

Original Research Article

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Exploring Phyllosphere Bacteria for Growth Promotion and Yield of Potato (*Solanum tuberosum* L.)

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ABSTRACT

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Plant growth promoting bacteria (PGPB) are plant associated micro-organisms known to induce plant defences and confer beneficial effects such as increased plant growth and low susceptibility to diseases caused by pathogens. Plant growth promoting bacteria are capable of significantly altering the growth phenotype of inoculated plants. Plants inoculated with PGPBs are capable to assimilate minerals and synthesize plant hormones. A total of 46 bacteria isolates were obtained from phyllosphere of different crops (Wheat, Pearl millet, Cotton, Mungbean and Potato). All the bacterial isolates were characterized for various plant growth promoting traits i.e., NH₃ excretion, IAA production, P-solubilization and Nitrate reductase. Seven isolates (POK-3, WHN-2, WHN-1, PMK-3, WHK-2, COJ-4, and MUK-1) were applied as foliar spray in different treatments. The plants inoculated with the phyllospheric bacterial isolates recorded an improved plant growth in terms of plant height and potato yield. Therefore, it can be concluded that application of PGPB has immense potential to be used as agricultural crop inoculants as they promote plant growth as well as improve the health and yield of the plants.

Introduction

Potato is an economical food, which belongs to genus *Solanum* (family Solanaceae) that consists of about 2,000 species and it provides a source of low cost energy to human diet. For vegetable purposes, potato has now become one of the most popular crops in India and cultivated in almost all states and under very diverse agro climate conditions. The states of Uttar Pradesh, West Bengal, Punjab, Bihar

and Gujarat accounted for more than 80 per cent share in total production. At world level, China, India, Russian Federation, USA, Ukraine, Germany and Poland shared more than 62 per cent of total global production potato. India ranks second, next to China in global production of potato (Anonymous, 2016). Plant-associated bacteria colonize the rhizosphere (rhizobacteria), the phyllosphere (epiphytes) and inside of plant tissues (endophytes). Phyllosphere bacteria can

promote plant growth and both suppress or stimulate the colonization and infection of tissues by plant pathogens (Lindow and Brandl, 2003). Phyllosphere microbiology has much to offer to the field of microbial ecology and promises to contribute the more effective and less environmentally damaging means of plant protection. Indole acetic acid (IAA) is one of the most physiologically active auxins. IAA is a common product of L-tryptophan metabolism by several microorganisms. Also, indole-3-acetic acid (IAA), the major naturally occurring auxin, is a signalling molecule in microorganisms because IAA affects gene expression in some microorganisms (Spaepen and Vanderleyden, 2016). They can also be involved in plant protection, which is due to direct interactions of microorganisms through the production of antibiotic compounds and competition for resources (Berg, 2009). Nitrogen is one of the most important nutrients required for plant growth and productivity as it forms an integral part of proteins, nucleic acids and other essential biomolecules (Bockman, 1997). Biological nitrogen fixation involves the conversion of nitrogen to ammonia by microorganism using a complex enzyme system identified as nitrogenase (Kim and Rees, 1994). Biological nitrogen fixation fixes about 60% of the earth's available nitrogen and represents an economically beneficial and environmentally sound alternative to chemical fertilizers (Ladha and Kundu, 1997). Plant growth-promoting rhizobacteria that fix nitrogen in non-leguminous plants are diazotrophs that form a non-obligate interaction with the host (Glick *et al.*, 1999). Phosphorus plays an indispensable biochemical role in photosynthesis, respiration, energy storage and transfer, cell division, cell enlargement and several other processes in the living plant. Phosphate solubilizing microorganisms are known to improve solubilization of fixed soil phosphorus and applied phosphates resulting in higher crop yields. Phosphate solubilizing

bacteria are a low cost solution that gives a thrust to economic development without disturbing ecological balance. Bio fertilizers are the substances that involve living microorganisms which are when applied to seed, plant and soil surfaces, colonizes the rhizosphere or the interior of the plant and then help to promote plant growth by increasing the supply or availability of nutrients to them (Vessey, 2003). Bio fertilizers add nutrients to the plants through the natural processes of biological nitrogen fixation, phosphorus solubilization, and synthesizing of growth-promoting substances. These biofertilizers can be used to reduce the use of chemical fertilizers and pesticides. So, the internal and external foliar microbiota and their likely key roles in plant performance, growth and health, current studies to understand better plant functioning and its responses and effects in a changing world, the importance and role of bacteria in phyllosphere making it available to the plants.

Materials and Methods

Isolation of bacteria from the phyllosphere of different crops

Bacteria were isolated from the phyllosphere of different crops (Wheat, Potato, Cotton, Mung bean and Pearl millet) grown at CCS HAU research farm by using serial dilutions method.

