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A Note on Agricultural Importance of PHAs Producing *Bacillus* sp. on Plant Growth Promoting Activities

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Abstract: Polyhydroxyalkanoates (PHAs) producing microbes which have been reported to exist in several ecological niches those generally exposed to abundant organic matter or growth limited (essential nutrients limitation) conditions such as rhizospheric region of plants, agricultural wastes and activated sludges of treatment plants. Some of the PHAs producers also synthesize extracellular by-products like bio-surfactant (rhamnolipids), extracellular polymeric substances and also help in plant growth by showing PGPR activities. PHAs producer contributes good shelf life period to the microbes. So, bacteria accumulating such fatty acid granules (PHAs) with PGPR activities can be considered as best biofertilizer in Agricultural industry. In this study we are focusing on few strains of *Bacillus* sp. that bestow the presence of PHAs granules in cytosol and as well as plant growth promoting activities.

Introduction

Polyhydroxyalkanoates (PHAs) are accumulated as intracellular granules in the cytoplasm of microbial species isolated from various natural locations. Depending on the production, PHAs produced sep-arated from the phase of microbial growth after de-pletion of a growth-essential nutrient with excess carbon source or occurs in parallel with biomass synthesis even at balanced nutritional conditions. Storage and utilization of poly-β-hydroxyalkanoate polymers are important for the shelf life of the bacteria in production of bioinoculants, products containing bacterial cells in a suitable carrier for agricultural use [17]. This nutritional environment proliferate a good microbial interaction in the rhizospheric region of plant and subsequently promotes the plant growth. Though accumulation of PHA granules enhances bacterial generation time as well as contributes to the shelf life period of its growth. Thus, PHA producers can also be used as a best model organism for formulation of biofertilizer which ultimately increase the agricultural productivity. Moreover, yield can be increased by focusing those microbes which are having plant growth promoting (PGP) activities that not only promotes growth but also increases the crop productivity. Due to the negative environmental impact of chemical fertilizers with their increasing costs, sustainable approach for organic produce has persuaded search for plant growth promoting rhizobacteria (PGPR) which involves use of microorganisms as bioinoculant. Plant Growth Promoting Rhizobacteria (PGPR) is the group of free living bacteria that increases plant growth via various growth promoting substances [8] by different mechanisms like phosphate solubilization, nitrogen fixation,

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repression of soil pathogens by the production of hydrogen cyanide, making nutrients available for the plant, siderophore and phytohormones production such as indole-3-acetic acid 1. It was found that treatments with PGPR increase germination percentage, seedling vigor, root and shoot growth, total biomass of the plants, seed weight, early flowering, grains, fodder and fruit yields etc. In view of this an attempt was made to the present investigation and to asses the potential PHA producers for their intrinsic ability to produce plant growth promoting substances.

Material and methods

Screening for presence of PHAs Granules

Identified Bacterial isolates such as *Bacillus* sp. P1, *Bacillus* sp. P2, *Bacillus* sp. P3 and *Bacillus* sp. P4 [11] were revived using Nutrient Broth medium (Hi-media laboratories private limited, Mumbai) which were previously isolated from rhizospheric soil region of sweet potato during pre-harvesting period in the field of CTCRI, Bhubaneswar, Odisha. After two years of preservation, pure cultures of these isolates were again screened for presence of PHA granules. Existence of PHA granule was determined by Sudan Black B staining. Prior to staining bacterial isolates were inoculated to GM medium with high carbon and low nitrogen ratio for induction of PHA granules [12].

Screening for PGPR activities of PHAs producers

The PHAS producing isolates were screened for Plant growth promoting activities and the isolates were streaked on Pikovaskaya's agar medium for Phosphate solubilization [14]. These isolates were also tested for auxin production (IAA) following the method of [9]. Bacterial isolates were tested for the production of ammonia following the method elsewhere [3]. Siderophore production was checked on solid CAS universal blue agar plates [18]. Isolates were further streaked on nutrient agar medium containing 4.4 g/ l of glycine for their HCN producing abilities and finally incubated at 35 ± 2°C for 4 days [4].

