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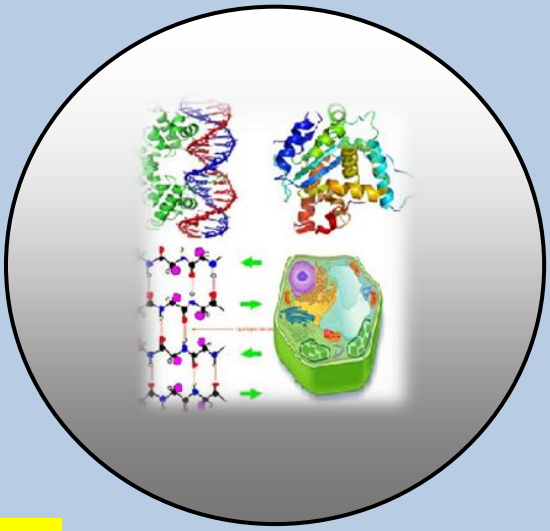
ISSN 2319-3077 Online/Electronic
ISSN 0970-4973 Print

Journal Impact Factor: 4.275
Global Impact factor of Journal: 0.876
Scientific Journals Impact Factor: 3.285
InfoBase Impact Factor: 2.93
Index Copernicus International Value
IC Value of Journal 6.01 Poland, Europe

J. Biol. Chem. Research
Volume 32 (2) 2015 Pages No. 934-944



Journal of Biological and Chemical Research

An International Peer Reviewed / Refereed Journal of Life Sciences and Chemistry

Indexed, Abstracted and Cited in various International and National Scientific Databases

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 32, No. 2: 934-944, 2015
(An International Peer Reviewed / Refereed Journal of Life Sciences and Chemistry)
Ms 32/2/125/2015

ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)

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RESEARCH PAPER
Received: 17/10/2015 Revised: 05/11/2015 Accepted: 09/11/2015

Rhizosphere and Rhizoplane Bacteria Isolated from Subtropical Region of Jazan in Saudi Arabia

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ABSTRACT

On quantitative basis, the number of bacterial isolates was less in rhizoplane region than rhizospheric region. Bacterial isolates from soil of Sorghum bicolor field were Microbacterium barkeri, Pseudomonas alcaligenes, Brevundimonas vesicularis, Pseudomonas syringae pv aceris and Pseudomonas syringae pv atrofaciens. All bacterial isolates M. barkeri , P. alcaligenes, B. vesicularis, P. syringae pv aceris and P.syringae pv atrofaciens were present in the rhizosphere and not found on the root surfaces (rhizoplane) exept P. alcaligenes and B. vesicularis. P. alcaligenes can coexist as a rhizosphere and rhizoplane bacterium represented with 24.6 and 40.7% occurrence Frequency respectively while occurrence frequency of M. barkeri was 15.4 %. Future studies will determine the potential application of these isolates in plant growth promotion for enhancing plant productivity
Key words: Rhizosphere, Rhizoplane, Bacteria, Subtropical Region and Saudi Arabia.

INTRODUCTION

Rhizosphere is a zone between the root surface and the soil adjacent to the roots. The bacteria inhabiting in the rhizosphere are called rhizobacteria. They live on the surface of roots in the soil and form a barrier to the root infecting parasites (bacteria, fungi, nematodes etc). Rhizobacteria excrete antibiotic substances, thereby protecting the roots from plant parasites by the toxic effects (Ritesh and Prasad 2014). The boundary between rhizoplane and rhizosphere is very thin and therefore this habitat is largely considered as a continuum (Johri et al., 2003). Knief et al. (2011) reported that the microorganisms that colonize the aerial parts (phyllosphere), the root surface (rhizoplane) as well as the zone around the root (rhizosphere). Studying the coexistence of microorganisms with plants is

very important for agricultural development and can be exploited in plant biotechnology; for example, plant growth enhancement, diseases-fighting plants, immune system resistance enhancement, useful compound extraction and siderophore production (Choudhary and Johri 2009). The rhizosphere is an area around the plant root that is occupied by a unique population of useful bacteria known as the PGPR (Kamilova et al., 2006).

