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# Altitudinal Variation in *Azospirillum* Species Collected from the Rhizosphere and Roots of *Zea mays* (L.)

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**Abstract:** The rhizosphere soil and roots of maize (*Zea mays* L.) plants were collected from plain land of Islamabad, from Durgai and from the Shringal Campus of Malakand University. *Azospirillum* was isolated, identified and characterized by physiological and biochemical tests using QTS (Quick Testing System) miniatured identification system from the 24 h old bacterial culture grown on Lb (liquid broth) and NFM (nitrogen free medium) suspended in saline solution. The rhizosphere and roots of maize plants collected from plain land of Islamabad at the altitude of 800 m contained *Azospirillum lipoferum* whereas both the roots and rhizosphere of maize plants collected from Durgai at the altitude of 3500 m and Shringal campus of Malakand University at the altitude of 4000 m contained *Azospirillum brasilense*.

**Key words:** *Azospirillum*, altitudinal variation

## INTRODUCTION

Since its rediscovery by J. Dobereiner and her collaborator in 1970 the species *Azospirillum* has gained the reputation of being the most studied plant-associative bacterium. Apart from direct agricultural application, *Azospirillum* is an excellent model for genetic studies of plant-associative bacteria in general. As the most researched associative bacterium. *Azospirillum* has also been used in production of Poly B-hydroxybutyrate (PHB) for medical use, degradation of pollutants, vitamin production and purification of residual water are slowly being introduced into this field.

Two species of the genus *Azospirillum lipoferum* and *A. brasilense* were identified by DNA/DNA homology experiments with a large number of strains.

*Azospirillum* species occur in soil and are enriched in root surface of many different plants. *A. lipoferum* and *A. brasilense* could be isolated from 30-90% of soil samples collected all over the world (Dobereiner *et al*., 1995; Del Gallo and Fabbre 1991).

The beneficial effects of *Azospirillum* on plants are enhanced when coinoculated with other microorganism. Apparently, coinoculation allows plants to have a more balanced nutrition and the absorption of Nitrogen and phosphorus and other mineral nutrients are improved. Combination with both Rhizobia and *Azospirillum* gave the highest yield (Hassouma *at al*., 1994).

*Azospirillum* can produce *in vitro* the phytohormones IAA, gibberellins, cytokinins, (Tien *et al*., 1979; Bashan, 1991) and ethylene. Sometime external application of synthetic hormones initiated the positive effect of Azospirillum on root development morphology. Nitrogen fixation was the first mechanism proposed to explain improved plant growth following inoculation with *Azospirillum*. Present investigation is aimed to isolate, characterize and identify *Azospirillum* species from the roots and rhizosphere of maize plants collected from 3 different altitudes.

## MATERIALS AND METHODS

Fresh maize soil from the rhizosphere and maize roots were collected from Islamabad (plain land, altitude 800 m), Durgai (altitude 3500 m) and Shringal campus of Malakand University( altitude 4000 m). The soil samples were shaken vigorously for 10-15 min in a test tube with hand. The soil suspension was centrifuged. Then serial dilution were made for the supernatant. Three vials of LB (liquid broth) and three vials of NFM were inoculated with diluted soil extracted from series of maize rhizosphere soil. After inoculation these vials were incubated at 30°C for 48-72 h. Then a thin layer of pellicle was observed. For rapid multiplication, the culture was grown in liquid media of LB and NFM and these LB and NFM media were inoculated from the vials that contain growth in a thin layer of pellicle.

After inoculation these media were kept in shaker at 28°C for 24 h. These media of LB and NFM were processed for QTS.

Physiological and biochemical tests were performed using QTS (24) miniaturized identification system (DESTO laboratories Karachi Pakistan) following the method of

Table 1: QTS (Quick Test System) for *Azospirillum* collected from rhizosphere of maize plants from three different places

