



ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(3): 1829-1835
© 2022 TPI

www.thepharmajournal.com

Received: 13-12-2021

Accepted: 21-02-2022

Hemant Dasila

Department of Microbiology,
G.B. Pant University of
Agriculture and Technology,
Pantnagar, India

VK Sah

Department of Agronomy, G.B.
Pant University of Agriculture
and Technology, Pantnagar,
India

Vandana Jaggi

Department of Microbiology,
G.B. Pant University of
Agriculture and Technology,
Pantnagar, India

Manvika Sahgal

Department of Microbiology,
G.B. Pant University of
Agriculture and Technology,
Pantnagar, India

Phosphate solubilizing bacteria (PSB) a potential tool to enhance soil health and wheat vigor parameters in pot trial experiment

Hemant Dasila, VK Sah, Vandana Jaggi and Manvika Sahgal

Abstract

In the present study, attempts were made to Analyze the effect of 3 Phosphate solubilizing bacteria (PSB) recovered from forest ecosystem of Pantnagar, India on wheat plant vigor. For this, a pot trial in a Completely Randomized Design (CRD) with a superior wheat genotype (CBW 38) was carried out in glass house conditions. The response was measured in terms of two soil enzyme activities e.g. fluorescein di acetate (FDA) hydrolysis and alkaline phosphatase (AP). Plant health was measured in form of plant vigor parameters (shoot length, root length, shoot fresh weight, root fresh weight and grain yield). All three PSB strains showed positive for phosphorous and zinc solubilization activity as well as FDA and AP enzyme activities. During pot trial it was also observed that PSB strains promoted development of wheat plant thereby enhancing the crop yield. Among the three PSB strain, *Pseudomonas putida*-P3 was found to be most effective and show maximum phosphate (3.4 cm); zinc (3.0 cm) and inorganic phosphate ($72.48 \mu\text{g mL}^{-1}$) solubilisation index with a drop of pH to 4.6 along with maximum FDA (78.6) and AP ($62.6 \mu\text{g mL}^{-1} \text{h}^{-1}$) activity. All these parameters lead to increase in crop yield from. 24 Kg ha^{-1} (under uninoculated conditions) to 2.4 Kg ha^{-1} in P3 inoculated crop. Therefore *Pseudomonas putida*-P3 could be developed as potential bioinoculant.

Keywords: Genotype, soil enzyme, grain yield and completely randomized design (CRD)

Introduction

Phosphorus (P) is considered as one of the essential macronutrient and part of important structural element such as nucleic acid (DNA and RNA) and adenosine tri phosphate (ATP) which is considered as energy currency of cell. P is also involved in regulating protein synthesis and energy transfer. In plants P plays very important role in root development and thus improves yields of crops [4]. Soil deficient in P limits the plant growth and causes reduction of upto 15% in crop yield [18]. The main cause P deficiency in plants is due to its unavailability in soil as plants takes up P in form of primary (H_2PO_4^-) and secondary orthophosphate (HPO_4^{2-}) and their retention in soil is so high that most of the time they are unavailable to plants. To address soil P unavailability in soil input of inorganic fertilizer in soil either alone or in combination increases which up to some extent increase crop growth and productivity but with passing time P use efficiency (PUE) of crops is reduces significantly due to fixation of P into soil cations. For example, P fertilizers rapidly react with some divalent cations such as iron (Fe), aluminium (Al) and calcium (Ca) to form insoluble mineral form of P [3]. Hence to improve PUE in terms of crop yield and nutrient uptake remains a big challenge. Considering this, the role of promising phosphate solubilizing microorganisms (PSM) in mitigating soil p unavailability becomes very prominent. PSB posses the potential to solubilize immobilized form of inorganic or organic P into most available form to plants in an eco-friendly manner. PSM inoculation is reported to enhanced mineralization or solubilization of immobilized P in soil [18]. About 1-50% of bacterial and 0.1-0.5% of fungal communities posses p solubilization potential [11]. Amongst PSM major group of phosphate solubilizing bacteria (PSB) includes *Agrobacterium spp.*, *Pseudomonas spp.*, *Azotobacter*, *Bacillus circulans*, *Bradyrhizobium*, *Burkholderia*, *Erwinia*, *Kushneria*, *Paenibacillus*, *Ralstonia*, *Rhizobium*, *Rhodococcus*, *Serratia*, *Salmonella*, *Sinomonas*, and *Thiobacillus* [18; 23]. PSB group like *Rhizobium* and *Pseudomonas* when use individually or in combination reported to increase P solubilization rate which resulted in higher crop yield [17]. One of the most important mechanisms that is involved in P solubilization is proton (H^+) extrusion which cause lowering of pH in an environment. Acidification of the medium via H^+ extrusion involves

Corresponding Author:

Hemant Dasila

Department of Microbiology,
G.B. Pant University of
Agriculture and Technology,
Pantnagar, India

mineralization of both organic and inorganic form of P by alkaline or acid phosphatase activity [2].

