# **Research Article**

# Formulated Biofertilizer Improves Wheat Growth, Yield and Nutrient Uptake

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## Abstract

Inoculation of phosphorus solubilizing microorganisms improves the availability of phosphorus in soil. Under unfavorable environmental conditions, success of such nutrient solubilization is mainly depending on the capacity of the bioinoculant to survive. In this context, liquid biofertilizer technology is gaining importance and is becoming an alternative way with longer shelflife and field performance. In the present study, an experiment was conducted to check the effect of phosphate solubilizing bacterial (PSB) bioinoculant formulated with different cell protectants which supports growth and promote survival of PSB inoculants. Seeds were inoculated with 90 days old PSB bioinoculants with various treatments. PSB inoculants formulated by different cell protectants or additives significantly improves yield, plant growth, nutrient content and their uptake in wheat at 75 days of growth as compared to uninoculated control/treatment.

**Keywords:** Bioinoculants, Cell protectants, Glycerol, Gum arabic, Poly vinyl pyrollidone, *Triticum aestivum* L.

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# Introduction

To maximize crop yield agricultural systems depends on fertilizer application to maintain soil nutrient levels however, in many agricultural areas, the lack of available nutrients limits crop yield [1]. Overuse of fertilizers can cause environmental degradation and pollution through various pathways. Worldwide interest in the use of biological agents, such as biofertilizer including phosphorus solubilizing microorganisms (PSM) is increasing day by day because they significantly improve crop growth and development. A direct contribution by P-solubilizing bacteria to improved growth of maize crop [2]. Other recent studies report increased crop yields in response to inoculation with soil microorganisms associated with improved crop nutrition [3, 4].

Next to nitrogen, phosphorus is the most important plant macronutrient and it plays a major role in many aspects of plant structure and function. The majority of phosphorus in soil is immobile or sparingly soluble due to its reactive nature by some chemical and physical factors, making it unavailable to plant. The low P availability can be the limiting factor and causing crop yield loss [5-7]. One can increase the uptake of P and yield of crops by increasing P concentration and by enhancing activity of soil microbes. There are many ways to increase availability of P in the rhizospheres, but primary method involve root growth promotion or rhizosphere acidification through hydrolytic enzymes, organic acids and siderophores [8]. PSB are capable of solubilizing accumulated phosphatic compound sources in soil and make the P available to the plants [9]. These PSB are one of the important components of integrated nutrient management as these are cost effective and renewable source of plant nutrients to replace the chemical fertilizers for sustainable agriculture. The most efficient phosphate solubilizing microorganisms (PSMs) belong to genera Aspergillus and Penicillium amongst fungi and Bacillus and Pseudomonas amongst bacteria. Inoculation of plants with PSMs generally results in enhance plant growth and yield, in particular, under glasshouse conditions [10, 11]. Recently, [3] and [4] had reported improves plant growth and yield of mung bean and wheat by the application of formulated *Rhizobium* and phosphorus bioinoculants. Consequently, the aim of the present work was to investigate the effect of formulated bioinoculats or biofertilizer with different cell protectants on wheat growth and phosphorus uptake.

## **Materials and Methods**

Bacterial strains such as *Azotobacter chrococcum* Mac 27 and PSB (phosphate solubilizing bacteria, *Pseudomonas* sp. strain P-36) isolates collected from Department of Microbiology, CCS Haryana Agricultural University, Hisar and seeds of wheat (*Triticum aestivum* L.) cv Raj 3765 was obtained from Department of Plant Breeding, CCS HAU, Hisar.

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Jensen broth [12] was used as basal medium for *Azotobacter chrococcum* Mac 27 and Nutrient broth (NB) (Beef extract 3.0, Peptone 5.0, NaCl 5.0, Dextrose 10.0, pH 6.8–7.0) was used as a basal medium for liquid phosphate solubilizing bacterial inoculants formulation with selected appropriate concentrations of different additives [13].

