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PHENOTYPIC IDENTIFICATION AND BIOCHEMICAL STUDIES OF BACTERIA ASSOCIATED WITH RHIZOSPHERIC SOIL OF GINGER (*ZINGIBER OFFICINALE* L.)

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A B S T R A C T

Zingiber officinale L. is a common condiment for various foods and a long history of important traditional Medicine herb for the treatment of ailments and body disorders**.** Soil microorganisms are very important as almost every chemical transformation taking place in soil involves active contributions from soil microorganisms. In contrast, other soil microorganisms are pathogenic to plants and may cause considerable damage to crops. Large numbers of pathogenic microorganisms are routinely found in the soil and many can infect the plant through the roots. Through the current study describes the ginger rhizome associated soilborn bacterial strains. Rhizosphere bacteria were isolated from soil around the root zones of ginger fields. This study reports results concerning the isolation and identification of bacteria on the morphological basis and biochemical analysis. Ten species of bacteria belonging to eight different genera viz., *Acidovorax temperans*, *Arthrobacter* sp., *Burkholderia pseudomallei, B. cepacia* , *Aeromonas hydrophila, A. veronii, Cupria-viridis* sp., *Bacillus* sp.*, Flavobacterium odoratum, Oscillospira* sp. were isolated and processed for identification by their enzymatic activity and carbohydrates utilization.

Keywords: Identification, biochemical analysis, rhizoshere, *Z. officinale*, enzymatic activity.

INTRODUCTION

The bacterial species are very fragile and can be killed by slight changes in the soil environment. Other species are extremely tough, able to withstand severe heat, cold or drying. Some can lie dormant for decades waiting for favorable conditions. Others can extract nitrogen directly from the air or break down some toxic substances (Laskar and Sharma, 2013). Populations of microbes can boom or bust in the space of a few days in response to changes in soil moisture, soil temperature or carbon substrate. To gain advantage in this process, many microbes release antibiotic substances to suppress particular competitors. In this way some species can suppress other disease-causing microorganisms (Reinhardt *et al*., 2008). Some bacteria inhabiting in the rhizosphere are called rhizobacteria. The root system, which was thought to provide anchorage and uptake of nutrients and water, in fact mediates numerous underground interactions (Badri *et al.,* 2009). From this point of view the rhizosphere is a very complex environment in which the effects of the plant on soil microorganisms and the effects of the microorganisms on the plant are interacting and are interdependent (Danhorn and Fuqua, 2007).

Plant root exudates attract microbes and feed them and, in turn, the plants often benefits from the microbes (Smalla *et al.,* 2001). They live on the surface of roots in the soil and form a barrier to the root infecting parasites (bacteria, fungi, actinomycetes, nematodes etc.). Rhizobacteria excrete substances that protect the roots from plant parasites by the toxic effects (Shamima and Rahman, 2007). In exchange they get their nutrition from the root exudates. Thus symbiotic relationship is established between the host plant roots and the rhizosphere bacteria. It was recognized that the rhizosphere microbial population might be depend on the kind of plant, the age and stimulation by roots to different microbial types (Kuklinski-Sobral *et al.,* 2004).

It was observed that microflora in rhizosphere soil is higher than the soil without rhizosphere indicates the influence of living roots in the soil. Many bacteria are

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intimately associated with plant roots. Rhizo-deposition of various exudates provides an important substrate for the soil microbial community and there is a complex interplay between this community and the quantity and type of compounds released (Kandeler *et al*., 2002; Marschner and Baumann, 2003). The present work has been undertaken to isolate and characterize the bacteria from the ginger field rhizosphere soil.

MATERIALS AND METHOD

Collection and Processing of Soil Sample: For isolation of bacteria 10 rhizospheric soil samples of ginger field were collected from different areas of Lahore city during September to November 2013. The upper layer of ginger rhizospheric soil was important as most of the microbial activity enhance bacterial population. Soil samples (approximately 5g) were collected using some clean dry and sterile polythene bag along with sterile spatula. 1g of the soil sample was dissolved in 10ml of water to make soil suspensions by serial dilution technique (Harley, 2008).