Wheat (*Triticum aestivum* L.) is the world's most important cereal crop which contributes about 30% of protein and 45% of energy in human diet and also an important livestock feed [23]. Wheat production in India rise to 110 million tons from 100 in year 2021 and is expected to rise above 110 million tons as the population increase and thus to meet wheat demand with growing population without compromising soil health role of PSB as biofertilizers in agricultural systems provides a better alternative than regular inorganic fertilizer. There are studies reporting increase in wheat production upon inoculation of phosphate solubilizing *Bacillus*, *Pseudomonas*, and *Streptomyces* [15]. Promising PSB strain when used in combination with commercially available inorganic P results in higher grain yield e.g. 27% as compared to inorganic P

when applied alone in wheat plants [13]. Keeping this in mind we aim to maximize wheat development via using PSB strain which were isolated from *Dalbergia sissoo* Roxb. Forest ecosystem in a pot trial experiment. After comprehensive statistical analysis we further planned them to use in field conditions.

Material and Methods

Isolation and screening of PSB

Four PSB strains were retrieved from Department of Microbiology culture collection, G.B.Pant University of Agriculture & Technology, Pantnagar. These strains were previously recovered from *Dalbergia sissoo* forest ecosystem from Pantnagar (29°3'0"N latitude and 79°31'0"E longitude) regions of Uttarakhand [9]. List of treatments given below in Table 1.

Table 1: Details of PSB strains with mentioned code that used in pot trial along with their accession number

S. No.	PSB treatments used in pots	NCBI Gen Bank Accession no.
1	<i>Pseudomonas humanensis</i> - P1	MG966346
2	<i>Pseudomonas putida</i> - P3	MG966348
3	<i>Pseudomonas plecoglossicida</i> - P4	MG966349

Qualitative estimation of phosphorous

Qualitative estimation of P was taken by measuring phosphate solubilizing index (PSI) of 3 PSB. Spotting was done on pikovaskya agar (Hi-Media) plates by taking loop full of active cultures of 3 PSB strain in which tri calcium phosphate (TCP) serves as inorganic P source and PSI was measured by the formulae.

$$PSI = \frac{\text{Total diameter (Colony diameter + halozone diameter)}}{\text{diameter of colony}}$$

Qualitative Zinc (Zn) estimation of PSB

Qualitative Zn solubilizing potential of 3 PSB strain was done by spotting loop full of active culture of 3 PSB strain onto Zn solubilizing agar (Hi-Media) plates in which zinc oxide e.g. ZnO serve as a inorganic Zn source and dextrose act as carbon source. Qualitative Zn potential response was measured as Zinc solubilizing index (ZSI)

$$ZSI = \frac{\text{Total diameter (Colony diameter + halozone diameter)}}{\text{diameter of colony}}$$

Quantitative estimation of phosphorous

Quantitative test of 3 PSB strain was done to measure how much P released in the medium during solubilization of inorganic P via acidification. For this 3 PSB strains were inoculated in National Botanical Research Institute's Phosphate (NBRIP) broth medium in which tri-calcium phosphate (TCP) serve as inorganic P source. pH of the medium was adjusted to 7.0 ± 0.1 prior to sterilization. PSB strains were inoculated in NBRIP broth medium and kept at $25 \pm 2^\circ$ C with shaking speed of 120 rpm in incubator shaker

for 4-5 days. After attaining log phase bacterial cultures were subjected to centrifuge at 4000 rpm for 10 min at 5° C to obtain cell free supernatants. Standard P solution ($100 \mu\text{g mL}^{-1}$) was prepared by dissolving 0.429g of KH_2PO_4 in one litre of distilled water (TDW) and solution was further diluted to get concentration of 15, 45, 60, 90 and $100 \mu\text{g mL}^{-1}$ absorbance (640 nm) was plotted against standard P solution for plotting standard curve. Soluble P was then measured as reduced phosphomolybdic acid (blue color). To calculate amount of P released, graph was plotted against absorbance (640 nm) and concentration KH_2PO_4 standard solution [5]. The pH of NBRIP medium was also monitored at 5 days post inoculation DPI through pH meter.

Pot Experiment

Pot experiment with 3 PSB strain was used against wheat genotype (CBW 38) planted in glass house condition relatively at controlled condition e.g. at temperature ($18-35^\circ$ C) and humidity (40-80%) with completely randomized block design (CRD) having 3 replicates per treatment. Uninoculated treatment serves as control (C). Each pot was filled with 2-3 kg of sterilized soil and was added in combination with recommended dose fertilizer (RDF) of NPK e.g. Nitrogen (N-80 Kg ha^{-1}), Phosphorous (P-40 Kg ha^{-1}) and Potassium (K-40 Kg ha^{-1}). Plant sampling was done at 45 and 60 days after transplanting (DAP) and plants samples were analyzed for plant vigor parameters which include shoot length (SL), root length (RL), shoot fresh weight (SFW), root fresh weight (RFW) and grain yield (GY). Soil samples were also analyzed for two analyze soil enzyme activity, i) fluorescein di acetate hydrolysis (FDA) and ii) soil alkaline phosphatase activity (AP).

