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ISOLATION AND CHARACTERIZATION OF BACTERIAL SPECIES ASSOCIATED WITH WEED PLANTS

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ABSTRACT

During the current investigation, the presence of different bacterial strains were identified from four weeds viz. Parthenium hysterophorus (whitetop), *Convolvulus arvensis (lehli), Euphorbia esculenta (dhodak) and Chenopodium album (bathu). Weeds were collected from diiferent areas of Punjab university and main canal road sides as these are local and dominant agricultural weeds of Pakistan. Present study reported information concerning the isolation and identification of bacteria on the morphological and biochemical analysis. Thirteen species of bacteria were isolated in this study belonging to eight different family viz. Burkholderiaceae, Bacillaceae, Peptococcaceae, Comamonadaceae, Enterobacteriaceae, Corynebacteriaceae, Rhodospirillaceae and Moraxellaceae. Identification was further confirmed by their enzymatic activity and carbohydrates utilization. Although, E. esculenta and* P. hysterophorus *were the weeds found to be carrying maximum percentage of bacterial species. Cupriavidus sp., Bacillus sp., Peptococcus sp., Acidovorax facilis, Klebsiella sp., Yersinia ruckeri, Corynebacterium minutissimum, Bacillus farraginis, Enterobacter agglomerans, Azospirillum lipoferum, Acinetobacter lwoffii, Cedecea davisae, and Curtobacterium albidum were the identified bacterial species.*

Key words: Bacteria, biochemical tests, common weeds, identification.

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INTRODUCTION

The plant surface and their internal tissue colonized by different microorganisms including fungi, bacteria, yeast, protozoans, thereby occupying distinct habitat and behavior (Andrews, 1996; Petrini,

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1991). Nearly all plants retain a microbiota and some microbial colonizers take up residence in the tissues of plants (Bascom *et al*., 2009). Different microorganisms such as fungi and bacteria spend their life by invading the living tissues of plants and cause unapparent and asymptomatic infections entirely within plant tissues, but cause no symptoms of disease (Muzzamal *et al*., 2012).

It is note worthily that nearly 3000 weed species exist on the earth and each individual weed plant is host to one or more microorganisms. Only few of these weed plants have ever been completely studied relative to their bacterial flora. Therefore, the chance to find new and interesting microorganisms among weed plants in different environment and ecosystem is significant. However, little information is available about microbiota of different weeds in Pakistan.

Furthermore, Tanvir *et al*. (2013) isolated 42 strains of *Streptomyces* spp. from *P. hysterophorus* strains. In addition, majority of the strains were obtained from the roots probably because a morphologically, physically, and chemically complex microcosm is formed in the root tissues. Present investigation was aimed to isolate and characterize different bacterial species on the basis of their family from four local weeds collected from Lahore.

MATERIALS AND METHODS

Collection of samples

Common weeds were collected from main canal side and vicinity of Punjab University, Lahore, Pakistan and were easily identified, as they are common weeds. Table-1 shows the list of common weeds used in this study. Properly labeled samples with sample type, collection site, date of collection etc. were transported to the laboratory.

Table-1. Weeds listed with their Botanical name and diseased parts used

S. No.	Botanical Name	Diseased part used
1.	*Parthenium hysterophorus* L.	Stem
2.	*Convolvulus arvensis* L.	Leaves
3.	*Euphorbia esculenta* L.	Leaves
4.	*Chenopodium album*L.	Stem

Isolation of bacterial species

Plant parts were surface sterilized with 1% sodium hypochlorite and then rinsed in three different times of distilled water. The segments of the diseased portion were then plated on solidified Luria Bertani Agar (LBA) and Nutrient Agar (NA) media plated aseptically

(Ali and Naseem, 2011). The inoculated plates were labeled properly for easy identification and then incubated at 37ºC for 24hours. Purification was done by streak plate technique, transferred the emerging colonies on fresh media petriplates under aseptic conditions (Beishir, 1991). Pure bacterial cultures were stored in 20 % sterile glycerol at -20 °C until further analysis.

Identification of bacterial species

First morphological and cultural features of the bacterial colony/cells were recorded then certain biochemical tests were performed as the routine steps of bacterial identification.

Morphological Features

Morphological parameters recorded for identification were cell shape, gram type, capsule stain, motility and pigmentation. Growth on osmotic medium i.e containing 2% NaCl was also observed. Finally the ability of bacteria to grow at 25 ^{o}C and 40 ^{o}C was also studied Konem *et al*., 1997.