Microbacterium barkeri strain 2011-R4 is a Gram-positive epiphyte which has been confirmed as a biocontrol agent against several plant pathogens (Liu et al.,2012). *Microbacterium barkeri* is one of the species in the genus *Microbacterium*, which belongs to the high-GC-content phylum *Actinobacteria* (Morohoshi et al., 2011). It has been reported that *Microbacterium* strains can cause human, animal, and plant diseases (Kaku et al., 2000). However, *Microbacterium* spp. have frequently been isolated from the soil and used as biocontrol agents (Barnett et al., 2006; Pereira et al., 2007; Sartori et al., 2012). Some reports have demonstrated that *Microbacterium barkeri*strains are strong in plant colonization (Rau et al., 2009) and play a very important role in biocontrol. *Halophilic Microbacterium barkeri* was isolated from mangroves rhizosphere and the surrounding sediments (Yateem and Al-Sharrah 2011).

Root colonization of rapeseed by *Pseudomonas alcaligenes* in rhizosphere microcosms was investigated (Hu and Liu 2002). The effects of *Pseudomonas alcaligenes* on the root-rot disease complex caused by the root-knot nematode *Meloidogyne incognita* and the root-rot fungus *Macrophomina phaseolina* in chickpea was assessed (Akhtar and Zaki 2008) by quantifying differences in the shoot dry mass, pod number, nodulation, and shoot content of chlorophyll, nitrogen, phosphorus and potassium. Inoculation of plants with *P. alcaligenes* increased shoot dry mass, pod number, and content of chlorophyll, nitrogen, phosphorus and potassium in plants inoculated with pathogens over that in the uninoculated control plants. In plants inoculated with antagonist, *P. alcaligenes* reduced galling and nematode multiplication. *P. alcaligenes* was isolated from the rhizospheres plants of the Families Solanaceae and Legumonosae, and exhibited antagonistic activity against Fusarium oxysporum f.sp. lycopersici, the cause of tomato wilt disease (Ketut et al., 2013).

Brevundimonas vesicularis is non-lactose-fermenting environmental Gram-negative bacilli previously assigned to the genus *Pseudomonas* (Gilad 2000). *B. vesicularis* has been implicated in rare cases of human infections (Gilligan et al., 2003). *B. vesicularis* has been isolated from soil and water, hospital instruments, and also from human cervical specimens (Szymanska 2007) and from metal contaminated soil (Resmi et al., 2012). The genus Brevundimonas was proposed by reclassification of two Pseudomonas species as *B. diminuta* and *B. vesicularis* by Segers et al. (1994). Several species, including *Brevundimonas alba*, *B. aurantiaca*, *B. bacteroides*, *B. intermedia*, *B. subvibrioides*, *B. terrae* and *B. variabilis*, were transferred from the genus Caulobacter to the genus Brevundimonas (Abraham et al., 1999). The species *Pseudomonas syringae* is heterogeneous and is divided into 57 pathovars (Gardan et al., 1997). *P.syringae* is a Gram negative, rod shaped bacterium with polar flagella causing disease in most of the plant species. Recently Prasanth et al. (2015) stated that using the comparative analysis of 16S rRNA, the fluorescent, poly-betahydroxybutyrate negative pseudomonads associated with the type species, P.aeruginosa, and including P.syringae and related species, are now included in δ-proteobacteria (Young 2010).

As a pathogen, P.syringae affects large group of plant species and mostly symptoms of the diseases are similar. There is a great deal of specialization, within the species, with respect to plants with which individual strains are likely to interact. *P. syringae* group appears to be the best adapted for epiphytic growth, defined as an increase of bacteria populations on apparently healthy external parts of the shoot (leaves, buds, pods, etc.) (Hirano and Upper, 2000). Strains of P. syringae cause diseases in nearly every cultivated plant and on an unknown number of wild plant species, and they have been classified into some 51 different pathovars depending on their host range (Young et al., 1996). The main objectives of this study were to isolate and identify rhizosphere and rhizoplane bacteria from *Sorghum bicolor* field in Jazan, Saudi Arabia for further investigation for their ability to promote plant growth.