Table 2: Details of PSB treatments with mentioned dose of inorganic NPK fertilizers

S. No.	PSB treatments
1	<i>Pseudomonas humanensis</i> - P1 + (N-80 Kg ha^{-1} / P-40 Kg ha^{-1} / K-40 Kg ha^{-1})
2	<i>Pseudomonas putida</i> - P3 + (N-80 Kg ha^{-1} / P-40 Kg ha^{-1} / K-40 Kg ha^{-1})
3	<i>Pseudomonas plecoglossicida</i> - P4 + (N-80 Kg ha^{-1} / P-40 Kg ha^{-1} / K-40 Kg ha^{-1})
4	Uninoculated control-C + (N-80 Kg ha^{-1} / P-40 Kg ha^{-1} / K-40 Kg ha^{-1})

Seeds collection and sterilization

Seeds of superior wheat genotypes e.g. CBW 38 were collected from Seed Processing Center (SPC), G.B. Pant University of agriculture & Technology, Pantnagar followed by sterilization using sodium hypochlorite (NaOCl), ethanol (C₂H₅OH) and mercuric chloride (HgCl₂). Seed sterilization proceed in 3 stage, i) the seeds were pre sterilized with NaOCl solution for 3 min and then further washed for 4-5 times with autoclaved distilled water, ii) In second stage, pre sterilized seed was rinsed with ethanol (95%) for 1 min, iii) in final stage the seed was sterilized with 0.1% HgCl₂ solution for 2 min, followed by 8-10 washings with autoclaved DW [14].

Seed bacterization

Seeds of CBW 38 genotype was bacterized with microbial inoculum by dipping 10g of seeds in three separate 50 mL flask containing log phase (1x10⁸ cells/mL⁻¹) culture of 3 PSB strain containing 1% carboxy methyl cellulose (CMC) followed by 30 min of shaking at 28±2° C for 4h. Seeds dipped in sterilized distilled water without PSB strain was served as a control [16].

Soil enzyme assays

Two soil enzyme activities e.g. FDA and AP were done. Fluorescein di-acetate (FDA) hydrolysis activity was assessed to measure total microbial activity in soil and was done according to method [7]. For FDA, 1 g of moist soil was taken in 50 mL flask further mixed with 1 mL of FDA solution and 15 mL of phosphate buffer. The flasks were then shaken for 20 min on rotary shaker at room temperature after which 10 mL of acetone (CH₃COCH₃) was added for extraction. Finally absorbance (A₀) of filtered samples were measured at 490 nm. Soil AP activity was done to evaluate how much P was released due to phosphatase activity which convert insoluble inorganic P in soil to available P of bacteria and was done according to method [24]. For AP 1 g of soil was placed in a 50 mL flask, to which 4 mL of Modified Universal Buffer (MUB), 1 ml of p- nitrophenyl phosphate (PNPP) and 0.25 mL of toluene was added and incubated at 37 °C for 1 h. Finally 1 mL of 0.5M calcium chloride (CaCl₂) and 4 mL of 0.5M sodium hydroxide (NaOH) were added. Suspension was thoroughly mixed and further filtered and finally A₀ was taken at 400nm.

Estimation of chlorophyll and carotenoid content

Chlorophyll and carotenoid estimation was done to estimate the effect of PSB on nitrogen (N) uptake in wheat plants. Test tubes were filled with 10 mL of di methyl sulfoxide DMSO followed by addition of 50 mg of leaves in DMSO further test

tubes were kept at water bath for 3 h at 60° C until the leaves turned colorless. Finally A₀ of leaf extract was measured at 663nm and 645nm using a spectrophotometer after keeping leaf extract at room temperature for 1 h.

To calculate chlorophyll a, b and total chlorophyll following formulae were used [6]

$$\text{Chlorophyll a (mg/g)} = \frac{(12.7 \times A_{663} + 8.02 \times A_{645})}{1000 \times W} \times V$$

$$\text{Chlorophyll b (mg/g)} = \frac{(22.9 \times A_{645} + 8.02 \times A_{663})}{1000 \times W} \times V$$

$$\text{Total chlorophyll (mg/g)} = \frac{(20.2 \times A_{645} + 8.02 \times A_{663})}{1000 \times W} \times V$$

Carotenoid content leaf sample was estimated with above procedure only difference was that in this A₀ of leaf extract was measured at 480nm [10].

$$\text{Carotenoid (mg/g)} = \frac{[(A_{480} + 0.11 \times A_{663} - 0.638 \times A_{645})]}{1000 \times W} \times V$$

Where, W= weight of leaf sample and V= volume of leaf extract.

