Symbiosis

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ISSN Online: 2475-4714

Research Article | Journal of Advanced Research in Biotechnology | Open Access

Isolation and Characterization of Haloalkaliphilic Bacteria from the Rhizosphere of *Dichanthium annulatum*

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Received: May 25, 2018; Accepted: July 27, 2018; Published: July 31, 2018

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Abstract

Diversity of haloalkaliphilic bacteria from the rhizosphere of halophytes is a crucial determinant of plant health and productivity. The main objective of this study is the identification and characterization of haloalkaliphilic bacteria from the rhizosphere, rhizoplane and root endosphere of *D. annulatum* collected from Khewra Salt Mine, Pakistan. A total of 41 bacterial strains were isolated and identified on the basis of morphological and biochemical characterization. Twenty two strains were selected for phylogenetic analysis based on 16S rRNA gene sequences. About 41% bacterial strains were identified as different species of *Bacillus. Exiguobacterium, Kocuria, Citricoccus* and *Staphylococcus* were dominant genera identified in this study. Most of the bacterial strains characterized in this study were alkaliphilic, moderately halophilic and mesophilic in nature. Mostly strains were considered as a good source of hydrolytic enzymes because of their ability to degrade proteins, carbohydrates and lipids. Results for screening of hydrolytic enzymes showed that more than 90% strains had ability to produce at least three enzymes screened in this study. These results showed that haloalkaliphilic bacterial diversity identified in this study had great biotechnological potential.

Keywords: Haloalkaliphilic bacteria; rhizosphere; 16S rRNA gene; *Dichanthium annulatum*; hydrolytic enzymes

Introduction

Hypersaline environments are widely distributed across the globe as salt mines, saline lakes, salt marshes and marine water [1, 2]. Halophytes such as *Atriplex, Salsola, Dichanthium,* kallar grass and para grass may contribute significantly to the developing world's supply of food, fiber, fuel and fodder. For areas where farm land has been salinized by poor irrigation practices or that overlie reservoirs of brackish water or for coastal desert regions, these plants could be successfully grown [3, 4].

The rhizosphere of halophytes harbors an impressive array of halophilic and alkaliphilic microorganisms. Poly extremophilic organisms have ability to tolerate two or more extreme conditions, such as haloalkaliphiles, halothermophiles and alkalithermophiles [5]. Haloalkaliphiles are organisms that require high salinity (3-30%) and an alkaline pH (pH 9-13) for their growth [6]. These organisms have been isolated and characterized from a number of environments such as saline-sodic lakes, acid mines, hypersaline saline soils, salt mines, marine environments and salt marshes [7, 8]. Haloalkaliphiles usually use small organic molecules (osmolytes, e.g., ectoine, betaine and proline) and intracellular enzymes (α-galactosidase) to maintain their osmotic balance and pH ranges near neutral to survive under extreme saline and basic environments [9, 10]

Haloalkaliphiles have a wide range of applications in biodefense, bioenzymes and biofuel production [11, 12]. These organisms provide a good source of novel alkaliphilic and halophilic enzymes such as proteases, gelatinase, amylases, lipases, cellulases and xylanases [13, 14]. Enzymes isolated and characterized from haloalkaliphiles have ability to function properly even at high pH and salinity. These enzymes can be used in industrial applications such as detergent industry, food stuffs, paper and pulp and pharmaceuticals industry [15, 16].

Though a number of studies have been reported on the isolation of haloalkaliphiles from different environments but this study is the first report on characterization of haloalkaliphilic bacteria from the rhizosphere of *Dichanthium annulatum* (halophyte) collected from Khewra Salt Mines, Pakistan. In the present study, haloalkaliphilic bacteria were isolated from rhizospheric soil and roots and identified on the basis of 16S rRNA gene sequence analysis. Selected haloalkaliphilic bacterial strains were further characterized for their biotechnological potential and ability to produce different industrially important enzymes (cellulases, proteases, amylases, xylanases and lipases).

Material and Methods

Sampling site

Khewra Salt Mine is the world second largest salt mine, It is located near Jhelum District, Punjab, Pakistan (32° 38′ North latitude, 73°10′ East longitude). It is classified as thalassic hypersaline environment because it is derived from evaporation of sea water [17]. It has Na+ and Cl- dominating ions and the pH is near neutral to slightly alkaline. Vegetation of this area is classified as sub-tropical dry evergreen forest. Plants like *Suaeda,*

Salsola, Atriplex, Dichanthium, Justica, Lantana, and *Chrysopogon* are dominant genera found here.

