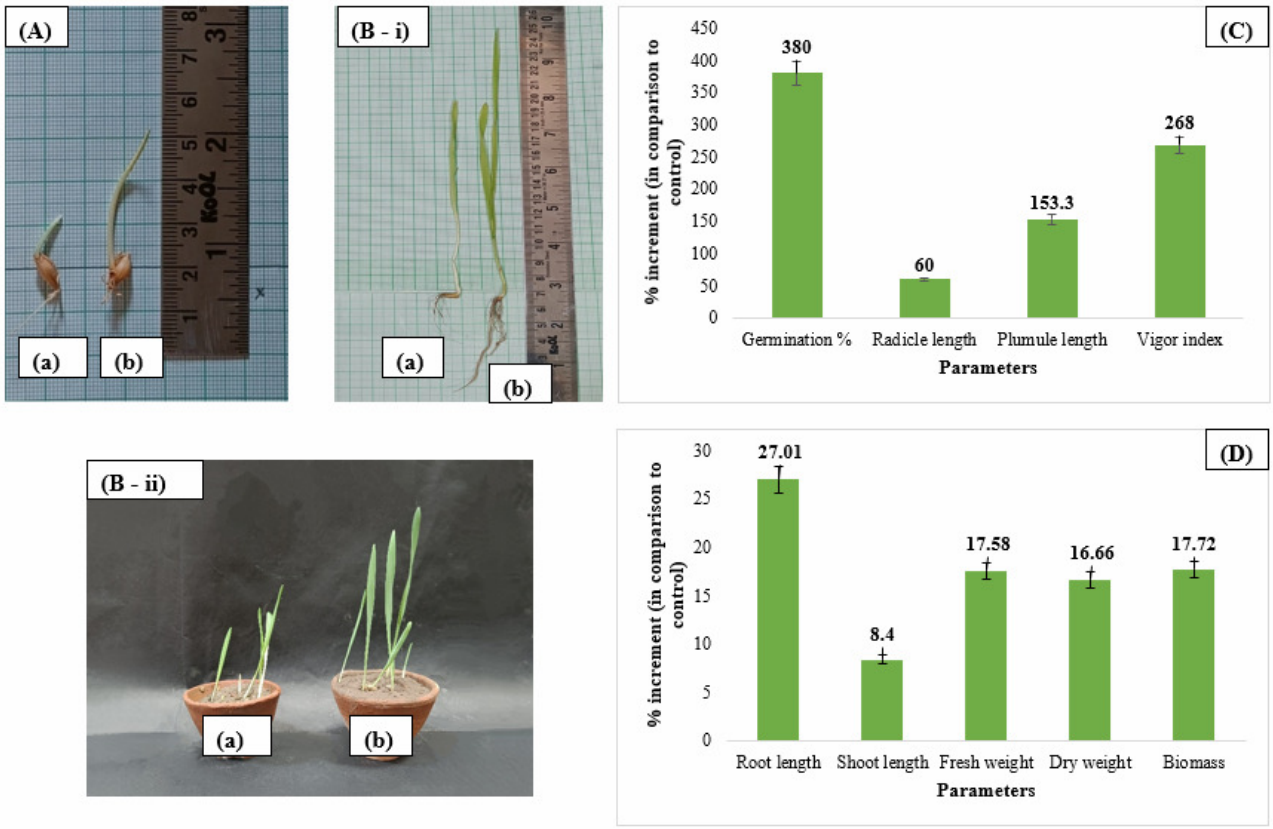

Fig 1: (A) Effect of AUPPB02 on seed germination of *Hordeum vulgare L.* with respect to control - (a) Control and (b) AUPPB02; (B) Effect of AUPPB02 on *Hordeum vulgare L.* (a) Control and (b) AUPPB02 after 15 days of treatment; (C) Impact of AUPPB02 on seed germination of *Hordeum vulgare* L. and (D) Impact of AUPPB02 on growth of *Hordeum vulgare* L.

Discussion

To determine plant growth promoting traits, researchers perform qualitative and quantitative tests to evaluate microorganisms for their positive effects. Different PGP traits associated with PGPB are N_2 fixation, P and K solubilization, siderophore production (Barbaccia *et al*., 2022). Through this study, it was found that AUPPB02 fixed atmospheric N_2 (20.5 mm), solubilized P (7 mm) and K (17 mm). Similar to our result, *B. amyloliquefaciens* RHFS18 exhibited halozone in the range of 5-10 mm on PVK media as reported by Castaldi *et al*. (2021). As per report of Rajkumar *et al*. (2024) *B. amyloliquefaciens* LAS10 quantified P at a concentration of 155 µg ml^{-1} which, was much higher than our findings. For K solubilization, similar result was observed by Luo *et al*. (2022) in *B. amyloliquefaciens* B9601-Y2. Our strain produced IAA with halozone of 16.5 mm. In the respective reports, Shao *et al*. (2015); Jamali *et al*. (2018) in *B. amyloliquefaciens* SQR9 and *B. amyloliquefaciens* AH53 also showed the ability to produce IAA. Also *B. amyloliquefaciens* cha43 quantified 22.20 ± 2.61 µg ml^{-1} IAA in Trp^{+} and 29.44 ± 2.93 µg ml^{-1} IAA in Trp^{-} (Lotfi *et al*., 2022) while, *B. amyloliquefaciens* AH53 quantified 125.24 ± 1.14 µg ml^{-1} IAA (Jamali *et al*., 2018). AUPPB02 showed the appearance of greenish blue color for GA production, also *B. amyloliquefaciens* MBNC showed similar results. AUPPB02 produced 577.41 µg ml^{-1} GA whereas, contrary to our finding, *B.*