Statistical analysis

Principal component analysis (PCA) analysis was performed two reasons i) to determine the most influenced agronomic variable after PSB inoculation. Analysis of variance (ANOVA) was done to find overlap of significance amongst three PSB strain.

Results

Qualitative estimation of P

Three PSB strains (*Pseudomonas hunanensis*- P1, *Pseudomonas putida*- P3 and *Pseudomonas plecoglossicida*-P4) were tested positive on pikovaskia agar plates and the response was measured in PSI. Highest PSI was observed in P3 (3.4 cm), followed by P1 (2.8 cm) and P4 (2.78 cm) respectively (Fig.1).

Qualitative estimation of Zn

Three PSB strains (*Pseudomonas hunanensis*- P1, *Pseudomonas putida*- P3 and *Pseudomonas plecoglossicida*-P4) were also tested positive on Zn agar plates and the response was measured in ZSI. Highest ZSI was observed in P3 (3 cm), followed by P4 (2.64 cm) and P1 (2.52 cm) respectively (Fig.1).

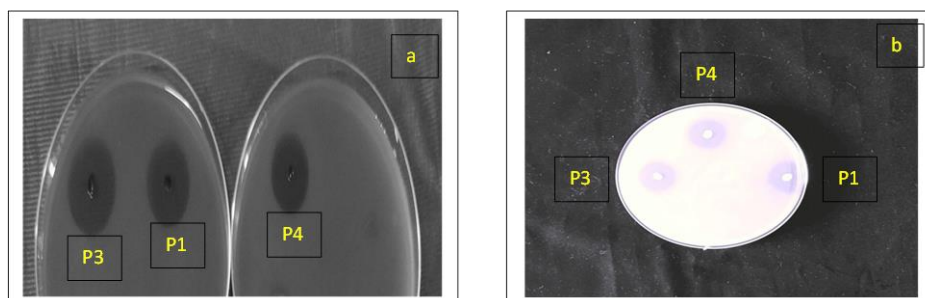


Fig 1: PSB strain showing P solubilization (a) and Zn solubilization strain (b) showing positive halozone formation around the colony

P-quantification of inorganic P

The release of soluble P in the NBRI-BPB medium was highest in *Pseudomonas putida*- P3 (74.6 $\mu\text{g mL}^{-1}$) followed by *Pseudomonas hunanensis*- P1 (72.48 $\mu\text{g mL}^{-1}$) and *Pseudomonas plecoglossicida*- P4 (70.48 $\mu\text{g mL}^{-1}$)

respectively. It was also observed that 3 PSB strain also acidified the medium which may be due to H^+ extrusion as a result of which pH of the medium decreased from pH 7 to 4.6. Highest drop of pH was observed in P3 (4.6) followed by P1 (4.8) and P4 (4.92) (Fig. 2).

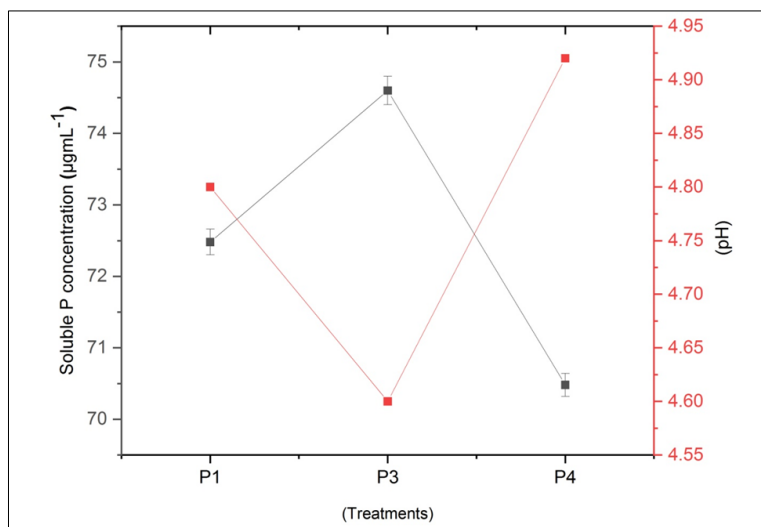


Fig 2: Quantitative estimation of PSB strain in NBRIP broth medium with drop of pH

