Screening of Epiphytic Isolates from Different Crops for Plant Growth Promoting Traits

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A B S T R A C T

The role of Zn as micronutrient in healthy growth of plants is very important. The microorganisms present in phyllospheric environment have capability to increase micronutrient availability to plants. Total forty eight isolates were obtained from five different crops (wheat, mustard, cotton, pearl millet and moong). All isolates were characterized for different plant growth promoting attributes i.e., production of ammonia, IAA, and solubilization of Zinc, Phosphate. Among all isolates total 87.5 % showed IAA and Ammonia production in different range. Total 72.91 % Zn solubilization and 60.41 % P solubilization showed by isolates respectively. These plant growth promoting attributes are helpful in enhancing crop productivity. So these can be used as bioinoculant or as spray in crops.

Keywords: Zn, Epiphyte, Crop, IAA, Ammonia, P

Introduction

Microorganisms are present in soil, near and above the roots and also present on aerial part of plants like leaves. Ruinen (1961) introduced term phyllosphere for plant leaf surface. The termed epiphytes used for inhabitants of phyllosphere. The diverse range of bacteria, filamentous fungi, yeasts and algae which are important for plant health and growth are present on phyllosphere (Whipps et al., 2008; Vorholt, 2012). The total count of microbes approximately is about 6.4 x 10^8 km^2 (Morris and Kinkel, 2002) in which each km^2 is estimated to harbor 10^{16}–10^{18} bacteria (Bulgerelli et al., 2013) on leaf surface. Phyllospheric microbes have important role in growth promotion of plants due to their ability to produce different plant growth hormone; production of antibiotic compounds which role in plant protection (Berg, 2009). Zinc as micronutrient have also significant role in different metabolic processes i.e., carbohydrate, lipid, nucleic acid and protein synthesis as well as their degradation. Zn is an integral component of many enzyme structures and also present in all six enzyme classes, i.e. oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases. Zinc is also role in control synthesis of indole
acetic acid, chlorophyll synthesis and carbohydrate formation. It is part of phosphatase enzyme structure so its role in native phosphorous nutrient mobilization in rhizosphere also. Keeping in the view, the importance of zinc and Phyllospheric microbes this investigation was carried out. Isolation of phyllosphere microbes from different leaves of crops were carried out. These all isolates were checked for their plant growth promoting traits.

Materials and Methods

Isolation from phyllosphere of different crops

Different bacteria were isolated from the phyllosphere of different crops (wheat, mustard, cotton, moong and Pearl millet) grown at CCS HAU research farm. Different type of medium i.e., nutrient agar, Luria Bertani and Jensen’s N₂ free medium were used for isolation. Based on the morphological appearance, different bacterial colonies were selected, maintained and purified on respective medium slants. Cultures were stored at 4°C for further studies.

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 μg ml⁻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). Solubilisation of zinc were assessed according to the procedure of Saravanan et al., (2007) using nutrient agar medium containing 0.1 % insoluble zinc compounds [ZnO, ZnS, Zn₃(PO₄)₂ and ZnCO₃], respectively solubilization index was calculated. Phosphorus solubilization was 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±2°C 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:

\[
\text{Solubilization index} = \frac{(0.5 \ H)^2}{(0.5 \ A)^2}
\]

A = Colony diameter
H = Diameter (Colony + Halo zone)

Results and Discussion

Isolation of bacteria from the phyllosphere of different crops

Total forty eight isolates from leaves of wheat, mustard, cotton, pearl millet and moong were isolated. These isolates obtained on three types of medium nutrient agar, Luria Bertani and Jensen’s N₂ free media were used (Table 1). Phyllosphere population include all type of microbes like different genera of bacteria, filamentous fungi, yeasts, algae, and less frequently, protozoa and nematodes. These have important role in plant health and growth promotion (Whipps et al., 2008; Vorholt, 2012). The epiphyte population is dominated by bacteria ranging from 10⁵ to 10⁷ cells per gram of plant material (Yadav et al., 2005).

Screening of epiphytic isolates for plant growth promoting traits

Total 87.5 % isolates were showed ammonia and IAA production. The range of Ammonia production vary from 0.109 - 4.890 μg/ml. Highest ammonia excretion was observed in
isolate COJ1 (4.890 μg/ml) followed by WHN2 (3.208 μg/ml) isolate (Table 2).

Ammonia is the immediate product of nitrogen fixation in nitrogen fixing microorganisms. 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. Inoculation of maize and wheat with ammonia excreting bacteria (Azotobacter and Azospirillum) increased plant growth, nutrient uptake, yield and development of lateral roots (Rolfe et al., 1997; Dobbeleare et al., 2001; Ozturk et al., 2003).