A pot experiment was conducted in relation plant P content, height, shoot weight and root weight to evaluate the efficacy of PSB inoculants on wheat crop. Seeds of wheat (*Triticum aestivum* L.) cv Raj 3765 were treated with different liquid inoculants stored for 90 days at room temperature and in a refrigerator. Seeds after treatment were sown in the pots. Plants were uprooted at 75 d of growth and observations on plant height, shoot weight, root weight and plant phosphorus content were taken. Total plant P content was estimated by the method [14] and completely randomized design (CRD) was used for experimental data analysis.

#### **Results and Discussion**

To find the role of additives in maintaining shelf life of PSB liquid inoculants and their subsequent role in plant growth and P- uptake, a pot experiment was conducted. All growth parameters (plant height, plant P content, shoot and root biomass) of wheat were better in inoculated treatments as compared to control (Uninoculated) (T1 and T2). In some of the treatments inoculated with 90 days old inoculants + additives (added after the growth), showed significantly better response with respect to all growth parameters compared to treatment inoculated with 90 days old inoculants (T7).

At 75 days, among the treatments inoculated with 90 days old inoculants stored under refrigerated conditions, treatment inoculated with the inoculant amended with 2% GA showed more shoot biomass (i.e. 2.790 g) and phosphorus content (1.009 mg P plant<sup>-1</sup>) followed by the inoculants amended with 2% PVP ( 2.783g shoot biomass and 0.999 mg P plant<sup>-1</sup>) and 1% GA (2.682 g shoot biomass and 0.985 mg P plant<sup>-1</sup>) as compared to 50 % RDF (2.519 g shoot biomass and 0.891 mg P plant<sup>-1</sup>) (**Figure 1**). Among the treatments inoculated with 2% GA showed more shoot biomass (i.e. 2.785g) and phosphorus content (0.939mg P/ plant) followed by inoculants amended with 2% GA showed more shoot biomass (i.e. 2.785g) and phosphorus content (0.939mg P/ plant) followed by inoculants amended with 2% PVP (2.587 g shoot biomass and 0.934 mg P plant<sup>-1</sup>) and 1% GA (2.576 g shoot biomass and 0.931 mg P plant<sup>-1</sup>) as compared to 50 % RDF (2.519 g shoot biomass and 0.591 mg P plant<sup>-1</sup>) and 1% GA (2.576 g shoot biomass and 0.931 mg P plant<sup>-1</sup>) as compared to 50 % RDF (2.519 g shoot biomass and 0.931 mg P plant<sup>-1</sup>) as compared to 50 % RDF (2.519 g shoot biomass and 0.891 mg P plant<sup>-1</sup>) (**Table 1**).



**Figure 1** Effect of 90 days old liquid PSB inoculants (stored in a refrigerator) on the growth of wheat plant (T4 - 50% RDF; T6 - 50% RDF + *Azotobacter chroococum* Mac 27 + fresh PSB; T14 - 50% RDF + *Azotobacter chroococcum* Mac 27 + 90 days old PSB inoculant (control); T18 - 50% RDF + *Azotobacter chroococcum* Mac 27 + 90 days old PSB inoculant (amended with 1% PVP); T20 - 50% RDF + *Azotobacter chroococcum* Mac 27 + 90 days old PSB inoculant (amended with 2% GA))

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Enhanced grain yield, nutrient availability and uptake in wheat crop by the application of *Azotobacter* and liquid PSB inoculant over control observed by [15]. Similar results were observed by [4] who found increase in the yield of wheat by the use of combined inoculation of *Azotobacter* and formulated liquid PSB inoculant. During several other studies, significant effects of combined inoculation of PSB and non symbiotic bacterium *Azotobacter* on yield, nitrogen (N) and phosphorous (P) accumulation of different crops have been demonstrated [16, 17]. Among the inoculated treatments, the treatments inoculated with inoculants amended with additives showed more shoot biomass and plant P content as compared to their respective controls.