Growth promoting effect of PSB inoculation on wheat

Plant agronomic parameters of wheat genotypes (CBW 38) were monitored at 45 and 60 days post inoculation (DPI). PSB inoculation positively influenced overall plant vigor of wheat plants (Fig. 3). Overall there was 1-2 fold increase in agronomic parameters. Highest plant vigor parameters was observed in *Pseudomonas putida*- P3 (SL-32cm, RL-12.4cm, SFW-0.36g, RFW-0.18g, GY-2.4 Kg ha^{-1}) followed by

Pseudomonas hunanensis- P1 (SL-30.8cm, RL-12cm, SFW-0.32g, RFW-0.12g, GY-2.1 Kg ha^{-1}) and *Pseudomonas plecoglossicida*-P4 (SL-30cm, RL-11.4cm, SFW-0.31g, RFW-0.14g, GY-1.96 Kg ha^{-1}) respectively which was found to be much higher than uninoculated control (C) in which SL-22.4 cm, RL-5.6 cm, SFW-0.18g RFW- 0.88g and GY-1.24 Kg ha^{-1} respectively (Fig 3).

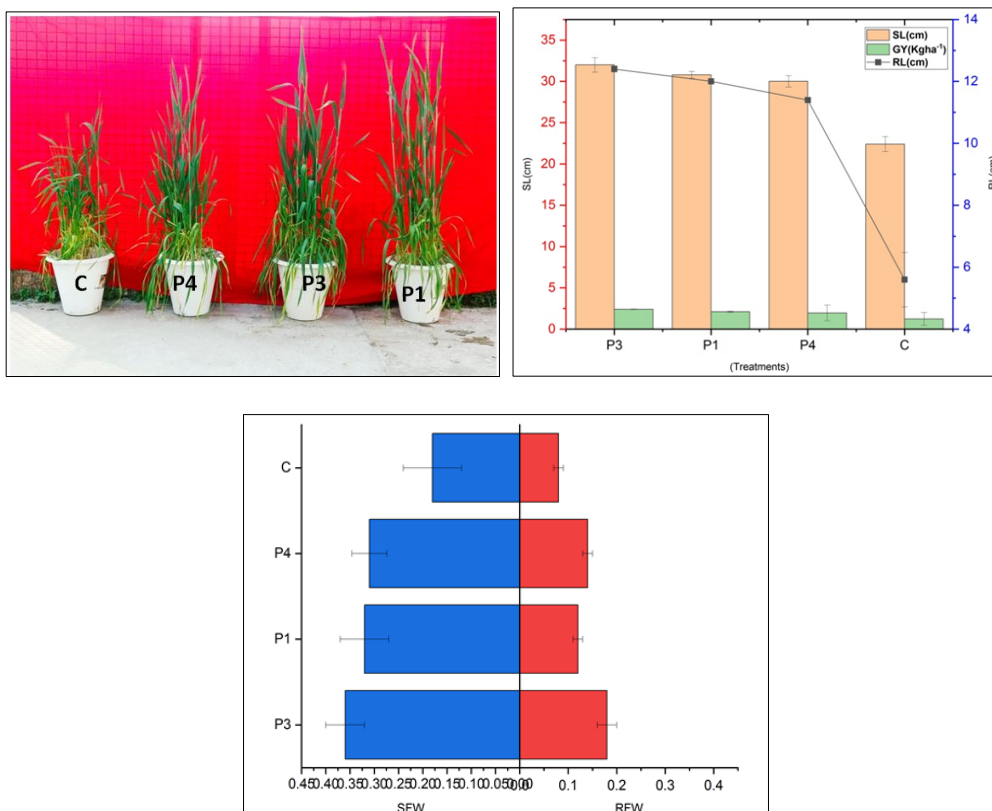


Fig 3: Pot trial of PSB strain in CBW 38 wheat genotype plant a), agronomic plant parameter variation in terms of SL, RL and GY b), and SFW and RFW of PSB treated wheat plants c)

Soil enzymes analysis

Soil enzyme activity is considered as a marker for soil health. Rhizospheric soil was assessed for FDA and AP to evaluate the impact of PSB inoculation on soil health. Overall, there was a positive impact of inoculation of 3 PSB on soil health as compared to uninoculated control (C). Highest FDA and AP was observed in *Pseudomonas putida*- P3 in which FDA and AP was found to be 78.6 $\mu\text{g mL}^{-1}\text{h}^{-1}$ and 62.6 $\mu\text{g mL}^{-1}\text{h}^{-1}$

respectively followed by *Pseudomonas humanensis*- P1 (FDA-72.6 $\mu\text{g mL}^{-1}\text{h}^{-1}$ and AP-58.4 $\mu\text{g mL}^{-1}\text{h}^{-1}$) and *Pseudomonas plecoglossicida*- P4 (FDA-68.36 $\mu\text{g mL}^{-1}\text{h}^{-1}$ and AP-56.4 $\mu\text{g mL}^{-1}\text{h}^{-1}$) respectively. Uninoculated PSB wheat plants serve as control which have significantly lower FDA (58 $\mu\text{g mL}^{-1}\text{h}^{-1}$) and AP (44.6 $\mu\text{g mL}^{-1}\text{h}^{-1}$) as compared to PSB treated strains (Fig 4).