Biochemical Analysis

Using the commercially available bacteria identification kit, MicrogenTM GnA+B-ID Identification System (Microgen Bioproducts Ltd, Surrey, UK), pure colonies were differentiated by biochemical test. Initially preference of carbon source of isolated bacteria was analyzed by providing a wide range of carbohydrate sources that include glucose, lactose, sucrose, inositol, sorbitol, mannitol and xylose while sterile water was used as control. Other biochemical analysis 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). Bacteria were identified by providing the results of all above mentioned biochemical tests to Microgen Identification System software.

RESULTS AND DISCUSSION

In present investigation, a total of thirteen bacterial species were isolated from four different weed plants. However maximum percentage of bacterial species was obtained from *E. esculenta* and *P. hysterophorus*. Different species of bacteria isolated in this study belonging to eight different family viz. Burkholderiaceae, Bacillaceae, Peptococcaceae, Comamonadaceae, Enterobacteriaceae, Corynebacteriaceae, Rhodospirillaceae and Moraxellaceae (Fig. 1). Thirteen bacterial isolates were sorted in two gram-positive bacteria and eleven gram-negative by gram staining technique. Each isolate was given a reference number (W1-W13) that was used throughout this study to represent the results of that particular bacterium. In this study, all bacterial strains have rod shaped cell except W3 strain

consequently all strains were unable to produce pigment except W10. Furthermore only W4 and W6 managed to grow at 25 °C although all strains showed similar pattern of growth at 37 °C. In case of W7 strain, capsule was observed around the cells using capsule staining. Data recorded while studying the morphology of bacteria is presented in Table-2 and 3. Naturally different bacterial strains have different metabolic path ways (Sathishkumar *et al*., 2008). Results demonstrated that all isolates gave negative results with the carbohydrates (glucose) except W8, W9 and W11. In addition, W1, W2 and W13 have the ability to ferment lactose also while others did not while isolates W2 and W4 exhibited negative result with 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 use monosaccharides, for example glucose, as energy source while few prefer disaccharides or polysaccharides (Richard *et al*., 2011). Furthermore, Fish (2002) reported that bacteria commonly secrete different chemicals into their surroundings to modify it according to them and these secreted chemicals are mostly proteins and might proceed as enzymes that digest some variety of food present in the environment. Therefore each pathogen has a distinctive range of interactions with its hosts. Biochemical tests actually exhibit the ability of an enzyme to utilize different substrates. Such ability can be assessed by the presence of products in a biochemical reaction. Results of these tests help in identification of bacteria (Harley, 2008).

Capacity of different bacterial strains to use various carbon compounds as energy source and enzymatic activities of bacterial isolates are tabulated in Table-2 and -3. The identification of bacteria is essential in microbiology in various aspects (Nitesh *et al*., 2011). Identification was made by using the Microgen Identification System software. Cultural and biochemical data recorded for the isolates (Table-2 and -3) was entered in the software to key out the unknown bacteria. *Cupriavidus sp., Bacillus sp., Peptococcus sp., Acidovorax facilis, Klebsiella sp., Yersinia ruckeri, Corynebacterium minutissimum, Bacillus farraginis, Enterobacter agglomerans, Azospirillum lipoferum, Acinetobacter lwoffii, Cedecea davisae, Curtobacterium albidum* were the identified species. Identified bacterial species were deposited in First Fungal Culture Bank of Pakistan (FCBP). All species versus their reference no as well as their FCBP accession numbers are given in Table-4. Percentage of each bacterial strain in each weed sample is also shown in Fig. 1. The microscopic examination of infected areas gave important information on the occurrence of the bacterial flora on

different parts of weeds (Table-2 to -4). The bacterial strains isolated from tested weeds varied significantly. In addition, the number and species of bacterial isolates also differ with their host weed plants. Bacteria are known to be the principal microbial population and these microbes can be found both as epiphytes on the plant surface and as endophytes within plant tissues (Arnold *et al.,* 2000; Inacio *et al.,* 2002; Lindow and Brandl, 2003; Stapleton and Simmons, 2006). It is known from studies that specific bacterial population has associated with different host. Furthermore Adams and Kloepper (2002) and Elvira-Recuendo and van-Vuurde (2000) also studied the different bacterial population isolated from cotton cultivars. Generally, these bacterial species are widely reported as common primary saprobes from attached leaf surfaces of large variety of plants all over the world (Pandey, 1990; Andrews, 1996; Osono, 2006).