| | | Plain land of Islamabad (800 m) | Durgai (3500 m) | Shringal campus (4000 m) |
|---|---|---|---|---|
| Test | Reaction | *Azospirillum lipoferum* | *Azospirillum brasilense* | *Azospirillum brasilense* |
| ONPG | ONPG | + | - | - |
| CIT | Sodium citrate | + | + | + |
| MALO | Sodium malonate | - | + | + |
| LDC | Lysine decarboxylase | + | + | + |
| ADH | Arginine dihydrolase | + | + | + |
| ODC | Orthinine decarboxylase | - | + | + |
| $H_2S$ | $H_2S$ production | - | - | - |
| URE | Urea hydrolysis | + | + | + |
| TDA | Tryptophane deaminase | + | + | + |
| IND | Indole | - | - | - |
| VP | Voger Proskaur (Acetion) | - | - | - |
| GEL | Gelatin hydrolysis | + | + | + |
| GLU | a) Acid from glucose | | | |
| | b) Nitrate reductase | | | |
| | c) $N_2$ Gas | - | + | + |
| MAL | Acid from maltose | - | - | - |
| SUC | Acid from sucrose | - | - | - |
| MAN | Acid from mannitol | - | + | + |
| ARA | Acid from arabinose | - | + | + |
| RHA | Acid from rhamnose | - | - | - |
| SOR | Acid from sorbitol | - | - | - |
| INO | Acid from inositol | - | - | - |
| ADON | Acid from adonitol | - | - | - |
| MEL | Acid from melibiose | - | - | - |
| RAF | Acid from raffinose | - | - | - |
| MOT | Motility | + | + | + |
| CO | Coytocrome Oxidase | + | + | + |

MacFaddin (1980). The bacterial cultures (24 h old) grown on LB (liquid broth) and NFM (nitrogen free medium)plates were suspended in saline solution.

Oxidase test was performed according to Steel (1961). The catalase test was performed according to the procedure of MacFaddin (1980).

Petri plates and vials containing the growth in the form of pellicles and colonies were observed under microscope. A very characteristic pellicle appeared that grow rapidly in to dense white undulated pellicles. These pellicles were easily recognized against blue medium. Simultaneously petri plates contain discrete colonies that differ in appearance on each of the agar plate culture of LB and NFM. The vials and petri plates containing microorganisms growth were observed under the microscope. *Azospirillum* species were very motile characterizing wiggling rods. After observation of growth of *Azospirillum* species, from first culture further subculture were made, in order to obtain pure colonies for reconviction of more transparent growth of *Azospirillum* species. This observation was repeated for all the rhizosphere of maize plants of different altitudes.

For isolation of *Azospirillum* from roots of maize plants, the roots were washed in tap water to remove adhering soil particle, thereafter surface sterilized in 10% chlorox and successively washed in sterile water and the roots were crushed in sterile water. The suspension was centrifuged and serial dilutions were made as described earlier.

QTS 24 is based on 25 biochemical tests in addition to cytochromes oxidase test and results revealed that the isolates obtained from rhizosphere of maize collected from plain land of Islamabad differ from those of isolates collected from Durgai and Shringal Campuses in biochemical tests of ONPG, Sodium malonate, ornithine decarboxylase, acid from glucose, nitrate reductase, acid from mannitol and acid from sucrose (Table 1). These results were compared with Bergeys Manual of Determinative Bacteriology (Holt *et al*., 1994).

## RESULTS AND DISCUSSION

According to the Bergeys Manual of Determinative Bacteriology (Holt *et al*., 1994), on the basis of biochemical test results (Table 2) indicated that the roots and rhizosphere of maize plant, collected from the plain land of Islamabad at the altitudes of (800 m) contained *Azospirillum lipoferum*, rhizosphere of maize plants collected from Durgai at the altitudeof 3500 m and Shringal campus of Malakand University at the altitude of 4000 m contained *Azospirillum brasilense*.

Table 2: QTS (Quick Test System) showing difference between *Azospirillum lipoferum* and *Azospirillum brasilense* and the selected biochemical reaction from the QTS test are represented for showing the differences in the identification of *Azospirillum lipoferum* and *Azospirillum brasilense* from maize root

| *Azospirillum lipoferum* | | | *Azospirillum brasilense* | | |
|---|---|---|---|---|---|
| Test | Reaction | | Test | Reaction | |
| ONPG | ONPG | + | ONPG | ONPG | - |
| MALO | Sodium malonate | - | MALO | Sodium malonate | + |
| ODC | Orthinine decarboxylase | - | ODC | Orthinine decarboxylase | + |
| GLU | a) Acid from glucose | | GLU | a) Acid from glucose | |
| | b) Nitrate reductase | | | b) Nitrate reductase | |
| | c) $N_2$ Gas | - | | c) $N_2$ Gas | + |
| MAN | Acid from mannitol | - | MAN | Acid from mannitol | + |
| ARA | Acid from arabinose | - | ARA | Acid from arabinose | + |

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