Plant growth promoting traits

Quantitative estimation of the indole acetic acid (IAA) was carried out by inoculating 1 ml of the respective bacterial suspension in 10 ml respective broth containing L-tryptophan (100 μgml^{-1}), and incubating it in the dark for 96 h. The concentration of IAA in the culture supernatant was estimated by the procedure of Salkowski's method (Glickmann and Dessaux, 1995). All the isolates were tested for their

ability to excrete ammonia by growing the cultures in 30 ml respective broths. Ammonia released in the supernatant was determined according to the method of Chaney and Marbach (1962). Phosphorus solubilization were tested on Pikovskaya's agar plates. Five µl of each culture broth was taken and spotted on these plates. These plates were incubated at 28±2⁰C for 4 days in a BOD incubator and observed for P-solubilization zone. Zone of solubilization and colony diameter were measured. Solubilization index was calculated using the following formula:

$$\text{Solubilization index} = (0.5 H)^2 / (0.5 A)^2$$

A = Colony diameter

H = Diameter (Colony + Halo zone)

Nitrate Reductase activity in bacterial isolates was determined by the method given by MacFaddin (1980). All isolates were inoculated in 30 ml nitrate broth and were incubated at 30⁰C for 8 days under stationary conditions of growth. Colour intensity was estimated at 540 nm using UV-vis spectrophotometer against a reagent blank within 5-10 seconds, after adding second reagent.

Selection of bacterial isolates for pot house studies in potato crop

On the basis of plant growth promoting traits, seven bacterial isolates were selected for the pot house studies. Isolate PMK3 (from Pearl millet phyllosphere) showed highest ammonia excretion along with all other traits, WHK2 showed maximum IAA production, COJ4 was showing highest phosphate solubilization and POK3, MUK1 and WHN2 showed all traits positively (Table 1).

Effect of foliar spray on growth of potato

Nine kg sandy loam soil was used to fill up the plastic pots. Seeds of potato crop (*Solanum*

tuberosum L.) var. Kufri bahar were sown in pots. After 20 days of sowing, foliar spray of selected bacterial isolates and *Azotobacter chroococcum* Mac27 were done according to the treatments. Plants were uprooted after 90 days of crop and observations on plant height and potato yield were recorded.

Statistical analysis

The experimental data were analysed using OPSTAT software available on university home page.

Results and Discussion

A total of 46 bacterial isolates were retrieved from five phyllospheric leaf samples (wheat, potato, cotton, pearl millet and mung bean) using dilution plating on Nutrient agar media, King's B media and Jensen's N₂ free media. Bacterial isolates were characterized for ammonia excretion.

All the 46 isolates were capable of excreting ammonia under shaking conditions ranging from 0.86 – 5.29 µg/ml. Maximum ammonia excretion was showed by PMK3 (5.29 µg/ml) followed by WHN2 (Table 1). Ammonia is the immediate product of nitrogen fixation in nitrogen fixing micro-organisms. Narula *et al.*, (1981) and Chandna (1982) reported that wild strains of *A. chroococcum* are able to release ammonia in medium containing 1% sucrose as a carbon source. Lakshaminarayana *et al.*, (2000) reported that diazotroph like *Azotobacter* had good capability of conversion of nitrogen to ammonia. Banik *et al.*, (2016) reported that thirty-five isolates from four rice genotype possessed the intrinsic PGP traits like indole (0.44-7.4 mg/ml), ammonia (0.18-6 mmol/ml), nitrite (0.01-3.4 mol/ml) and siderophore (0.16-0.57 mmol/ml) production. Inoculation of rice (cv. Swarna) seedling with selected isolates had a positive impact on plant growth.

Table.1 Plant growth promoting traits of bacterial isolate selected for pot house studies

Isolate no.	NH ₃ excretion (µg ml ⁻¹)	IAA production (µg ml ⁻¹)	Nitrate reductase activity (µg nitrite ml ⁻¹)	Phosphate solubilization index
POK3	4.80	15.98	64.46	2.89
PMK3	5.29	23.82	119.05	2.64
COJ4	3.73	6.94	52.32	19.18
MUK1	5.29	14.47	148.63	3.45
WHK2	5.29	31.06	59.15	-
WHN1	3.60	24.42	122.09	16.00
WHN2	4.81	17.79	131.19	12.96

Table.2 Effect of different treatments on plant height and yield of potato under pot-house conditions at 90 DAS

Treatment	DETAILS	Plant height (cm.)	Potato weight (kg.)
T1	CONTROL	21	0.083
T2	75% RDF (Recommended dose of fertilizer)	23.1	0.126
T3	RDF (Recommended dose of fertilizer)	23.9	0.132
T4	75% RDF + Mac27 (Tuber treatment)	25.3	0.131
T5	RDF + Mac27 (Tuber treatment)	27.4	0.152
T6	75% RDF + Mac27 (Foliar treatment)	28.2	0.132
T7	RDF + Mac27 (Foliar treatment)	30.4	0.148
T8	75% RDF + Bacterial isolate POK-3 spray	32.2	0.134
T9	RDF + Bacterial isolate POK-3 spray	30.8	0.156
T10	75% RDF + Bacterial isolate WHN-2 spray	27.6	0.146
T11	RDF + Bacterial isolate WHN-2 spray	28.5	0.156
T12	75% RDF + Bacterial isolate WHN-1 spray	29.9	0.137
T13	RDF + Bacterial isolate WHN-1 spray	30.6	0.150
T14	75% RDF + Bacterial isolate PMK-3 spray	35.5	0.160
T15	RDF + Bacterial isolate PMK-3 spray	36.0	0.194
T16	75% RDF + Bacterial isolate WHK-2 spray	26.7	0.149
T17	RDF + Bacterial isolate WHK-2 spray	28.5	0.155
T18	75% RDF + Bacterial isolate COJ-4 spray	30.5	0.160
T19	RDF + Bacterial isolate COJ-4 spray	31.7	0.173
T20	75% RDF + Bacterial isolate MUK-1 spray	26.07	0.145
T21	RDF + Bacterial isolate MUK-1 spray	26.7	0.159
CD at 5%		1.66	0.03

