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MORPHOLOGICAL AND BIOCHEMICAL STUDIES ON BACTERIAL MICROFAUNA FROM LAHORE SOILS

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A B S T R A C T

Bacteria have direct influence on humans and their environment. Conservation of local bacterial fauna is therefore crucial for many purposes. Routinely, identification of bacteria is carried out on the basis of morphological or cultural characters combined with biochemical analyses. During present study, bacteria were isolated from rhizospheric soils, canal water and sugarcane stem. Five species of bacteria belonging to three different genera *viz., Acidovorax temperans, A. facilis, Burkholderia pseudomallei, B. cepacia* and *Ensifer adhaerens* were isolated and identified on the basis of morphological features and metabolic processes.

Keywords: Identification, metabolism, enzyme activity, carbohydrate, morphology.

INTRODUCTION

First Fungal Culture Bank of Pakistan (FCBP) was established in June 2003 as a research centre within the Institute of Mycology and Plant Pathology (renamed Institute of Agricultural Sciences) University of the Punjab Lahore, Pakistan. The objectives of this highly valuable project were conservation, identification and preservation of local microbial cultures, to supply these to researchers and collaborate with world microbial culture collection centers. FCBP is registered with World Data Centre for Microorganisms (WDCM), World Federation of Culture Collection (WFCC) and Microbial Research Center (MRCEN). Currently FCBP owns over 1200 fungal cultures and 400 bacterial isolates. We are aimed to upgrade the Fungal Culture Bank of Pakistan into a National Microbial Culture Centre. For this, we would like to add viruses and nematode cultures as well.

Bacteria are ubiquitous in nature and have the ability to colonize a wide variety of substrates (Cleenwerck and Paul, 2008). Correct identification of bacterial strains is beneficial for researchers and physicians in many aspects especially for their control or treatment (Hasibe and Dilek, 2011). In the absence of molecular data, morphological features combined with certain biochemical tests are performed as the part of identification procedure. In this way, sufficient data is obtained to suggest the identity of unknown bacteria (Ali and Naseem, 2012). Present study aimed to identify unknown bacterial isolates in the form of pure cultures.

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MATERIALS AND METHODS

Identification of bacteria is carried out following the standardized procedure starting with the colony and cell morphology followed by Gram staining and finally testing the metabolic activities of unknown strain.

Sampling and isolation of bacterial species: For each type of substrate, ten samples were selected randomly from different areas of Lahore and its vicinity. Such samples were placed in separate sterile bottles/polythene bags and stored in a refrigerator at 4 °C till use.

The Luria Bertani Agar (LBA) and Nutrient Agar (NA) media were prepared following the manufacturer's instructions for isolations. Using appropriate isolation technique (Ali and Naseem, 2011; Beishir, 1991; Hampton *et al.,* 1990), inoculation of bacteria was carried out under aseptic conditions. Inoculated petriplates were incubated at 37°C for 2-3 days. Single colonies were sub-cultured for purification. After 24 hours of incubation at 37°C, single colonies were

streaked on fresh media plates.

Identification of bacterial species: Identification of bacterial species was done by recording colony morphological features (Konem *et al.,* 1997) (color, shape, size, texture, margins and odor etc.) and cell microscopic characters (color, cell wall, contents, shape, arrangement, material, Gram stain, capsular material and motility).

The pure colonies were differentiated by biochemical tests (Holt *et al.,* 2000; Benson, 1996). All the biochemical tests were carried out using Microgen™ GnA+B-ID Identification System (Microgen Bioproducts Ltd, Surrey, UK). Such biochemical analysis includes certain enzymatic activities (citrate, nitrate reductase, oxidase, catalase, urease, malonate and gelatinase etc) and carbohydrate (inositol, sorbitol, glucose, mannitol, xylose, sucrose, lactose etc.) fermentation. Also arginine, lysine as well as hydrogen sulphide (H_2S) metabolism was studied. The interpretation of above mentioned biochemical tests results was carried out using Microgen Identification System software.

RESULTS AND DISCUSSION

During present study, a total of five different isolates were purified from various sources. Each isolate was given a reference number (B1 - B5) that was used throughout this study to represent the results of that particular bacterium. Detail of these isolates is summarized in (Table 1).

Table 1. Detail of the substrates used to isolate the bacterial strains.

