# Isolation and characterization of endophytic bacteria from endorhizosphere of sugarcane and ryegrass

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**Citation**

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**Abstract**

The present study was conducted with a view to isolate and characterize endophytic bacterial diversity from endorhizosphere of sugarcane (Saccharum sp.) and rye grass (Lolium perenne). Eight bacterial isolates from sugarcane and seven from rye grass were obtained and identified as Azospirillum, Bacillus, E. coli and Pseudomonas. S1 and R1 (Azospirillum) sugarcane and ryegrass isolate exhibited maximum nitrogenase activity of 1221.0 and 1168.3 nM $C_2H_4h^{-1}$ $mg^{-1}$ protein respectively while the maximum IAA production was recorded in S5 (E. coli) and R7 (Bacillus) i.e. 19.3 and 20.0 mg $ml^{-1}$ respectively. The maximum siderophore production was observed in S6 (E.coli) and R6 (Pseudomonas) i.e. 2.4 and 3.0 mg $ml^{-1}$ respectively. Only S5 (E.coli) was observed to be solubilize phosphate (21 mg P). The endorhizosphere of sugarcane and rye grass exhibited endophytic bacterial diversity not only in terms of different types of isolates but also in terms of functional diversity.

## INTRODUCTION

Endophytes are of agronomic interest as they can enhance plant growth in non-leguminous crops and improve their nutrition through nitrogen fixation (Boddey et al 2003). Endophyte being a broader term includes fungal, actinomycetal as well as bacterial forms. Endophytic bacteria reside within the interior of plants without causing disease or forming symbiotic structures. Thus, they inhabit various tissues of seeds, roots, stem and leaves (Johri 2006). Majority of them are non-specific regarding their host preference which holds greater promises for plant growth promotion and increased yield in agriculturally important grasses such as sugarcane (Saccharum sp), rice (Oryza sativa), wheat (Triticum aestivum), sorghum ((Sorghum bicolor), maize (Zea mays), Panicum maximum, Brachiaria spp and Pennisetum purpureum. Endophyte-infected plants often grow faster than non-infected ones which may partly by due to endophytic production of phytohormones such as indole-3-¬acetic acid (IAA), cytokinin, and other plant growth-promoting substances such as production and secretion of siderophores (Fe chelating ligands) and/or partly owing to the fact that endophytes could have enhanced the host's uptake of nutritional elements such as nitrogen and phosphorus.

Thus, study was conducted with a view to isolate endophytic bacteria and to asses functional potentialities in relation to plant growth promoting activities i.e. IAA, phosphate solubilizer, nitrogenase activity and siderophore production. These can be recommended as bioinoculants for non-leguminous crops, which can help to reduce dependence on chemical fertilizers and provide a step forward towards sustainable agriculture.

## MATERIALS AND METHODS

Sugarcane and ryegrass root samples (eight each) were collected from different areas of Ludhiana i.e Jagraon, Gujjarwal, Laddowal, Samrala and Punjab Agricultural University. Isolation of bacterial diversity was done by using Reis method (Reis et al 1994). Morphological characterization of all the isolates was done on the basis of colony colour, appearance, motility and gram staining. Biochemical characterization includes acid production, catalase production and sugar utilization tests. The intrinsic antibiotic resistance spectra was deciphered using 7 antibiotics Amikacin, Ampicillin, Choramphenicol, Gentamycin, Oxytetracycline, Pencillin G and Streptomycin in a disc assay. Commercially available discs impregnated with known ppm of antibiotic were used and zone of inhibition around the disc was observed to infer the results. Functional characterization was done by performing acetylene reduction assay for nitrogenase activity (Hardy et al 1973). Qualitative screening of phosphate solubilizing isolates was done by using NBRI-BPB medium (Nautiyal

1999). Quantitative characterization of isolates for phosphate solubilization was done by Jackson's method (1973) by using Ammonium molybdate-Ammonium vandate reagent. Siderophore production was estimated by using Arnow's method. IAA production of isolates was performed by method of Gorden and Weber (1951)

## RESULTS AND DISCUSSION

## MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION

Eight isolates from sugarcane (S1-S8) and seven isolates from ryegrass (R1-R7) were obtained from endorhizosphere of both the crops. The growth of cultures S3, S4 (sugarcane) and R1, R2 (rye grass) on respective media was recorded to be circular and transparent in colour. Morphologically, the cells appeared to be gram negative, motile vibroids. Sugarcane (S1) and rye grass isolate (R6) exhibited formation of yellow colored irregular colonies and cells were motile, gram negative rods as observed under microscope. Lindberg and Granhall (1984) reported similar morphological characteristics in dinitrogen fixing bacteria from rhizosphere of temperate cereals and forage grasses.