MATERIAL AND METHODS

Isolation of Rhizosphere bacteria

Soil samples were collected using the methods of Dongmo and Oyeyiola (2006). Each rhizosphere soil sample was collected of *Sorghum bicolor* field in Jazan, Saudi Arabia by carefully uprooting a plant and shaking the soil adhering to the roots into a sterile polythene bag.

Isolation of Rhizosplane bacteria

Some *Sorghum bicolor* roots were carefully uprooted from soil and taken to the laboratory in sterile containers. The roots were manually shaken to remove loose soil particles. The roots were cut into about 2mm segments and 5 g representative samples shaken into 95 ml of sterile distilled water. Serial dilutions were made from this stock and plated for isolation of bacteria. The isolation frequency (Fr) of species were calculated according to Gonzalez *et al.,* (1995) as follows: Fr (%) = Number of samples with a species or genus/ Total number of samples X 100.

Identification of bacterial isolates

Bacterial isolates were cultivated on King's B Agar containing per liter of distilled water: 10 g peptone, 10 ml glycerol, 1.5 g K_2HPO_4, 1.5 g $MgSO_4$, 20 g agar, pH 7.2 and were characterized and identified based on their morphological, cultural, physiological and biochemical characteristics

RESULTS and DISCUSSION

In the present study the obtained results showed that on quantitative basis, the number of bacterial isolates was less in rhizoplane region than rhizospheric region (Table 1). Isolated bacteria from soil of *Sorghum bicolor* field were *Microbacterium barkeri*, *Pseudomonas alcaligenes*, *Brevundimonas vesicularis, Pseudomonas syringae* pv aceris and *Pseudomonas syringae* pv atrofaciens identified according to morphological (Table2) and biochemical tests (Table 3 & Fig.1). All bacterial isolates *M. barkeri* , *P. alcaligenes*, *B. vesicularis, P. syringae* pv aceris and *P.syringae* pv atrofaciens were present in the rhizosphere and not found on the root surfaces (rhizoplane) exept *P. alcaligenes*, *B. vesicularis*. This results were agreement with Eze and Amadi (2014), rhizosphere soil contained a great spectrum of bacteria whereas there were no bacteria isolated from the rhizoplane. The association between microorganisms and roots can be beneficial, neutral or harmful, but often the effects depend on the soil conditions (Ownley et al., 2003).

Microflora in rhizosphere soil is higher than the soil without rhizosphere indicates the influence of living roots in the soil. One such a physiological group, the ammonifying bacteria related to the capacity of mineralizing the nitrogenous materials by bringing about a rapid decomposition of organic nitrogen in the rhizosphere. Bacteria in the rhizosphere influence plant growth because they affect soil chemical properties and interact with plant roots, where the influence can be beneficial, neutral, or deleterious (Sakai et al. 2004; Ritesh and Prasad 2014).

Rhizosphere bacteria isolated from the soil were tested for its biochemical (Table 2) and morphological characterization. Based on these studies conducted, the bacterial species was identified as *B. vesicularis*. It is a gram negative, rod shaped and has motility. These bacteria can assimilate D-Glucose and Maltose (Table 3). Growth parameters were also observed,it has aerobic respiration. *B. vesicularis* encountered in this work was previously reported as Endophytic isolate obtained from three *Phaseolus vulgaris* cultivars (Costa, et al., 2012).

In the current study *P. syringae* was isolated as a Gram negative and rod shaped bacterium in rhizoplane, although many reports stated that *P. syringae* causing disease in most of the plant species. Baltrus et al. (2011) reported that *P. syringae*, an important pathogen of many plant species, is a diverse assemblage of strains isolated from different host plants as well as from the environment. These findings are consistent with those of Costa *et al*. (2012) who reported that, some bacterial species considered pathogenic for certain plant species have been isolated as endophytic in other species. Ulrich et al. (2008) isolated endophytes with high similarity to known plant pathogens, such as *Pseudomonas syringae* and *Xanthomonas populi*. In few decades a large number of bacteria from the rhizosphere soil have been isolated and identified among which the common species of Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligens, Arthobacter, Burkholderia, Bacillus and Serratia have reported (Glick, 1995).