Strain No.	Substrate	Locality
B1	Soil from canal	Lahore
B2	Rhizospheric soil of pea plant	Lahore
B3	Soil from canal	Lahore
B4	Rhizospheric soil of maize plant	Lahore
B5	Rhizospheric soil of citrus plant	Lahore

Cultural characters help in identification as well as classification of bacteria in different taxonomic groups (Harley, 2008). All the strains studied for present work were rod-shaped, Gram negative and motile bacteria. Moreover all strains were unable to grow on osmotic medium (containing 2 % NaCl) as well as at high temperature i.e. 40 °C. Only B1 managed to grow at 25 °C although all strains showed maximum growth at 37 °C. Morphological observations for the strains are compiled in Table 2.

Table 2. Morphological and cultural features of bacteria studied.

Feature	B1	B2	B3	B4	B5
Cell shape	rods	rods	rods	rods	rods
Gram type	-	-	-	-	-
Capsule stain	-	-	-	-	-
Motility	+	+	+	+	+
Growth on 2% NaCl	-	-	-	-	-
Growth at 25 ^oC	+	-	-	-	+
Growth at 40 ^oC	-	-	-	+	-

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 use monosaccharides, for example glucose, as energy source while few prefer disaccharides or polysaccharides (Richard *et al.,* 2011). Presence of different sets of enzymes in different bacteria enables them to utilize respective carbohydrate that can be found out simply by checking the presence of its byproducts (Bednarski, 2006). Results demonstrated that B1 has the ability to ferment lactose, sucrose and mannitol while three bacteria, B2, B3 and B4 could not show positive results with any of the carbohydrate compound tried. Finally B5 has the metabolic machinery to utilize glucose, lactose as well as sorbitol. Abilities of different isolates studied during present study to use various carbon compounds are given in Table 3.

Being metabolically different, bacteria can be identified on the basis of biochemical tests. Such tests actually show the ability of a specific enzyme to utilize a bio-

molecule as substrate (amino acids, proteins, lipids etc). Catalysis of these bio-molecules results in the production of by-products that can be determined. Results of these tests aid in identification of unknown bacteria (Harley, 2008). Enzymatic activities of bacterial isolates are recorded in Table 4.

Table 3. Carbohydrate source preference analysis of bacterial isolates.

Sugar compound	B1	B2	B3	B4	B5
Glucose	-	-	-	-	**+**
Lactose	**+**	-	-	-	**+**
Sucrose	**+**	-	-	-	-
Inositol	-	-	-	-	-
Sorbitol	-	-	-	-	**+**
Mannitol	**+**	-	-	-	-
Xylose	-	-	-	-	-

Results of citrate test suggested that none of the isolate was capable of citrate transport into the cell for fermentation. Nitrite was detected for strain B2, B3 and B5 in the reaction tube that was an indicative of nitrate conversion to nitrite by nitrate reductase (Reisner, 1978). Next to nitrate reductase, bacteria were analyzed for their ability to produce cytochrome oxidase i.e oxidase activity. Oxidase activity was positive for B1, B2 and B5. When the catalase test was performed, it revealed that only B4 has the ability to breakdown hydrogen peroxide. Positive urease activity was only recorded for B5 that is an indicative of low or no urea breakdown by rest of the strains B1, B2, B3 and B4. Results of gelatinase test demonstrated that only B4 contain gelatinase enzyme and hence has the ability digest the gelatin whereas only B1 has the ability to metabolize ferrous ammonium sulfate and produce hydrogen sulfide as byproduct. None of the bacteria showed to have malonate, arginine and lysine functional metabolisms (Table 4).

Table 4. Enzymatic activities of bacterial isolates.

Biochemical test	B1	B2	B3	B4	B5
Citrate	-	-	-	-	-
Nitrate reductase	-	+	+	-	+
Oxidase	+	+	-	-	+
Catalase	-	-	-	+	-
Urease	-	-	-	-	+
Malonate	-	-	-	-	-
Gelatinase	-	-	-	+	-
Hydrogen sulfide	+	-	-	-	-
Lysine	-	-	-	-	-
Arginine	-	-	-	-	-

Table 5. Species identified.

Strain Ref. No	Species Identified	FCBP accession No.
B1	*Acidovorax temperans*	FCBP211
B2	*A. facilis*	FCBP330
B3	*Burkholderia pseudomallei*	FCBP080
B4	*B. cepacia*	FCBP340
B5	*Ensifer adhaerens*	FCBP335

The identification of microorganisms including bacteria is essential for the advances in microbiology and medicines (Nitesh et al., 2011). During present experiment, a series of cultural and biochemical studies was conducted to identify bacteria. Upon compiling the data obtained from morphology and biochemical

analysis of unknown bacteria, results were generated using Microgen Identification System software. Identified bacterial cultures were deposited in FCBP. Identifies species versus their reference no as well as their FCBP accession numbers are given in Table 5.

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