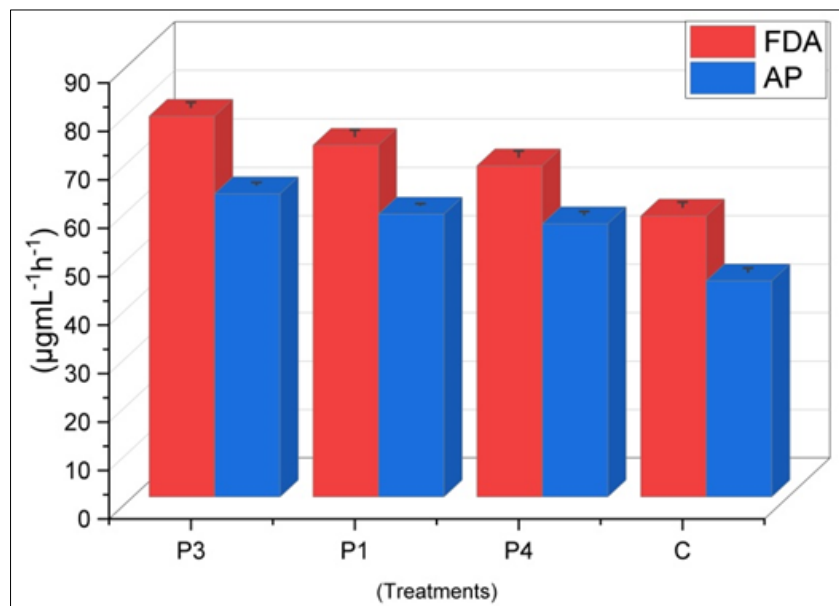


Fig 4: FDA and AP soil enzyme activities of PSB treated soil along with untreated control (C)

Chlorophyll estimation

Chlorophyll and carotenoid content in plants is directly linked to amount of nitrogen (N) present in plant sample. PSB inoculation on treated wheat plants promotes the average chlorophyll a, b total chlorophyll and carotenoid content wheat genotype CBW 38. Maximum chlorophyll a (2.6 mg/g), b (0.04 mg/g), total chlorophyll (2.32 mg/g) and carotenoid (0.5 mg/g) found respectively was observed in P3

PSB inoculated wheat plants followed by P1 and P4 which were significantly higher as compared to uninoculated PSB wheat plants which serve as control where chlorophyll a (2 mg/g), b (0.03 mg/g), total chlorophyll (1.9 mg/g) and carotenoid (0.55 mg/g) found respectively. These results in addition to Phosphorous mobilization, PSB inoculation also promotes N content in wheat genotypes (Fig. 5).

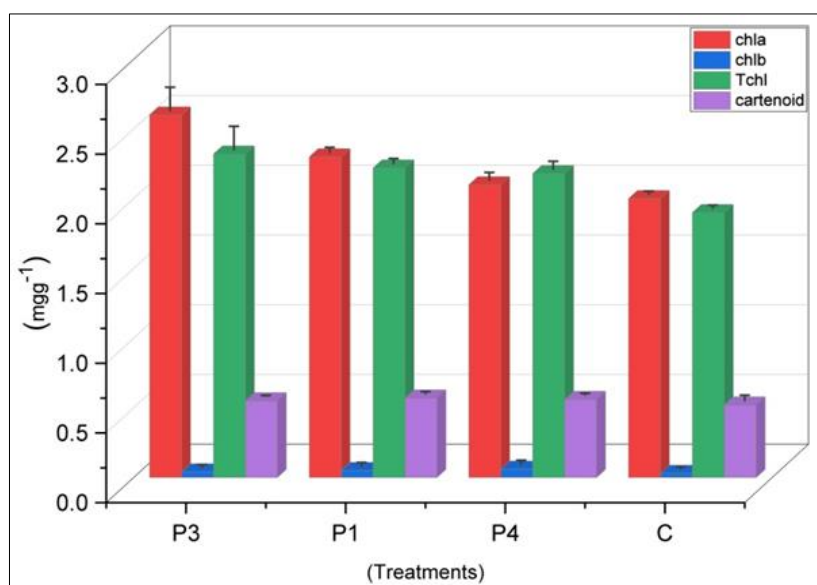


Fig 5: Chlorophyll a, b, total chlorophyll and carotenoid of wheat treated with PSB strain and uninoculated PSB control (C)