It is evident from these results that the *Microbacterium barkeri* was isolated as rhizospheric bacterium , their Occurrence Frequency (%) in rhizosphere was 15.4 % (Table 1) These findings are consistent with those of Rau et al. (2009) who reported that *M. barkeri*s are strong in plant colonization. These results suggested that *M. barkeri* may be useful to further explore the commercial potential for biofertilizer

The present study demonstrated that *Pseudomonas alcaligenes* can coexist as a rhizosphere and rhizoplane bacterium represented with 24.6 and 40.7% occurrence Frequency respectively (Table1) and can be used as a biofertilizer. Previeowisly studies demnostrated that inoculation of plants with *P. alcaligenes* significantly increased shoot dry mass, pod number, and content of chlorophyll, nitrogen, phosphorus and potassium in plants (Akhtar and Zaki 2008). Several studies found that Pseudomonads may improve plant growth by suppressing parasitic and nonparasitic root pathogens through the production of biologically active substances or the conversion of unavailable minerals and organic compounds into forms that are available to plants (Hu and Liu 2002; Ketut et al., 2013; Akhtar and Zaki 2008). The bacteria isolated from rhizosphere soils, have proved to be beneficial to the plants by directly having an effect on nitrogen fixation (Han et al., 2005), solubilization of nutrients, production of growth hormones, 1-amino- cyclopropane-1-carboxylate (ACC) deaminase (Correa et al., 2004) and indirectly by antagonizing pathogenic fungi by the production of siderophores, -1,3-glucanase, antibiotics, fluorescent pigments and cyanide (Pal et al., 2001).

Table 1. Table Frequency of Occurrence (%) of Bacteria in Rhizosphere and Rhizoplane.

Bacterial Isolate	Occurrence Frequency (%) of bacterial isolate from	
	Rhizosphere	Rhizoplane
M. barkeri	15.4±04	0.0±00
P. alcaligenes	24.6±13	40.7±03
B. vesicularis	15.2±09	59.3±10
P. syringae pv aceris	19.6±07	0.0±00
P. syringae pv atrofaciens	25.2±01	0.0±00

Table 2. Morphological characteristics and oxygen reqiurement for bacterial isolates

Characteristic	*P. syringae* pv aceris	*P. syringae* pv atrofaciens	*M. barkeri*	*B. vesicularis*	*P. alcaligenes*
Gram staining	Gr-ve	Gr-ve	Gr+ve	Gr-ve	Gr-ve
Cell Shape	Rods	Rods	Rods	Rods	Rods
Motility	Motile	Motile	Non	Motile	Motile
Endospore	-	-	-	-	-
O_2 requrement	+	+	+	+	+

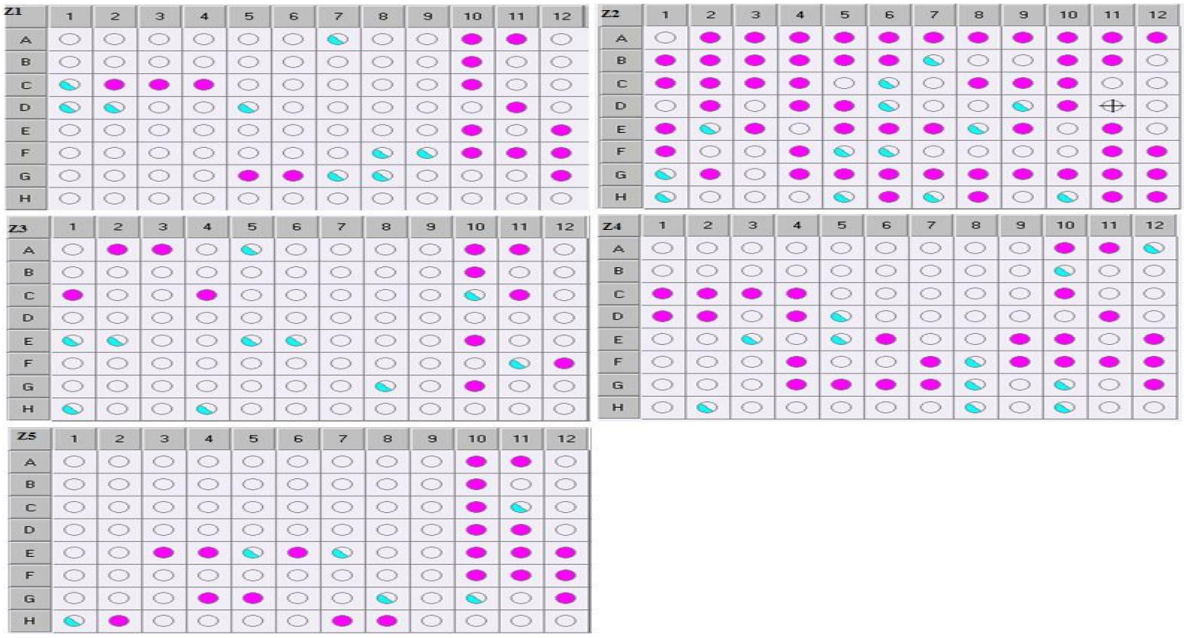
Table 3. Physiological and biochemical characteristics for bacterial isolates.