Isolation of Bacteria: The media used in the present investigations was nutrient agar medium and Luria Bertani Agar (LBA). Small Portions of the soil suspension were inoculated aseptically on the nutrient agar and LBA media by streaking method and were incubated at 37°C for 24 hours. After which colonies were appeared and transferred to freshly prepared petriplates for purification. Pure bacterial cultures were stored in 20 % sterile glycerol at -20 °C until further analysis.

Morphological Features: Morphological parameters recorded for identification were cell shape, Gram type, motility and pigmentation. Growth on osmotic medium i.e containing 2% NaCl was also observed. Finally the ability of bacteria to grow at 25°C and 40°C was also studied (Konem *et al.,* 1997). Colony growth on nutrient agar and LBA was gram stained in accordance with standard gram staining procedure described by Prabhat *et al.* (2010).

Biochemical Tests: Biochemical tests were conducted for differentiation in morphological characters (or any other can be mentioned) of pure colonies by using the commercially available bacteria identification kit, MicrogenTM GnA+B-ID Identification System (Microgen Bioproducts Ltd, Surrey, UK). The isolates were also examined for fermentation of the various sugars including glucose, lactose, sucrose, inositol, sorbitol, mannitol and xylose. Sterlized distilled water was used as control (Holt *et al.,* 2000; Benson, 1996). 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 morphological as well as biochemical tests to Microgen Identification System software.

RESULTS AND DISCUSSION

During present study, a total of ten different bacterial species were isolated and identified from rhizospheric soil of ginger field. Soil is considered as a store house of microbial activity. Microorganisms are very important constituents of the soil. Hardly there is any soil without microorganisms, mainly the bacteria (Badri *et al.,* 2009). Soil samples were collected from different field areas of Lahore city. Mostly the bacteria are responsible for the degradation of organic and inorganic compounds present in soil. They derive their nutritional requirement from the compounds present in the vicinity of particular plant because Plant roots excrete organic substances, although in smaller amounts, in the soil around the roots. Bacteria are able to synthesize their enzymes, metabolic intermediates, structural proteins, lipids and nucleic acids from carbon compound in the feed.

This study reports the isolation and identification of the strains of *A. temperans*, *Arthrobacter* sp., *B. pseudomallei, B. cepacia*, *A. hydrophila, A. veronii, Cupria-viridis* sp., *Bacillus* sp*., F, odoratum* and *Oscillospira* sp. from the rhizospheric soil of ginger. The reference number (GS1 – GS10) was assigned to each isolate, which was used throughout this study to represent the results of that particular bacterium.

Identification as well as classification of bacteria in different taxonomic groups is mostly based on the phenotypic characters (Harley, 2008). All the strains studied for present work were rod-shaped. The study showed majority of isolates were gram negative bacteria and few gram positive. Both motile and non-motile bacteria are identified from rhizospheric soil of ginger. Most of the strains were unable to grow on osmotic medium (containing 2 % NaCl) while four strains i.e, GS1,GS7,GS9 and GS10 showed good growth in osmotic medium. Strains GS5, GS6 and GS7 showed good growth at high temperature while others couldn't tolerate such high temperature i.e. 40 °C and fail to grow. On the other hand GS1, GS3, GS7 and GS10 couldn't manage to grow at low (25 °C) as well as high temperature. Morphological observations for the strains are presented in Table 1.

Table 1. Phenotypic characters, enzymatic activities and Carbohydrate source preference analysis of bacterial isolates.

