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# CHARACTERIZATION OF GROWTH PROMOTING RHIZOBACTERIA OF LEGUMINOUS PLANTS

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**A B S T R A C T**

The present investigation deals with the morphological and biochemical identification of rhizobacteria having plant growth promoting characteristics, with a focus on the family Papilionaceae, whose representatives are widespread in nature as leguminous crops. Eleven bacterial strains were isolated from the rhizoplane of pea (*Pisum sativum* L.), chickpeas (*Cicer arietinum* L.) and clover (*Trifolium pretense* L.). The media used to isolate the rhizosphere bacteria were Luria Bertani (LB) agar and Nutrient agar (NA). Rhizosphere bacteria were identifiedbased on different characteristic i.e. phenotypic and biochemical characters. The isolated strains were characterized and assigned to various genera and species, such as *Enterobacter aerogenes*, *Aureobacterium flavescens*, *Pseudomonas fluorescens*, *Kurthia*sp., *Acidovorax facilis*, *Bordetella pertussis*, *Corynebacterium* sp., *Bacillus* sp., *Curtobacterium albidum* and *Microbacterium lacticum*. All identified species are given FCBP accession numbers and were stored for further use.

**Keywords**: Bacterial isolation, biochemical tests, chickpea, clover, morphological characters, pea.

## INTRODUCTION

Papilionaceae is a large plant family in the dicotyledons consisting of about 16,400 species in 657 genera. Plants belonging to this family are found throughout the world, growing in many different environments and climates.The characteristic feature of the members of this family is the symbiosis with the bacteria genera *Rhizobium* and *Brady rhizobium*. A large number of important agricultural and food plants including pea, chickpea and clover belong to this family. Many free-living and rhizospheric bacteria have been isolated from leguminous plant (Markova *et al.,* 2005). Plant growth promoting rhizobacteria (PGPR) are a group of free-living saprophytic bacteria that can be found in the rhizosphere in association with root system and enhance the growth and development of plant either directly or indirectly (Arzanesh*et al.,* 2011; Amkraz*et al.,* 2010; Deepa *et al.,* 2010; Gutierrez *et al.,* 2010; Chen *et al.,* 2000). Plant growth promoting rhizobacteria belong to several genera including *Azospirillum*, *Azotobacter*, *Bacillus*, *Pseudomonas* and *Herbaspirillum* (Park*et al.*, 2008). The current research work was concerned with the isolation, phenotypic and biochemical identification of rhizobacteria having plant growth promoting characteristics, with a focus on the family Papilionaceae, whose representatives are widespread in nature as leguminous crops.

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## MATERIALS AND METHODS

**Sample collection:** The root free soil used for bacterial isolation was collected from the rhizosphere of *P. sativum*, *C. arietinum*and *T. pretense*. Rhizospheric soil samples were taken randomly from selected locations with the help of spade. Collected samples were placed in separate sterile labeled polythene bags and stored in a refrigerator at 4 °C till use.

**Isolation of bacteria:** Collected rhizospheric soil (10 g) was suspended in 90 ml of distilled water. An aliquot (100 µl) from serial dilutions was inoculated on LBA and NA medium (pH 6.5), incubated at 28±2 °C. Colonies formed after 24 hours were counted and single colonies were transferred to new plates for purification and further study.

**Identification of bacterial species:**First, morphological and cultural features of the bacterial colony were observed and recorded, followed by several biochemical

tests as the routine steps of bacterial identification.

**Morphological Features:** Morphological characteristics i.e., cell shape, Gram type, capsule stains, motility and pigmentation were recorded for identification. Growth ability on osmotic medium (containing 2% NaCl) and at 25°C and 40 °C were also observed.