Sample collection

Rhizospheric soil samples were collected by gently removing the plants and obtaining the soil attached the roots. Soil and root samples were collected four sites from different sites of Khewra Salt Mine. At each site, soil samples of approximately 500 g were collected in black sterile polythene bags. These samples were stored at 4^0C for further analysis.

Soil physicochemical parameters

Each soil sample (300 g) was thoroughly mixed and sieved through a pore size of 2 mm. Physical properties (pH, moisture content, salinity and temperature) of soil samples from different plants and non-rhizospheric soils samples were determined. Moisture (%); temperature and texture class were measured by Anderson method [18]; pH was measured by 1:2.5 (w/v) soil to water mixture and electrical conductivity (dS/m) was measured by 1:1 (w/v) soil to water mixture at 25^0C [19]. Organic matter (Corg) was calculated by Walkley-Black method [20]. Cation exchange capacity (CEC) is capacity to retain and release cations (Ca^{2+}, Mg^{2+}, K^+ and Na^+) and sodium adsorption ratio (SAR) is the measure of the sodicity of soil which is calculated as the ratio of the sodium to the magnesium and calcium.

Isolation of haloalkaliphilic bacteria

Haloalkaliphilic Medium (HaP) (Tryptone 5 g/l, Yeast Extract 1 g/l, NaCl 30 g/l, 5 g/l KCl, 10 g/l $MgSO_4$, 2 g/l K_2HPO_4 and pH 9.2) was used for the isolation and purification of bacteria present in saline environments [21]. Rhizosphere was fractionated into rhizosphere fraction (RS), rhizoplane fraction (RP) and root endosphere or histoplane bacterial fraction (HP) according to the method described by Malik et al. [22]. RS fraction indicates the soil adhering with the roots; RP fraction is the root surface and HP is the interior of roots. In case of RS, the soil was mixed thoroughly, sieved and then one gram representative soil sample was taken. Bacterial fraction from RP was isolated by shifting one gram of washed root to a falcon tube containing 9 ml saline along with some pebbles and incubated in a shaker for 30 minutes. For the isolation of HP bacteria roots was sealed at both ends with wax after washing with water. Sealed roots were surface sterilized by using 10% bleach for 10 min. After sterilization waxed ends of roots were removed and roots were macerated by using FastPrep® instrument (MP Biomedicals). The soil from each non-rhizospheric soil samples and brine lake-bank soil samples was mixed thoroughly, sieved and then one gram representative soil sample was taken. Serial dilutions (10^{-1}-10^{-10}) were made for all samples [23]. Dilutions from 10-3 to 10-6 were inoculated on HaP plates for counting colony forming units (CFU) per gram of dry weight. Plates were incubated at 30^0C until the appearance of bacterial colonies. Bacterial colonies were counted and number of bacteria per gram sample was calculated. The bacteria were purified by repeated sub-culturing of single colonies. Single colonies were selected, grown in HaP broth and stored in 33% glycerol at -80^0C for subsequent characterization.

Morphological and biochemical characterization of haloalkaliphilic bacterial isolates

For morphological characterization, colony morphology (colour, shape, elevation, size and margin) and cell morphology (shape, size, motility and Gram-staining) were studied. Halophilic bacterial strains were biochemically characterized to detect different enzymes (β-galactosidase, arginine deaminase, lysine decarboxylase, tryptophan deaminase, gelatinase, catalase and oxidase) and carbon sources (glucose, sucrose, mannitol, maltose, arabinose, lactose and sorbitol) utilization by using QTS 24 strips (DESTO Laboratories, Karachi, Pakistan).

Molecular characterization of haloalkaliphilic bacterial isolates

Genomic DNA was isolated from different bacterial isolates by CTAB method [24]. PCR amplification of 16S rRNA were performed by using universal forward and reverse primers P15(5'-GAGAGTTTGATCCTGGTCAGAACGAAC-3'),P65 (5'CGTACGGCTACCTTGTTACGACTTCACC-3') for prokaryotes [25]. A PCR reaction of 25 µl was prepared by using Taq polymerase (5U) 0.5 µl, Taq buffer (10X) 1 µl, MgCl2 (25 mM) 1.5 µl, dNTPS (2.5 mM) 2 µl, 2 µl each of forward and reverse primer (10 pmol), 16 µl of dd.H_2O and 2 µl of template DNA. First denaturation step at 95^0C for 5 min followed by 30 cycles of 94^0C for 1 min, 54^0C for 1 min and 72^0C for 2 min and a final extension step was at 72^0C for 10 min. PCR products were analyzed by using 1% agarose gel. PCR products were purified by using GeneJET PCR Purification Kit (K0702 - Thermo Fisher Scientific). Purified PCR products were sequenced by using forward and reverse primers (Eurofins, Germany).