All the bacterial isolates were characterized for IAA production (in the presence of tryptophan). All the bacterial isolates produced IAA ranging from 0.30–31.06 µg/ml respectively. The highest IAA production was observed in isolate WHK2 (31.06 µg/ml) followed by COK3 that produced (29.55 µg/ml) (Table 1). This is also supported by many research workers who

have reported that IAA production varies with species, strains and growth conditions (Tien *et al.*, 1979; Pathak *et al.*, 1995). Kurepin *et al.*, (2014) reported that inoculation of two potato cultivars with *Burkholderia phytofirmans* strain PsJN resulted in twofold to threefold increase in IAA and CK levels, expressed on a per plant basis. In the present piece of work, it was observed that fifteen isolates formed P-

solubilization zone on Pikovskaya's agar medium plates and their P- solubilization index (P-SI) varied from 1.44–19.18. COJ4 showed maximum P-SI (Table 1). In similar way, Shahab *et al.*, (2009) screened 28 bacteria isolated from the different soil samples of Karachi for P-solubilization and found that three strains CMG 860, CMG 854 and CMG 857 formed large haloes (20-40 mm within 4 days of incubation). Chandrasekeran and Mahalingam (2014) collected the rhizosphere soil samples and processed for the presence of phosphate solubilizing microorganisms. The maximum solubilization index and inorganic phosphorus production was with *Bacillus* sp. and *Micrococcus* sp.

The reduction of nitrate to nitrite is the best documented step in this system and is catalyzed by nitrate reductase enzyme. Nitrate reductase activity of all bacterial isolates was checked. All the 46 isolates showed nitrate reductase activity in nitrate broth ranging from 11.45-148.63 μg nitrite ml^{-1} . Isolate MUK1 showed maximum nitrate reductase activity (Table 1). Similarly, Dunn *et al.*, (1979) reported that at dissolved oxygen tension of 15mmHg (2kPa) and below, nitrate limited continuous culture of *Klebsiella* K3 12 synthesized nitrate reductase (NR) and excrete ammonia. Furina *et al.*, (2002) also tested *A. chroococcum* C8 and *A. indicum* 8, for the ability to reduce nitrate. Both cultures reduced nitrate to nitrite under anaerobic conditions. The nitrite amount produced by the *A. indicum* 8 was higher than the amount produced by *A. chroococcum* C8.

Effect of selected bacterial isolates on plant height was observed at the time of harvest being made after 90 days. Plant height ranged from 21.0 – 36.0 cm at 90 DAS. Maximum plant height was observed with foliar application of isolate PMK3 along with RDF i.e. treatment T15 (36.0 cm) followed by

foliar application of isolate COJ4 along with RDF i.e. treatment T19 (31.7cm) (Table 2). Yield of potato ranged from 0.083-0.194 kg/pot at 90 DAS. Highest potato yield was observed in foliar application of isolate PMK3 along with RDF i.e. treatment T15 which was 0.194 kg followed by foliar application of isolate COJ4 along with RDF i.e. T19 (0.173 kg) (Table 2).

Similarly, Javaid (2006) also observed highest shoot length, shoot and root dry weight in the foliar spray of effective microorganisms, which was significantly greater than the rest of the treatments. Salim *et al.*, (2013) observed that biofertilizers, micronutrients and potassium silicate treatments gave significant increase in shoot fresh and dry weights of wheat as compared to the control in both seasons.

Concerning shoot dry weight, the superiority was due to the *Azotobacter* and mycorrhiza treatments as compared to control and other treatments in both seasons. Singh *et al.*, (2016) observed that emergence of tuber, number of haulms, plant height, number of leaves and dry weight of plant was significantly improved with the various treatments of organic sources of nutrients and biofertilizer. The increase in growth parameter due to large amount of vermicompost and tuber were treated with *Azotobacter* and phosphobacteria improved the soil structure, availability of nitrogen and phosphorus in soil, also be improved the water holding capacity, soil aeration and supply of nutrients through the use of balance fertilizers, which resulted in better plant growth in comparison to control plots. Earlier as discussed, bacterial isolate PMK3 established well and helped in overall functioning of plant machinery. This results in the better growth and yield of potato plant, when applied aerially along with dose of fertilizer.

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