Discussion

As P is most important limiting factor in agriculture crop

production, solubilization of inorganic and organic P in soil via PSB provides an effective strategy to release unavailable P

in the soil to most available form of P to plants. Thus PSB can be used alone or in combination with inorganic commercially available chemical fertilizers to reduce input dosage of fertilizers. The present study was aimed to establish the role of PSB as potential agent for improving soil health and wheat plant vigor. All 3 PSB strain show positive qualitative phosphate and zinc solubilizing potential in which maximum P and Zn solubilization was observed in P3 (Fig 1). Quantitative P concentration was also highest in P3 (74.6 $\mu\text{g mL}^{-1}$) accompanied with drop of pH 4.6 and it may be due to H^+ extrusion which is one of the key mechanisms of inorganic P solubilization bacteria [25]. In pot trial inoculation of PSB promotes wheat plant vigor parameters (Fig 3) as compared to uninoculated control. Maximum response was observed in P3 followed by P4 and P1 respectively. Average GY was highest in P3 strain e.g. 2.4 Kg ha^{-1} which is significantly higher than uninoculated control (C) where GY was found to be 1.24 Kg ha^{-1} . Our study results have been supported by studies in which PSB bacteria belonging to genus *Pseudomonas* notably improved GY up to 20-60% respectively in wheat plants [9]. Promising PSB strain when use in combination with commercially available inorganic P results in higher GY upto 27% as compared to inorganic P when applied alone in wheat plants [13]. Two soil enzyme activities (FDA and AP) were done after harvesting of plants and there was a substantial increase in both FDA and AP soil enzyme activities in soil treated with PSB strains as compared to untreated soil which serve as control (Fig 4). FDA represents total microbial activity present in the soil and an increase in FDA activity could stimulates AP activity [4]. Increase in AP activity promotes solubilization of unavailable organic form of P to most available inorganic P form thus mobilize P pool from soil to plants which in turn promotes plant vigor in wheat. This theory has been supported by previous study in which an increase in wheat biomass was observed when soil was treated with bacteria that promoted soil AP activity [4]. Average chlorophyll a, b, total chlorophyll and carotenoid increase were observed in PSB inoculated wheat plants. Maximum response was observed in P3 which was higher as compared to uninoculated wheat plant e.g. controls plants. Chlorophyll content increase indicates that addition of PSB does not solubilize inorganic P in soil but it also promotes N content in plants as chlorophyll and carotenoid concentration directly correlates with N content present in wheat plants [1].

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

Authors are thankful to A.F.R.C (Agroforestry Research Center), Department of Microbiology, CBSH, G.B.P UAT, Pantnagar, Uttarakhand, India for providing Facilities.

References

- Akhter MM, Akbar Hossain, Jagadish Timsina, DA Silva, Jaime A. Teixeira and MS Islam "Chlorophyll meter-a decision-making tool for nitrogen application in wheat under light soils", 2016,289-302.
- Anand R, Ranjha L, Cannavo E, Cejka P. Phosphorylated CtIP functions as a co-factor of the MRE11-RAD50-NBS1 endonuclease in DNA end resection. *Molecular cell*. 2016;64(5):940-950.
- Barrow NJ. The effects of pH on phosphate uptake from the soil. *Plant Soil*. 2017;410:401-10. doi: <https://doi.org/10.1007/s11104-016-3008-9>.
- Dasila H, Anjul R, Damini D, Anamika R, Manvika S, Salil T. Interaction between *Dalbergia sissoo* Roxb. and *Pseudomonas koreensis* AS15 Strain is Cultivar Specific. *Inter J Curr Microbiol App Sci*. 2018;7(10):51,297-306. <https://doi.org/10.20546/ijemas.2018.710.031>.
- Elhassoufi W, Khourchi S, Ibnyasser A, Ghoulam C, Rchiad Z, Zeroual Y, *et al*. Phosphate solubilizing rhizobacteria could have a stronger influence on wheat root traits and aboveground physiology than rhizosphere P solubilization. *Front Plant Sci*. 2020;11:979. doi: <https://doi.org/10.3389/fpls.2020.00979>.
- Fiske CH, Subbarow Y. The colorimetric determination of phosphorus. *J. biol. Chem*. 1925;66(2):375-253,400. <https://dx.doi.org/10.1042%2Fbj0260292>.
- Hiscox JD, Israelstam GF. A method for the extraction of chlorophyll from leaf tissue without maceration. *Can. J Bot*. 1979;57(12):1332-1334.
- Inbar J, Chet I. Evidence that chitinase produced by *Aeromonas caviae* is involved in the biological control of soil-borne plant pathogens by this bacterium. *Soil Biol Biochem*. 1991;23(10):973-978. [https://doi.org/10.1016/0038-0717\(91\)90178-M](https://doi.org/10.1016/0038-0717(91)90178-M).
- Joshi S, Jaggi V, Tiwari S, Sah VK, Sahgal M. Multitrate phosphate solubilizing bacteria from *Dalbergia sissoo* Roxb. Rhizosphere in natural forests of Indian Central Himalayas. *Environ Ecol*. 2019;37(3A):894-908.
- Kaur G, Reddy MS. Effects of phosphate-solubilizing bacteria, rock phosphate and chemical fertilizers on maize-wheat cropping cycle and economics. *Pedosphere*. 2015;25:428-37. doi: [https://doi.org/10.1016/S1002-0160\(15\)30010-2](https://doi.org/10.1016/S1002-0160(15)30010-2).
- Kirk JTO, Allen RL. Dependence of chloroplast pigment synthesis on protein synthesis: effect of actidione. *Biochem. Biophys. Res. Commun*. 1965;21(6):523-530.
- Mehrvarz, SMR, Chaichi, Alikhani HA. "Effect of phosphate solubilizing microorganisms and phosphorus chemical fertilizer on forage and grain quality of barely (*Hordeum vulgare* L.). *Agric. Environ. Sci*. 2008;3(6):822-828.
- Mäder P, Kaiser F, Adholeya A, Singh R, Uppal HS, Sharma AK, *et al*. Inoculation of root microorganism for sustainable wheat-rice and wheat-black gram rotations in India. *Soil. Biol. Biochem*. 2011,43(3):609-619. <http://dx.doi.org/10.1016/j.soilbio.2010.11.031>.
- Manzoor M, Abbasi MK, Sultan T. Isolation of phosphate solubilizing bacteria from maize rhizosphere and their potential for rock phosphate solubilization mineralization and plant growth promotion. *Geomicrobiol J*. 2017;34:81-95. doi: <https://doi.org/10.1080/01490451.2016.1146373>.
- Ramakrishna N, Lacey J, Smith JE. Effect of surface sterilization, fumigation and gamma irradiation on the microflora and germination of barley seeds. *Int. J. Food Microbiol*. 1991;13(1):47-54.
- Rana A, Saharan B, Joshi M, Prasanna R, Kumar K, Nain L. Identification of multi-trait PGPR isolates and evaluating their potential as inoculants for wheat. *Ann Microbiol*. 2011;61:893-900. <https://doi.org/10.1007/s13213-011-0211-z>.
- Sagervanshi A, Priyanka K, Anju N, Ashwani K. Isolation and characterization of phosphate solubilizing