Characteristic		*Pseudomonas syringae pv aceris*	*Microbacterium barkeri*	*Brevundimonas vesicularis*	*Pseudomonas syringae pv atrofaciens*	*Pseudomonas alcaligenes*
A1	Negative Control	-	-	-	-	-
A2	Dextrin	-	+	+	-	-
A3	D-Maltose	-	+	+	-	-
A4	D-Trehalose	-	+	-	-	-
A5	D-Cellobiose	-	+	±	-	-
A6	Gentiobiose	-	+	-	-	-
A7	Sucrose	±	+	-	-	-
A8	D-Turanose		+	-	-	-
A9	Stachyose		+	-	-	-
A10	Positive Control	+	+	+	+	+
A11	pH 6	+	+	+	+	+
A12	pH 5	-	+	-	±	-
B1	D-Raffinose	-	+	-	-	-
B2	α-D-Lactose	-	+	-	-	-
B3	D-Melibiose	-	+	-	-	-
B4	β-Methyl-D-Glucoside	-	+	-	-	-

Characteristic		*Pseudomonas syringae pv aceris*	*Microbacterium barkeri*	*Brevundimonas vesicularis*	*Pseudomonas syringae pv atrofaciens*	*Pseudomonas alcaligenes*
B5	D-Salicin	-	+	-	-	-
B6	N-Acetyl-D-Glucosamine	-	+	-	-	-
B7	N-Acetyl-β-D-Mannosamine	-	±	-	-	-
B8	N-Acetyl-DGalactosamine	-	-	-	-	-
B9	N-Acetyl Neuraminic Acid	-	-	-	-	-
B10	1% NaCl	+	+	+	±	+
B11	4% NaCl	-	+	-	-	-
B12	8% NaCl	-	-	-	-	-
C1	α-D-Glucose	±	+	+	+	-
C2	D-Mannose	+	+	-	+	-
C3	D-Fructose	+	+	-	+	-
C4	D-Galactose	+	+	+	+	-
C5	3-Methyl Glucose	-	-	-	-	-
C6	D-Fucose	-	±	-	-	-
C7	L-Fucose	-		-	-	-
C8	L-Rhamnose	-	+	-	-	-
C9	Inosine	-	+	-	-	-
C10	1% Sodium Lactate	+	+	±	+	+
C11	Fusidic Acid	-	-	+	-	±
C12	D-Serine	-	-	-	-	-
D1	D-Sorbitol	±	-	-	+	-
D2	D-Mannitol	±	+	-	+	-
D3	D-Arabitol	-	-	-	-	-
D4	myo-Inositol	-	+	-	+	-
D5	Glycerol	±	+	-	±	-
D6	D-Glucose-6-PO4	-	±	-	-	-
D7	D-Fructose-6-PO4	-	-	-	-	-
D8	D-Aspartic Acid	-	-	-	-	-
D9	D-Serine	-	±	-	-	-
D10	Troleandomycin	-	+	-	-	+
D11	Rifamycin SV	+	-	-	+	+
D12	Minocycline	-	-	-	-	-
E1	Gelatin	-	+	±	-	-
E2	Glycyl-L-Proline	-	±	±	-	-