Seed germination test

The bacterial isolates such as *Bacillus* sp. P1, *Bacillus* sp. P2, *Bacillus* sp. P3 and *Bacillus* sp. P4) showed PGPR characters and were further tested for seed germination and plant growth under lab conditions. For seed germination, Mung bean seeds (*Vigna radiata*) were considered as experimental materials, surface sterilized with 0.1% $HgCl_2$ for 2 min, then rinsed ten times with sterile distilled water. Bacterial isolates were grown in nutrient broth on shaking incubator (180 rpm) at 35 ± 2°C for 24 h. Cell densities in the suspension were adjusted to 10^8 CFU ml^{-1}. The surface sterilized seeds of mung bean were inoculated in fresh culture for 30 minutes [6]. Germination tests were carried out by the roll towel method. For roll towel method, PGPR-treated seeds and control (untreated seeds) were seeded onto paper towels. For pot experiment, Mung bean seeds were soaked in inoculum for 30 minutes and seeds were sown at 2 cm depth in pot containing 250 g sterilized soil. A control was also maintained in the same manner with untreated seed followed by germination percentage calculation. After 45 days of seed sowing, Mung bean plants were harvested through separating of plants from soil. The plants were washed, shoot length (cm $plant^{-1}$) & root length (cm $plant^{-1}$) were recorded.

Results and discussion

The bacterial isolates showed PHAs positive after sudan black staining were further characterized for PGPR activites. All the isolates showed the development of halo zones on Pikovaskya agar of which *Bacillus* sp. P4 showed maximum zone of 22 mm (Table 1) as indicative of phosphatase activities. Zone size on Pikovaskya agar medium is directly correlated with the phosphatase activity of bacterial isolates [13]. It was also observed that increasing in the incubation time ultimately increases the zone size. The test isolates were observed to produce siderophore where *Bacillus* sp. P4 showed maximum zone of 20 mm (Table 1).

Similar observations have been reported by Loper and Henkels [10], who observed that iron is essential for all living organisms including phytopathogens. Growth promotion may be attributed to other mechanisms such as production of phyto-hormones in the rhizosphere. Two

Table 1. PGPR activities by the PHAs producing bacterial isolates

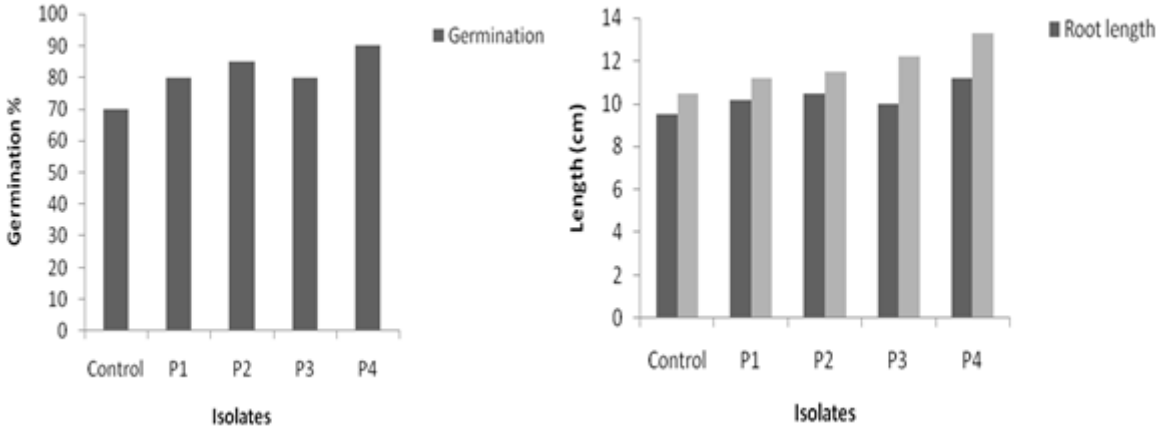
Isolates	Nitrate	Ammonia	IAA	Zone size (mm) Siderophore	Phosphate
Bacillus sp. P1	-	-	+	15mm	20mm
Bacillus sp. P2	-	-	-	12mm	18mm
Bacillus sp. P3	-	-	-	10mm	15mm
Bacillus sp. P4	+	+	+	20mm	22mm

isolates, *Bacillus* sp. P1 & P4 also produces plant growth promoting hormone (IAA). The ability of bacteria to produced phytohormone like Auxin i.e. IAA in the rhizosphere depends on the availability of precursors and uptake of microbial IAA by plant [1,15]. *Bacillus* sp.P4 also exhibited strong pro-duction of ammonia & nitrate which is another trait of PGPR and taken up by plants as a source of nitrogen for their growth [2].