- bacteria from and agriculture soil. *Int. J. Life Sci. Pharma Res.* 2012;23:256-266.
18. Satyaprakash M, Nikitha T, Reddi EUB, Sadhana B, Vani SS. Phosphorous and phosphate solubilising bacteria and their role in plant nutrition. *Int J Curr Microbiol Appl Sci.* 2017;6(4):2133-2144. <https://doi.org/10.20546/ijcmas.2017.604.251>.
 19. Scholz RW, Geissler B. Feebates for dealing with trade-offs on fertilizer subsidies: a conceptual framework for environmental management. *J Clean Prod.* 2018;189:898-909. doi: <https://doi.org/10.1016/j.jclepro.2018.03.319>.
 20. Selvi KB, Paul JJA, Vijaya V, Saraswathi K. Analyzing the efficacy of phosphate solubilizing microorganisms by enrichment culture techniques. *Biochemistry and Molecular Biology Journal.* 2017;3(1):1-7.
 21. Shenoy VV, Kalagudi GM. Enhancing plant phosphorus use efficiency for sustainable cropping. *Biotechnol. Adv.* 2005;23:501–513. <https://doi.org/10.1016/j.biotechadv.2005.01.004>.
 22. Shenoy VV, Kalagudi GM. Enhancing plant phosphorus use efficiency for sustainable cropping. *Biotechnol Adv.* 2005;23:501-13. doi: <https://doi.org/10.1016/j.biotechadv.2005.01.004>.
 23. Shewry PR. Wheat. *J Exp. Bot.* 2009;60(6):1537-1553. <https://doi.org/10.1093/jxb/erp058>.
 24. Siedliska A, Baranowski P, Pastuszka-Wozniak J, Zubik M, Krzyszczyk J. Identification of plant leaf phosphorus content at different growth stages based on hyperspectral reflectance. *BMC Plant Biol.* 2021;21:1-17. doi: <https://doi.org/10.1186/S12870-020-02807-4>.
 25. Tabatabai MA, Bremner JM. Use of p-nitrophenyl phosphate for assay 309 of soil phosphatase activity. *Soil. Biol. Biochem.* 1969;1(4):301-307. [https://doi.org/10.1016/0038-0717\(69\)90012-1](https://doi.org/10.1016/0038-0717(69)90012-1).
 26. Otieno, Nicholas, Richard D Lally, Samuel Kiwanuka, Andrew Lloyd, David Ryan, *et al.* Germaine, and David N. Dowling. "Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates." *Frontiers in microbiology.* 2015;6:745.