Features	GS-1	GS-2	GS-3	GS-4	GS-5	GS-6	GS-7	GS-8	GS-9	GS-10
Morphological Characters										
Cell shape	rods	rods	rods	rods	rods	rods	rods	rods	Rods	rods
Gram type	-ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve
Motility	-ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve
Growth at 2% NaCl	+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
Growth at 40 ^{o}C	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
Biochemical Tests										
Citrate utilization	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve
Hydrogen sulfide	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve
Lysine	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Nitrate reduction	-ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve
Oxidase	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve
Catalase	-ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve
Urease	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	+ve	-ve
Gelatine	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve
Malonate	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Enzymatic Activity										
Inositol	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Sorbitol	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve
Glucose	-ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve
Mannitol	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve
Xylose	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Sucrose	+ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve
Lactose	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	-ve
Arginine	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve

Findings of citrate test suggested that most of the isolate was capable of citrate transport into the cell for fermentation except single strain GS6. Nitrite was detected for five strains GS2, GS3, GS5, GS6 and GS9 in the reaction tube that was an indicative of nitrate conversion to nitrite by nitrate reductase (Reisner, 1978). Nitrate reductase, bacteria were further analyzed for their oxidase activity. Oxidase activity was positive for most of the strains except GS3, GS4 and GS10. Whereas only GS1, GS5 and GS7 have the ability to metabolize ferrous ammonium sulfates and produces hydrogen sulfide as byproduct. Results of gelatinase test demonstrated that only three strains GS6, GS9, GS10 contain gelatinase enzyme and hence has the ability to digest the gelatin. When the catalase test was performed, it revealed that most of the strains have the ability to breakdown hydrogen peroxide.

Positive urease activity was only recorded for GS6 and GS9 that is an indicative of low or no urea breakdown by rest of the strains. None of the bacteria showed to have lysine and malonate, functional metabolisms (Table 1).

Different bacteria behave differently for their metabolic activity, and can be identified on this basis. In this study none of the isolates were able to utilize inositol and xylose (Table 1) Results demonstrated that only three isolates GS5, GS6 and GS8 were able to utilize glucose. While in case of sucrose GS1, GS7 and GS8 has the metabolic machinery to utilize sucrose, an indication of the role played by these isolates in the formation of short chain fatty acids from carbohydrates or synthesis of amino acids (Cummings and Macfarlane, 1997).

Four isolates i.e, GS1, GS5, GS6 and GS7 exhibited the ability to ferment lactose also while others did not. Only two Isolate GS5 and GS6 exhibited positive result with of sorbitol among all species while in case of Arginine only GS5 revealed positive results .Enzymatic activities of bacterial isolate and abilities of different isolates studied to use various carbon compounds are recorded in Table 1. The data obtained from phenotypic and biochemical analyses of unknown bacteria, results were generated by using Microgen Identification System software. Identified bacterial cultures were deposited in FCBP. Identified species along their reference numbers as well as their FCBP accession numbers are given in Table 2. The isolate *Bacillus* sp., have been regarded as PGPR in earlier study (Laskar and Sharma, 2013), but *B. pseudomallei* and *B. cepacia,* as plant pathogen are also reported first time in our study. These two bacteria are reported as pathogenic and endophytes in many earlier study (Reinhardt *et al*., 2008).

Knowledge on phenotypic and functional traits of bacteria will help to determine their fitness for successful bio-fertilization and biological control. This study provides essential information to develop broad spectrum bio-control agent and bio-fertilizers.

Table 2. Isolated and identified bacterial strains

Strains ref no.	Isolates Identified	FCBP Accession no.
GS-1	*Acidovorax temperans*	*FCBP: 339*
GS-2	*Arthrobacter* sp.	*FCBP: 343*
GS-3	*Burkholderia pseudomallei*	*FCBP: 332*
GS-4	*Burkholderia cepacia*	*FCBP: 337*
GS-5	*Aeromonas hydrophila*	*FCBP: 331*
GS-6	*Aeromonas veronii*	*FCBP: 349*
GS-7	*Cupria-viridis* sp	*FCBP: 338*
GS-8	*Bacillus* sp.	*FCBP: 336*
GS-9	*Flavobacterium odoratum*	*FCBP: 376*
GS-10	*Oscillospira* sp.	*FCBP: 347*

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