The range of IAA production from 0.029 - 28.173 μg/ml vary. The highest IAA production was observed in isolate MNJ1 (28.173 μg/ml) followed by WHL6 that produced 25.199 μg IAA/ml after 4 days of incubation at 28±2°C (Table 2). The IAA production varies with species, strains and growth conditions (Pathak et al., 1995). Five major classes of phytohormones i.e., auxin, gibberlins, ethylene, cytokinins and abscisic acid are role in plant growth hormone. IAA is a member of auxin family, influences plant growth (Tsakelova et al., 2006).

**Fig.1** Zinc solubilization zone shown by bacterial isolates on MM agar plates containing zinc oxide

![Zinc solubilization zone](image1)

**Fig.2** Phosphate solubilization zone shown by bacterial isolates on Pikovskaya’s agar plates

![Phosphate solubilization zone](image2)
Table.1 List of bacteria isolated from phyllosphere of different crops

| Site of collection of leaves samples | Medium used for isolation | No. of isolates |
|-------------------------------------|---------------------------|-----------------|
| Wheat phyllosphere HAU research farm, Hisar | Nutrient agar, Luria Bertani, Jensen’s N₂ free medium | 23 |
| Mustard phyllosphere HAU research farm, Hisar | MUL1, MUL2, MUL3, MUL4 | 7 |
| Cotton phyllosphere HAU research farm, Hisar | COL1, COL2, COL3, COL4 | 8 |
| Pearl millet phyllosphere HAU research farm, Hisar | PML1, PML2 | 5 |
| Moong phyllosphere HAU research farm, Hisar | MNL1, MNL2 | 5 |
| Total no. of isolates | 14 | 19 | 15 | 48 |

Table.2 Characterization of bacterial isolates for plant growth promoting traits

| Isolate no. | Source (phyllosphere) | NH₃ Excretion (µg ml⁻¹) | IAA Production (µg ml⁻¹) | Zinc Solubilization index | Phosphate Solubilization index |
|-------------|------------------------|-------------------------|--------------------------|---------------------------|-------------------------------|
| WHL-1       | Wheat                  | 2.76                    | 13.77                    | -                         | -                             |
| WHL-2       | Wheat                  | 2.15                    | 14.35                    | -                         | -                             |
| WHL-3       | Wheat                  | 1.56                    | 6.79                     | 1.71                      | 2.94                          |
| WHL-4       | Wheat                  | 2.61                    | 8.31                     | 4.06                      | -                             |
| WHL-5       | Wheat                  | 0.10                    | 15.60                    | -                         | 3.45                          |
| WHL-6       | Wheat                  | 1.75                    | 25.20                    | -                         | -                             |
| WHL-7 | Wheat | 1.98 | 3.06 | - | - |
|-------|-------|------|------|---|---|
| MUL-1 | Mustard | - | 3.06 | - | - |
| MUL-2 | Mustard | 0.97 | 7.41 | - | - |
| MUL-3 | Mustard | 1.38 | 24.20 | 3.24 | - |
| MUL-4 | Mustard | 1.14 | 2.39 | 5.76 | 2.64 |
| COL-1 | Cotton | 0.50 | 3.44 | 4.00 | 16.00 |
| COL-2 | Cotton | 0.50 | 22.10 | 4.00 | 9.00 |
| COL-3 | Cotton | 0.98 | 3.90 | 2.12 | 12.96 |
| COL-4 | Cotton | 0.99 | 4.55 | - | - |
| PML-1 | Pearl Millet | 0.78 | - | - | - |
| PML-2 | Pearl Millet | 1.134 | 0.03 | - | - |
| MNL-1 | Moong | 1.12 | 10.18 | 0.90 | 2.09 |
| MNL-2 | Moong | 1.92 | 8.31 | 2.25 | 2.42 |
| WHJ-1 | Wheat | - | 2.33 | 8.16 | 1.44 |
| WHJ-2 | Wheat | 0.67 | 7.00 | 5.06 | - |
| WHJ-3 | Wheat | - | 8.49 | 5.29 | - |
| WHJ-4 | Wheat | 0.98 | 8.14 | 12.75 | 5.06 |
| WHJ-5 | Wheat | 0.98 | 2.48 | 4.84 | 2.25 |
| WHJ-6 | Wheat | - | 0.26 | - | - |
| WHJ-7 | Wheat | 0.20 | 0.44 | 6.53 | - |
| WHJ-8 | Wheat | 1.27 | 3.20 | 5.29 | 1.69 |
| MUJ-1 | Mustard | 1.27 | 3.27 | 5.76 | - |
| MUJ-2 | Mustard | 1.14 | - | 7.84 | 1.56 |
| COJ-1 | Cotton | 4.90 | 2.60 | 4.00 | 9.00 |
| COJ-2 | Cotton | 2.74 | 7.99 | 7.84 | 4.84 |
| PMJ-1 | Pearl Millet | 1.98 | 4.46 | 2.98 | 4.84 |
| PMJ-2 | Pearl Millet | 2.00 | 1.66 | 1.85 | 6.61 |
| PML-2 | Pearl Millet | 1.134 | 0.03 | - | - |
| MNL-1 | Moong | 1.12 | 10.18 | 0.90 | 2.09 |
| MNL-2 | Moong | 1.92 | 8.31 | 2.25 | 2.42 |
| WHJ-1 | Wheat | - | 2.33 | 8.16 | 1.44 |
| WHJ-2 | Wheat | 0.67 | 7.00 | 5.06 | - |
| WHJ-3 | Wheat | - | 8.49 | 5.29 | - |
| WHJ-4 | Wheat | 0.98 | 8.14 | 12.75 | 5.06 |
| WHJ-5 | Wheat | 0.98 | 2.48 | 4.84 | 2.25 |
| WHJ-6 | Wheat | - | 0.26 | - | - |
| WHJ-7 | Wheat | 0.20 | 0.44 | 6.53 | - |
| WHJ-8 | Wheat | 1.27 | 3.20 | 5.29 | 1.69 |
| MUJ-1 | Mustard | 1.27 | 3.27 | 5.76 | - |
| MUJ-2 | Mustard | 1.14 | - | 7.84 | 1.56 |
| COJ-1 | Cotton | 4.90 | 2.60 | 4.00 | 9.00 |
| COJ-2 | Cotton | 2.74 | 7.99 | 7.84 | 4.84 |
| PMJ-1 | Pearl Millet | 1.98 | 4.46 | 2.98 | 4.84 |
| PMJ-2 | Pearl Millet | 2.00 | 1.66 | 1.85 | 6.61 |