| Sr. No. | Treatments   | SH    | SDW                      | RDW                      | P content                 |
|---------|--|-------|--------------------------|--------------------------|---------------------------|
|         |  | (cm)  | (g plant <sup>-1</sup> ) | (g plant <sup>-1</sup> ) | (mg plant <sup>-1</sup> ) |
| T1      | Control  | 53    | 0.914                    | 0.201                    | 0.221                     |
| T2      | 100 % RDF  | 73    | 2.972                    | 0.898                    | 1.212                     |
| T3      | 75 % RDF   | 70.3  | 2.252                    | 0.602                    | 0.859                     |
| T4      | 50 % RDF   | 67    | 2.519                    | 0.473                    | 0.891                     |
| T5      | 50 % RDF + Ac  | 68.2  | 2.709                    | 0.586                    | 0.983                     |
| T6      | 50 % RDF + Ac + fresh PSB                            | 70.2  | 2.834                    | 0.671                    | 1.028                     |
| T7      | 50 % RDF + Ac + $90$ days old PSB                    | 65.2  | 2.534                    | 0.477                    | 0.902                     |
| T8      | 50 % RDF + Ac + 90 days old PSB (1 % glycerol)       | 69.5  | 2.519                    | 0.491                    | 0.905                     |
| T9      | 50 % RDF + Ac Mac 27 + 90 days old PSB (2% glycerol) | 66.3  | 2.52                     | 0.56                     | 0.896                     |
| T10     | 50 % RDF + Ac Mac 27 + 90 days old PSB (1% PVP)      | 66    | 2.534                    | 0.538                    | 0.916                     |
| T11     | 50 % RDF + Ac Mac 27 + 90 days old PSB (2% PVP)      | 68    | 2.587                    | 0.523                    | 0.934                     |
| T12     | 50 % RDF + Ac + 90 days old PSB (1% GA)              | 67.3  | 2.576                    | 0.474                    | 0.931                     |
| T13     | 50 % RDF + Ac + 90 days old PSB (2% GA)              | 69.3  | 2.785                    | 0.573                    | 0.939                     |
| T14     | 50 % RDF + Ac + $90$ days old PSB                    | 64.4  | 2.556                    | 0.533                    | 0.899                     |
| T15     | 50 % RDF + Ac + 90 days old PSB (1 % glycerol)       | 67.6  | 2.595                    | 0.593                    | 0.981                     |
| T16     | 50 % RDF + Ac +90 days old PSB (2% glycerol)         | 67.4  | 2.616                    | 0.633                    | 0.934                     |
| T17     | 50 % RDF + Ac + 90 days old PSB (1% PVP)             | 67.5  | 2.596                    | 0.601                    | 0.93                      |
| T18     | 50 % RDF + Ac + 90 days old PSB (2% PVP)             | 68    | 2.783                    | 0.602                    | 0.999                     |
| T19     | 50 % RDF + Ac + 90 days old PSB (1% GA)              | 67.8  | 2.682                    | 0.609                    | 0.985                     |
| T20     | 50 % RDF + Ac + 90 days old PSB (2% GA)              | 71.3  | 2.79                     | 0.666                    | 1.009                     |
|         | C.D. at 5%   | 1.618 | 0.063                    | 0.011                    | 0.19                      |

SH- Shoot height, SDW- Shoot dry weight, RDW- Root dry weight, TPP- Total plant phosphorus

Ac-Azotobacter chroococcum Mac 27

T7 - T13 - 90 days old PSB inoculants amended with different amendments stored at room temperature

T14 - T20 - 90 days old PSB inoculants amended with different amendments culture stored under refrigerated conditions

Evidently, the different additives added in inoculants provided some protection to bacteria on the seeds. PVP and GA have sticky consistency which might enhance cell adherence to the seeds and their dense nature may slow the drying method of the inoculation when applied to the seeds. These additives have conjointly reported to protect cells against harmful toxic seed coat factors [18]. Gum arabic has the ability to limit or minimize heat transfer and also has high water activities. These compounds are absorbed in a thin molecular layer on the surface of the individual mixture particles resulting in a stabilized suspension that prevents coalescence of cells which might block the oxygen and nutrient transfer from media to cells. These might be the mechanisms that improve the survival of microorganism cells on the seeds and provides rise to more plant growth.

Survival and shelflife of PSB inoculants may be maintained with the addition of additives GA and PVP. Plant growth, yield and plant P content of the wheat crop have been significantly affected by inoculants amended with PVP or GA. There was saving of about 25% phosphatic chemical fertilizer by the application of PSB inoculants and for obtaining maximum grain yield as well as profit from wheat, soil should be inoculated with *Azotobacter* with PSB inoculants amended with different additives.

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