**Biochemical Analysis:** Using the commercially available bacterial identification kit, Microgen TMGnA+B-ID Identification System (Microgen Bioproducts Ltd, Surrey, UK), pure colonies of bacteria was differentiated via the biochemical tests. Initially, preference for carbon source of the isolated bacteria was analyzed by providing a wide range of carbohydrate sources which include glucose, lactose, sucrose, inositol, sorbitol, mannitol and xylose. Sterile water was used as control (Holt *et al.,* 2000; Benson, 1996). Other biochemical tests included study of enzymatically catalyzed metabolic reactions such as citrate, Indole, Methyl red, nitrate reductase, oxidase, catalase, urease, malonate and gelatinase, hydrogen sulphide, arginine and lysine (Holt *et al.,* 2000; Benson, 1996). Identification of the isolates was performed by entering all the biochemical test results into the Microgen Identification System software.

**RESULTS AND DISCUSSIONS**

Microbial relation with their environment are multifaceted they may be harmful, beneficial and neutral and this symbiotic behavior effect the productivity of plant (Kennedy, 1998). However, researcher should focus to study of the favorable interactions of plants and microbes. Considerable attention has been given to the immense potential of using microbial strains for enhancing crop growth and yield in a sustainable manner.

The use of microbial technologies in agriculture is currently expanding quite rapidly with the identification of new bacterial strains, which are more effective in promoting plant growth. Free-living soil bacteria isolated from the rhizosphere, which have been shown to improve plant health or increase yield, are usually referred to as growth promoting rhizobacteria (Dastager *et al.,* 2010). This study on the bacterial flora from the rhizosphere of the family *Papilionaceae* has revealed that they possessed rather huge diversity of microorganisms. In recent investigation, different bacterial strains were isolated and identified by referring to the Bergey's Manual of Determinative Bacteriology, 9th edition.

Soil samples were collected from a root-free soil of rhizosphere of *P. sativum*, *C. arietinum*and *T. pretense*. Sampling was done in Lahore and Sargodha. In a present study, a total of ten different bacterial species were isolated from the rhizospheric soil (Table 1).

Table 1. Characterization of plant growth promoting rhizobacteria.

| Features | Reference Strains | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | R-1 | R-2 | R-3 | R-4 | R-5 | R-6 | R-7 | R-8 | R-9 | R-10 | R-11 |
| Morphological Characters | | | | | | | | | | | |
| Cell shape | rods | rods | rods | rods | rods | rods | rods | rods | rods | rods | rods |
| Gram type | -ve | +ve | -ve | +ve | -ve | -ve | +ve | +ve | +ve | +ve | +ve |
| Motility | -ve | +ve | +ve | +ve | +ve | +ve | -ve | +ve | -ve | +ve | +ve |
| Growth at 2% NaCl | -ve | -ve | -ve | -ve | +ve | -ve | -ve | -ve | -ve | -ve | -ve |
| Growth at 25 °C | -ve | +ve | -ve | -ve | -ve | -ve | -ve | +ve | +ve | -ve | -ve |
| Growth at 40 °C | -ve | -ve | -ve | +ve | +ve | +ve | -ve | -ve | -ve | -ve | -ve |
| Biochemical Tests | | | | | | | | | | | |
| Citrate utilization | -ve | -ve | -ve | -ve | -ve | +ve | -ve | -ve | -ve | -ve | -ve |
| Hydrogen sulfide | +ve | -ve | -ve | -ve | +ve | -ve | +ve | -ve | -ve | -ve | -ve |
| Lysine | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve |
| Nitrate reduction | -ve | +ve | +ve | -ve | +ve | +ve | -ve | -ve | +ve | -ve | -ve |
| Oxidase | +ve | +ve | -ve | -ve | +ve | +ve | +ve | +ve | +ve | -ve | -ve |
| Catalase | -ve | +ve | -ve | +ve | +ve | -ve | -ve | +ve | +ve | +ve | +ve |
| Urease | -ve | -ve | -ve | -ve | -ve | +ve | -ve | -ve | +ve | -ve | -ve |
| Gelatine | -ve | -ve | -ve | +ve | +ve | -ve | -ve | -ve | -ve | +ve | +ve |
| Malonate | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Enzymatic Activity | | | | | | | | | | |
| Inositol | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve |
| Sorbitol | -ve | -ve | +ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve |
| Glucose | -ve | -ve | -ve | -ve | +ve | +ve | -ve | +ve | -ve | -ve | -ve |
| Mannitol | +ve | -ve | -ve | -ve | -ve | -ve | +ve | -ve | -ve | -ve | -ve |
| Xylose | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve |
| Sucrose | +ve | -ve | -ve | -ve | -ve | -ve | +ve | +ve | -ve | -ve | -ve |
| Lactose | +ve | -ve | -ve | -ve | -ve | -ve | +ve | -ve | -ve | -ve | -ve |
| Arginine | -ve | -ve | -ve | -ve | +ve | -ve | -ve | -ve | -ve | -ve | -ve |