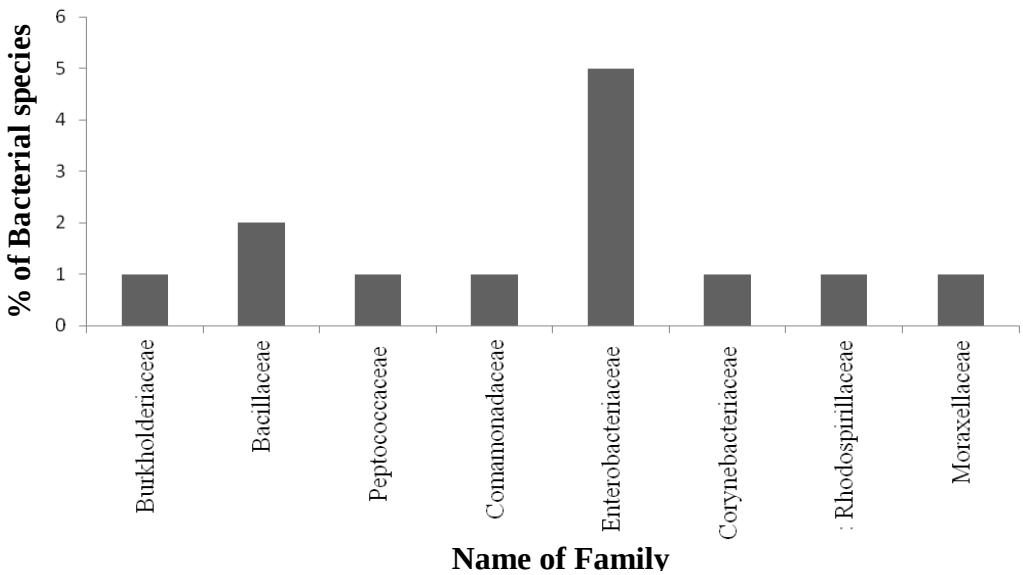

Figure 1. Percentage of Bacterial Strains Isolated from each weed sample

Table-3. Bacterial species identified from weeds plants

S. No.	Substrate	Ref.No. of Strain	Species Identified	FCBP accession No.
1	*P. hysterophorus*	W1	*Cupriavidus* sp.	FCBP222
		W2	*Bacillus* sp.	FCBP223
		W3	*Peptococcus* sp.	FCBP224
		W4	*Acidovorax facilis*	FCBP360
2	*C. arvensis*	W5	*Klebsiella sp.*	FCBP351
		W6	*Yersinia ruckeri*	FCBP352
		W7	*Corynebacterium minutissimum*	FCBP353
3	*E. esculenta*	W8	*Bacillus farraginis*	FCBP354
		W9	*Enterobacter agglomerans*	FCBP355
		W10	*Azospirillum lipoferum*	FCBP356
		W11	*Acinetobacter lwoffii*	FCBP357
		W10	*Cedecea davisae*	FCBP358
4	*C. album*	W13	*Curtobacterium albidum*	FCBP361

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Table-2. Phenotypic characters, enzymatic activities and Carbohydrate source preference analysis of bacterial species isolated from *P. hysterophorus, C. arvensis, E. esculenta* and *C. album.*

Features	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12	W13
Morphological Characters													
Cell shape	Rods	rods	cocci	rods	rods	Rods	rods	rods	rods	rods	Rods	rods	Rods
Gram type	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve
Motility	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
Capsule	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve
Spore formation	-ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve
Pigmentation	-ve	-ve	-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	-ve	-ve
Growth at 25 ^{o}C	-ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Growth at 40 ^{o}C	-ve	-ve	-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	-ve	+ve
Hydrogen sulfide	+ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve
Lysine	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Nitrate reduction	-ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve
Oxidase	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve
Catalase	-ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve
Urease	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve
Gelatine	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve
Malonate	-ve	-ve	-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	+ve	+ve
Sorbitol	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve
Glucose	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve
Mannitol	+ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve
Xylose	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Sucrose	+ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve
Lactose	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Arginine	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve

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