Characteristic		*Pseudomonas syringae pv aceris*	*Microbacterium barkeri*	*Brevundimonas vesicularis*	*Pseudomonas syringae pv atrofaciens*	*Pseudomonas alcaligenes*
E3	L-Alanine	-	+		±	+
E4	L-Arginine	-	-	-	-	+
E5	L-Aspartic Acid	-	+	±	±	±
E6	L-Glutamic Acid	-	+	±	+	+
E7	L-Histidine	-	+	-	-	±
E8	L-Pyroglutamic Acid	-	±	-	-	-
E9	L-Serine	-	+	-	+	-
E10	Lincomycin	+	-	+	+	+
E11	Guanidine HCl		+	-		+
E12	Niaproof 4	+	-	-	+	+
F1	Pectin	-	+	-	-	-
F2	D-Galacturonic Acid	-	-	-	-	-
F3	L-Galactonic Acid Lactone	-	-	-	-	-
F4	D-Gluconic Acid	-	+	-	+	-
F5	D-Glucuronic Acid	-	±	-	-	-
F6	Glucuronamide	-	±	-	-	-
F7	Mucic Acid	-	-	-	+	-
F8	Quinic Acid	±	-	-	±	-
F9	D-Saccharic Acid	±	-	-	+	-
F10	Vancomycin	+	-	-	+	+
F11	Tetrazolium Violet	+	+	±	+	+
F12	Tetrazolium Blue	+	+	+	+	+
G1	p-Hydroxy- Phenylacetic Acid	-	±	-	-	-
G2	Methyl Pyruvate	-	+	-	-	-
G3	D-Lactic Acid Methyl Ester	-	-	-	-	-
G4	L-Lactic Acid	-	+	-	-	+
G5	Citric Acid	+	+		+	+
G6	α-Keto-Glutaric Acid	+	+	-	+	-
G7	D-Malic Acid	±	+		+	
G8	L-Malic Acid	±	+	±	±	±
G9	Bromo-Succinic Acid	-	+	-	-	-
G10	Nalidixic Acid	-	+	+	±	±
G11	Lithium Chloride	-	+	-	-	-
G12	Potassium Tellurite	+	+	-	+	+

Characteristic		*Pseudomonas syringae pv aceris*	*Microbacterium barkeri*	*Brevundimonas vesicularis*	*Pseudomonas syringae pv atrofaciens*	*Pseudomonas alcaligenes*
H1	Tween 40	-	±	±		±
H2	γ-Amino-Butryric Acid	-	-	-	±	+
H3	α-Hydroxy- Butyric Acid	-	-	-	-	-
H4	β-Hydroxy-D,LButyric Acid	-	-	±	-	-
H5	α-Keto-Butyric Acid	-	±	-	-	-
H6	Acetoacetic Acid	-	+	-	-	-
H7	Propionic Acid	-	±	-	-	+
H8	Acetic Acid	-	+	-	±	+
H9	Formic Acid	-	-	-	-	-
H10	Aztreonam	-	±	-	±	-
H11	Sodium Butyrate	-	+	-	-	-
H12	Sodium Bromate	-	+	-	-	-



Color Guide: = Positive, = Intermediate (+/-), = Negative

Figure 1. Identification plate result of (Z1) *Pseudomonas syringae* pv aceris, (Z2) *Microbacterium barkeri* , (Z3) *Brevundimonas vesicularis*, (Z4) *Pseudomonas syringae* pv atrofaciens and (Z5) *Pseudomonas alcaligenes.*

CONCLUSION

Future studies will determine the potential application of these isolates in plant growth promotion for enhancing productivity.

ACKNOWLEDGEMENTS

Part of this study was conducted as part of the Project No. 30-G5-1434: "Modern integrated management of halothermophilic biofertilizers for enhancing agricultural soil fertility in Jazan", which is supported and funded by the Deanship of Scientific Research, Jazan University, KSA.

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