- = ND (Not Detectable)
Kannapiran and Ramkumar (2011) tested 17 bacterial isolates including *Azotobacter chroococcum*, *Azotobacter beijerinckii*, *Pseudomonas aeruginosa* and *Bacillus* sp. for indole acetic acid production in vitro. Among these isolates, amount of IAA produced by *Azotobacter chroococcum* and *Azotobacter beijerinckii* were 23.6 and 17.6 μg ml⁻¹ respectively.

All the isolates were screened for zinc solubilization on minimal medium (MM) agar plates containing insoluble zinc source i.e. zinc oxide. Solubilization potential for each isolate was determined by measuring zone of solubilization. Zinc solubilization index varied from 0.34 - 37.05 (Fig. 1). Isolate MNN1 showed highest Zn solubilization index (37.05) followed by MNN2 (31.94) (Table 2). Saravanan et al., (2003) also assessed zinc solubilizing ability of *Bacillus* sp. (isolated from zinc ore) and *Pseudomonas* sp. (isolated from paddy soil) using zinc oxide, zinc sulphide and zinc carbonate in both plate and broth assays. ZSB-O-1 isolate (*Bacillus* sp.) showed highest dissolution in the zinc sulfide (Sphalerite ore) and ZSB-S-2 (*Pseudomonas* sp.) showed more solubilizing ability in the zinc oxide. Sharma et al., (2014) also screened forty eight endophytic bacteria isolated from soybean and summer mungbean rhizosphere for zinc solubilizing ability in Tris- minimal medium separately amended with inorganic zinc compounds viz. zinc oxide (ZnO) and zinc phosphate Zn₃(PO₄)₂ by plate assay method and observed that only 2 isolates solubilized ZnO whereas 2 isolates solubilized Zn₃(PO₄)₂.

Indian soils are normally deficient in available phosphorus. A large portion of the soil phosphorus (P) is in unavailable form, which should be solubilized so that plants can use it. Also, phosphate solubilizing bacteria are able to mobilize more phosphorus to the plants and improve plant growth (Egamberdiyeva et al., 2004). Thus keeping in view importance of P for the plants, bacterial isolates were checked for phosphate solubilization. Twenty nine bacterial isolates solubilized phosphate with an index ranging from 0.53 - 18.78 (Fig. 2). Highest phosphate solubilization index was observed in isolate PMN1 (18.78) followed by isolate COL3 (12.96) (Table 2). Woo et al., (2010) isolated phosphate solubilizing bacterial isolates from the rhizosphere of Chinese cabbage and found that 10 strains which were having higher solubilization potential also solubilized insoluble ZnO. Duangpaeng et al., (2012) isolated five endophytic bacteria that solubilized tricalcium phosphate in Pikovskaya’s agar. Solubilization index of CHR3I01, BRR1I04, BRR3I01, CHR4I07 and CHR2I02 were 10.0, 7.0, 3.9, 3.2 and 2.7 respectively. 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.

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