Seed germination test

In this study, an increase in the plant growth by seed bacterization has been demonstrated. It is a well-established fact that overall plant growth and root development influenced by enhanced phosphorous nutrition [7]. A large number of evidence suggests that PGPR augment growth, seed emergence and crop yield [5,16]. The experimental results showed the effectiveness of PGPR isolates on seed germination percentage and growth of Mung bean. To see the effect of PGPR isolates on Mung bean, the seeds were pretreated with *Bacillus* sp. P1, *Bacillus* sp. P2, *Bacillus* sp. P3 and *Bacillus* sp. P4 which positively increase the germination in germination paper (Fig. 1). The PGPR isolates significantly increased the root & shoot length of Mung bean. Highest root & shoot elongation was recorded when seeds were pre-treated with *Bacillus* sp. P4 in germination paper over control (Fig. 1). Among the four bacterial isolates, *Bacillus* sp. P4 showed highest activities and thus it was selected for the application in pot culture method. Highest germination, root length & shoot length was also observed with *Bacillus* sp. P4 in pot culture method over control (Fig. 2). These results suggest that PHA producing plant growth promoting rhizobacteria are able to induce the production of IAA, solubilization of phosphorus, production of siderophore, ammonia & nitrate which helps in improving growth of plants [17].

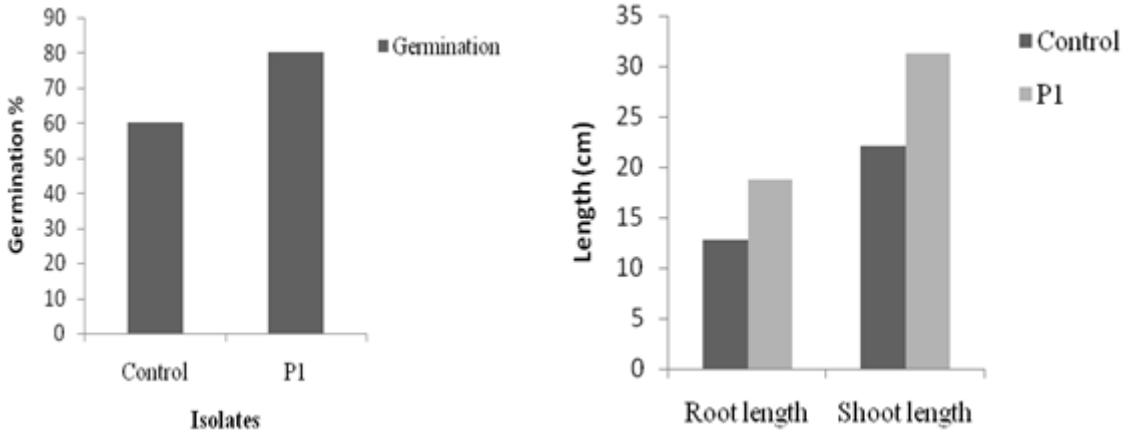
Hence in conclusion, it can be told that, though this investigation we have established the PGPR activity of PHA producing bacterial isolates



Seed germination Root & shoot length

Fig 1. Effect of PGPR isolates on Seed Germination, Root & Shoot length of Mung bean (Studied by Roll towel method)

(*Bacillus* sp. – P1,P2,P3,P4) of which *Bacillus* sp. P4 exhibited better activities, which can be aimed for agricultural uses. However, further scientific investigations are essential for evaluating the biotechnological properties of these soil bacteria.



Seed germination

Root & shoot length

Fig 2. Effect of PGPR isolates on Seed Germination, Root & Shoot length of Mung bean (Studied by Pot culture method)

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