Table 2.List of bacterial strains identified and their accession number.

| Reference strain no. | Source | Species Identified | FCBP accession no. |
|---|---|---|---|
| R-1 | Rhizosphericsoil of *P. sativum* | *Enterobacter aerogenes* | FCBP-326 |
| R-2 | | *Aureobacterium flavescens* | FCBP-327 |
| R-3 | | *Pseudomonas fluorescens* | FCBP-328 |
| R-4 | | *Kurthia sp.* | FCBP-329 |
| R-5 | | *Acidovorax facilis* | FCBP-330 |
| R-6 | Rhizospheric soil of *C. arietinum* | *Bordetella pertussis* | FCBP-232 |
| R-7 | | *Corynebacterium* sp. | FCBP-233 |
| R-8 | | *Bacillus* sp. | FCBP-234 |
| R-9 | | *Curtobacterium albidum* | FCBP-236 |
| R-10 | | *Microbacterium lacticum* | FCBP-261 |
| R-11 | Rhizospheric soil of *T. pratense* | *Microbacterium lacticum* | FCBP-371 |

However, a maximum percentage of bacterial species obtained was, five from *P. sativum* and *C. arietinum,* one species from *T. pretense* (Table 2). Overall, a total of 7 bacterial isolates that were categorized under 11 different gram-positive staining bacteria and 4 gram negative were identified from R1 to R11. All the studied strains in the present work were motile except for R-1, R-7 and R-9. In addition isolates from R4-R6 managed to grow at 40°C, while R-5 showed growth pattern at 2% NaCl. Data on the morphological study is presented in Table 1. Different bacterial strains have different metabolic path ways (Sathishkumar*et al.,* 2008). Results showed that all isolates gave positive results with the carbohydrates (glucose) except R-5, R-6 and R-8. Besides, R-1 and R-7 has the ability to ferment lactose also while others did not. Isolate R-3 has exhibited able to ferment among all species whereas all species showed negative results on fermentation of inositol.

Bacteria are referred to as individuals or groups based on their patterns of growth under various chemical (nutritional) or physical conditions.Like all other living organisms, different groups of bacteria utilize different sources of energy to generate ATP, required for their maintenance and reproduction. Most of the bacteria prefer monosaccharides, for example glucose, as energy source while a few may prefer disaccharides or polysaccharides (Richard *et al.,* 2011). Furthermore, capacity of different bacterial strains to use various carbon compounds as energy source and enzyme activities of these strains are summarized in Table 1. The identified bacteria were deposited in First Fungal Culture Bank of Pakistan (FCBP). The reference number for all the bacterial species as well as their FCBP accession numbers is given in Table 2. The ability of bacterial strains to colonize roots and survive in soil is often limited, reducing the expected growth promoting effect (Normander and Prosser, 2000).

Furthermore, a good selection of a growth promoting strain requires an understanding on the dynamic and composition of the bacterial communities colonizing the rhizosphere and the characterization of its related plant growth promoting properties. To date, information on the microbial diversity and dynamic of population in agricultural soil are limited (Dunbar*et al.,* 2000; Normander and Prosser, 2000; Smit *et al.,* 2001). Thus, extensive studies on the nature of these isolates are

required in order to harness their potential as bio-inoculants in agriculture.

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