Another kind of cultural characters were also observed for (S2) sugarcane and (R7) ryegrass isolates that appeared to have greyish white irregular colonies and morphologically were gram positive motile coccobacilli. (Table 1) These findings are similar to morphological characteristics of Bacillus sp. isolated from coconut palm (Prabhu et al 2000)

### Figure 1

Table1 Morphological characterization of bacterial isolates from sugarcane and rye grass

| Isolates | Colony color | Topography | Shape | Gram reaction | Motility |
|---|---|---|---|---|---|
| S1 | Yellow | Irregular | Rods | - | Motile |
| S2 | Greyish white | Irregular | Coccobacillus | + | Motile |
| S3 | Transparent | Small circular | vibroids | - | Motile |
| S4 | Transparent | Circular | vibroid | - | Motile |
| S5 | Creamish white | Irregular | Rods | - | Non-motile |
| S6 | Cream | Circular | Rods | - | Motile |
| S7 | Cream | Circular | Rods | - | Motile |
| S8 | Cream | Circular | Rods | - | Non-motile |
| R1 | Transparent | Small circular | vibroids | - | Motile |
| R2 | Transparent | Small circular | vibroids | - | Motile |
| R3 | Cream | Circular | Rods | - | Non-motile |
| R4 | Cream | Circular | Rods | - | Motile |
| R5 | Cream | Circular | Rods | - | Motile |
| R6 | Yellow | Circular raised | Rods | - | Motile |
| R7 | Greyish white | Irregular | Coccobacillus | + | Motile |

Acid production: Amongst sugarcane and rye grass isolates S3, S4, S5, S8, R4 and R7 exhibited acidic reaction on Norris media (Table2) However S1, S2, S6, S7 amongst sugarcane isolates and R1, R2, R3, R5, R6 amongst ryegrass isolates exhibited negative reaction for acid production. Standard Azospirillum strain showed acid production, which was also recorded in S3 and S4 (Azospirillum).

Catalase production: Out of eight sugarcane isolates only five (S2, S3, S4, S5 and S8) whereas six rye grass isolates (R1, R2, R4, R5, R6, R7) exhibited catalase positive reaction i.e. showed bubble formation on addition of hydrogen peroxide (Table 2). These results corroborate to findings of Lindberg and Granhall (1984) who reported catalase production in Azospirillum and E. coli isolated from temperate cereals and forage grasses. In similar report, Pseudomonas was found negative for catalase activity which shares similarity with sugarcane isolate S1 (Pseudomonas). Muthukumarasamy et al (2002) reported catalase production in an endophytic bacteria Gluconacetobacter sp. isolated from sugarcane endorhizosphere.

## CARBOHYDRATE UTILIZATION

Three sugarcane isolates S4, S7 and S8 and four ryegrass isolates R1, R2, R6 and R7 showed acid production in sugar fermentation broth supplemented with mannitol which indicates mannitol utilization as carbon source (Table2). Isolate S3 (sugarcane) and isolates R1, R2, R3, R4 (ryegrass) were able to utilize lactose. Galactose was utilized by sugarcane isolates (S5, S6) and ryegrass isolates (R1, R2, R3). Two ryegrass isolates i.e. R1 and R2 and two sugarcane isolates S2 and S3 were able to utilize xylose, which is a ketopentose. Majority of isolates among sugarcane isolates (S1, S2, S3, S5, S7) and ryegrass (R1, R3, R5, R6, R7) were able to utilize glucose indicating glucose to be a preferred carbon source. Lindberg and Granhall (1984) reported that E. coli was found to produce gas in fermentation broth in the presence of glucose and these findings were similar to our isolates i.e.S5, S7, R3 and R5 (E. coli). Muthukumarasamy et al (2002) reported that Gluconacetobacter sp. isolated from sugarcane were able to utilize galactose, xylose and mannitol.

**Figure 2**

Table1. Biochemical characterization of bacterial isolates

| Isolates | Acid production | Catalase acitivity | Sugar utilization test | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Mannitol | Glucose | Lactose | Sucrose | Galactose | Xylose |
| S1 | - | - | - | + | - | + | - | - |
| S2 | - | + | - | + | - | + | - | + |
| S3 | + | + | - | + | + | + | - | + |
| S4 | + | + | + | - | - | - | - | - |
| S5 | + | + | - | + | - | - | + | - |
| S6 | - | - | - | - | - | - | + | - |
| S7 | - | - | + | + | - | - | - | - |
| S8 | + | + | + | - | - | - | - | - |
| R1 | - | + | + | + | + | + | + | + |
| R2 | - | + | + | - | + | + | + | + |
| R3 | - | - | - | + | + | - | + | - |
| R4 | + | + | - | - | + | - | - | - |
| R5 | - | + | - | + | - | - | - | - |
| R6 | - | + | + | + | - | + | - | - |
| R7 | + | + | + | + | - | + | - | - |

\+ - Positive reaction
\- - Negative reaction

## ANTIBIOTIC RESISTANCE SPECTRA

All sugarcane isolates were sensitive to gentamycin, amikacin and streptomycin. Isolate S5 (Bacillus) was observed to be sensitive to all antibiotics used and a similar antibiotic resistance pattern was exhibited by isolate S3 and S4 (Azospirillum). All ryegrass isolates were sensitive to gentamycin and R6 isolate (Pseudomonas) was observed to be sensitive to all antibiotics used. R3 along with R5 exhibited same pattern of antibiotic resistance i.e. sensitivity to chloramphenicol, gentamycin, amikacin and resistance to pencillin G, ampicillin and oxytetracycline (Table 3).