amyloliquefaciens MBNC produced 35.15 µg ml^{-1} GA under neutral conditions and 26.66 µg ml^{-1} under acidic conditions (Chowdhury *et al*., 2022). As per *B. amyloliquefaciens* MH046937, exhibiting positive results for NH_3 production (Ashour *et al*., 2021), our bacterial strain also showed the appearance of yellowish brown color. The findings of Rajkumar *et al*. (2024) reported, *B. amyloliquefaciens* LAS10 to produce 5.2 ± 0.32 mg ml^{-1} NH_3, whereas AUPPB02 produced much less amount of NH_3 i.e., 46.40 µg ml^{-1}. Our strain produced siderophore with zone of 22 mm. In this context, similar optimistic results were reported by Ji *et al*. (2021) in different strains of *B. amyloliquefaciens* FZB42, B9601-Y2, Ba13 and UCMB5113. SU% for AUPPB02 was 77.69% while, Raut *et al*. (2021) observed 24.34 SU% in *B. amyloliquefaciens* RLS19 which, was much lower. AUPPB02 demonstrated HCN production by change in color of filter paper from yellow to brown and CMC production with a zone of 21.75 mm, respectively, comparing with an adequate HCN production in *B. amyloliquefaciens* MH046937 by Ashour *et al*. (2021). In accordance to our strain AUPPB02, Bhatt *et al*. (2024) also reported *B. amyloliquefaciens* OKB3, having potential of CMC production.

Illustrating bioremediation traits, AUPPB02 solubilised Zn, Mn, Co and Pb. In favour of our result, Zn, Mn, Co and Pb solubilization was detected in *B. amyloliquefaciens* Bam1 by Luo *et al*. (2022). According to report of Biswas *et al*. (2024) *B. amyloliquefaciens* MEBAphL4 was proved to be Pb solubilizer. *B. amyloliquefaciens* BAB-807 exhibited strong potential for bioaugmentation of Cd, Pb, Cu, Ni and Cd (Jayam and Sharmila, 2025).

Phytohormones play an important role in elevating the growth of plants. Our findings demonstrated significant production of IAA and GA by *B. amyloliquefaciens* AUPPB02. Apart from this, macronutrients like N, P and K are also essential for plant development, through the production of which, AUPPB02 positively directed the growth of *Hordeum vulgare* L. seedlings. Root and shoot lengths were increased by 27.01% and 8.4%, in respective manner, while fresh weight, dry weight and biomass were increased upto 17.58%, 16.66% and 17.72%, respectively. As reported by Liu *et al*. (2025) *B. amyloliquefaciens* Q1 under 0.16 M salinity condition, positively elevated the shoot length by 18.1% and dry weight by 23.3%, in barley seedlings, after 10 days of inoculation, which surpassed our findings. Introducing seeds with inoculum of *Bacillus* sp. depicted similar results in lettuce plants (Arkhipova *et al*., 2007).

Conclusion

The present study was associated with qualitative as well as quantitative analysis of plant growth promoting activities of the bacterial strain, i.e., *Bacillus amyloliquefaciens* AUPPB02 (OR187307.1). Qualitative screening revealed that it is a potent plant growth promoter, as it demonstrated positive results for N_2 fixation, P and K solubilization, production of IAA, GA, NH_3, siderophore, HCN and CMC. It presented optimistic results for bioremediation of heavy metals like Zn, Co, Mn and Pb. The outcomes of quantitative estimation also proved it to be an effective plant growth promoting bacteria (PGPB), as significant amount of N_2, P, IAA and siderophore was produced. By further employing it to test the growth of *Hordeum vulgare* L. seedlings, remarkable results were obtained, as it substantially enhanced the germination percentage and vigor index of the crop seed.

Application of PGPB stimulate crop growth and yield by supplying all the essential nutrients to crops, promoting the establishment and growth of beneficial soil microflora in the plant's rhizosphere. It solves the problem of bioremediation of heavy metals and prevents plant diseases by eliminating the harmful effects of phytopathogens. Due to their potential to demonstrate antimicrobial activity, PGPB can be used as biocontrol agents for crop protection. Also, they are environment friendly and are of low cost. Therefore, due to multiple advantages of PGPB over chemical fertilizers, it can be applied to stimulate plant growth and development.

Declarations

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Conflict of Interest : The authors declare that there is no conflict of interest.

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Declaration of Competing Interest : We declare that we have no conflict of interest with any parties whatsoever.

Ethics Statement : This article does not contain any studies on human participants or animals performed by any of the authors.

Data Availability : All datasets generated or analysed during this study are included in the manuscript.

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