**Figure 3**

Table3. Antibiotic resistance spectra of bacterial cultures

| Antibiotics (mcg/disc) | Sugarcane isolates | | | | | | | | Rye grass isolates | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | R1 | R2 | R3 | R4 | R5 | R6 | R7 |
| Chloramphenicol | - | + | + | + | - | - | + | + | + | + | - | - | - | - | - |
| Penicillin G | + | - | + | + | - | + | + | - | - | - | + | - | + | - | - |
| Gentamycin | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Amikacin | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Streptomycin | - | - | - | - | - | - | - | - | - | + | - | + | - | - | - |
| Oxytetracyclin | + | + | - | - | - | + | + | + | - | - | + | + | + | - | + |
| Ampicillin | - | + | + | + | - | + | - | + | + | + | + | + | + | - | - |

\+ resistant to antibiotic
\- sensitive to antibiotic

## PHOSPHATE SOLUBILIZATION

Out of all fifteen isolates only sugarcane isolate S6 (E. coli) was observed to solubilize phosphate. The amount of phosphate solubilized by S6 isolate was observed to be 21 mg P. Mikanova and Kubat (1994) reported high phosphate solubilizing activity (25-26 mg P) from strains of Rhizobium trifolii

## NITROGEN FIXATION ABILITY (ARA)

The range of nitrogenase ativity in case of sugarcane isolates was 217.3 -1221.0 nM $C_2H_4h^{-1}$ g $^{-1}$ protein with maximum value recorded by isolate S3 (Azospirillum) i.e. 1221.0 nM $C_2H_4h^{-1}$ g $^{-1}$ protein. The ryegrass isolates exhibited nitrogenase activity in range of 121.3- 1168.3 n$MC_2H_4h^{-1}$ g $^{-1}$ protein with R1 (Azospirillum) recording highest value i.e. 1168.3 n$MC_2H_4h^{-1}$ g $^{-1}$ protein (Table4).

## SIDEROPHORE PRODUCTION

All the isolates produced siderophore in range of 0.7-3.0 mg $l^{-1}$ . Out of eight isolates of sugarcane only six were observed to produce siderophore. S6 isolate (E. coli) produced the maximum amount (2.4 mg $l^{-1}$ ) whereas isolate S2 and S8 were found to be negative for siderophore production. Six ryegrass isolates (R1, R2, R3, R5, R6, R7) were found to be positive for siderophore production (Table4). Amongst ryegrass, isolate R6 (Pseudomonas) produced maximum amount (3.0 mg $l^{-1}$ ) of siderophore.

**Figure 4**

Table 4. Functional potentiality of bacterial isolates

| Sugarcane isolates | Nitrogenase activity (n$MC_2H_4h^{-1}\mu g^{-1}$ protein) | Siderophore production (mg/l) | Indole acetic acid (IAA) production µg/ml |
|---|---|---|---|
| S1 | 434.70 | 0.7 | 9.5 |
| S2 | 755.70 | - | 11.0 |
| S3 | 1221.0 | 2.2 | 4.0 |
| S4 | 1035.0 | 1.1 | 6.5 |
| S5 | 328.0 | 1.9 | 19.3 |
| S6 | 217.30 | 2.4 | 16.4 |
| S7 | 618.40 | 0.9 | 12.0 |
| S8 | 621.50 | - | 13.6 |
| R1 | 1168.0 | 0.8 | 4.5 |
| R2 | 923.10 | 1.8 | 6.5 |
| R3 | 253.80 | 1.4 | 14.0 |
| R4 | 773.10 | - | 16.0 |
| R5 | 241.30 | 0.7 | 19.5 |
| R6 | 121.30 | 3.0 | 9.0 |
| R7 | 316.0 | 3.3 | 20.0 |

## INDOLE ACETIC ACID PRODUCTION (IAA)

All the isolates were observed to produce the phytohormone IAA, which ranged from 4-19.3 g ml $^{-1}$ among sugarcane isolates. Isolate S5 (Bacillus) produced maximum IAA i.e. 19.3 g ml $^{-1}$ .The IAA production varied from 4.5-20 g ml $^{-1}$ for rye grass isolates. The maximum amount of IAA (i.e. 20 g ml $^{-1}$ ) was recorded for R7 (Bacillus) ryegrass isolate (Table 4). Hassan et al (1998) isolated Azospirillum from inoculated rice roots, which exhibited production of significant level of IAA (2.0-21.6 gml).

## CONCLUSIONS

The isolates were identified as Azospirillum (S3, S4, R1 and R2), E. coli (S6, S7, S8, R3, R4 and R5), Bacillus ( S2, S5 and R7) and Pseudomonas (S1 and R6). S6 was found to be a phosphate solubilizer and also produced sufficient IAA .Maximum nitrogen fixation was reported in S3 and R4 (Azospirillum). Pseudomonas was reported as higher siderophore producer.

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