Sequences of 16S rRNA gene were assembled and analyzed with the help of Chromus Lite 2.01 sequence analysis software (Technelysium Pty Ltd. Australia). The gene sequences were compared to those deposited in the GenBank nucleotide database using the NCBI BLAST program. Sequences were aligned using Clustal X 2.1 program and phylogenetic tree was constructed using neighbor-joining method. Bootstrap confidence analysis was performed on 1000 replicates to determine the reliability of the distance tree topologies obtained [26]. The evolutionary distances were computed using the Neighbor-joining method [27]. Phylogenetic analyses were conducted in MEGA7 [28]. There were a total of 1434 positions in the final dataset. Sequence of 16S rRNA gene from *Micrococcus luteus* was sued as outgroup. Bacterial strains identified in this study were submitted in GenBank under the accession numbers MH489029-MH489050.

Screening of haloalkaliphilic bacterial strains with respect to their salt, pH and temperature

Tolerance Ability

Bacterial isolates were grown in the presence of different salt

concentrations (3-12% NaCl), pH ranges of 4-12 and temperature ranges of 4-42^{0}C by using HaP broth medium. Isolates were cultured in 250 ml flasks at 30^{0}C with continuous rotatory agitation at 150 rpm for 72 h (hours) [29]. During incubation, bacterial growth in terms of optical density (OD 600) was measured after different time intervals (3, 6, 12, 24, 48 and 72 h).

Enzyme assays for haloalkaliphilic bacterial strains

Cellulose and amylase activities were identified by using 2% iodine solution and spotting single colony of the bacterial strains on CMC (carboxymethyl cellulose 1%) and starch (1%) supplemented LB agar plates respectively [30]. Protease activity was tested on the medium described by Kumar et al. [31]. Test for gelatin hydrolysis was performed by using the method described by Pitt and Dey [32]. Lipase activity was tested by using HaP medium with 1% butyrin and Tween 80 hydrolysis assay as described by Sierra [33]. Xylanase activity was tested by using HaP medium supplemented with 1% xylan [34]. The clear zones around the bacterial colonies after 4-12 days of incubation at 30^{0}C were considered as a positive result of protease, cellulose, xylan and lipase activities.

Results

Soil Physicochemical Analysis

Rhizospheric soil samples of four *D. annulatum* plants were analyzed and characterized on the basis of physicochemical properties such as soil pH, salinity, moisture, temperature, organic matter, NPK, CEC and SAR (Table 1). Soil pH ranged from 8.11 to 8.56 with the highest value in plant 3 and the lowest value in plant 1, electrical conductivity (EC1:1) ranged from 3.77 to 4.65 dS/m, values for soil moisture content ranged from 24.15 to 27.32%, temperature ranged from 29.23 to 32.52^{0}C (Table 1). The value for total organic matter was maximum in soil sample 1 (35.77) and minimum in soil sample 4 (32.29). The amounts of available P, K, Ca and Mg were maximum in soil sample 1 as compared to other soil samples. CEC and SAR values were maximum (73.61 mg.dm-3 and 13.51) for soil sample 2 (Table 1).

Table 1: Physical and chemical properties of rhizospheric soil samples of D. annulatum

Parameters	D. annulatum 1	D. annulatum 2	D. annulatum 3	D. annulatum 4
pH	8.11^a	8.29^{ab}	8.56^b	8.35^{ab}
$EC_{1:1}$ (dS/m)	4.14^{ab}	3.77^a	4.19^{ab}	4.65^b
Moisture (%)	25.83^{ab}	24.15^a	27.32^b	25.52^{ab}
Temperature (°C)	29.23^a	32.52^b	31.01^{ab}	30.82^{ab}
Texture class	Silty loam	Silty loam	Silty loam	Silty loam
OM (g.Kg^{-1})	35.77^b	33.15^a	34.55^{ab}	32.59^a
P (mg.kg^{-1})	3.99^{ab}	3.26^a	3.82^{ab}	3.59^{ab}
K (mg.kg^{-1})	0.76^a	0.58^b	0.65^b	0.49^a
Ca (mg.kg^{-1})	1.70^b	1.67^b	1.51^a	1.48^a
Mg (mg.kg^{-1})	1.28^b	1.15^a	1.26^b	1.19^a
NO^{-3} (mg.kg^{-1})	12.76^b	13.12^b	10.21^a	10.87^a
H+Al (mg.kg^{-1})	67.55^b	59.32^a	61.24^a	65.87^b
V (mg.kg^{-1})	4.13^b	3.87^a	4.18^b	3.76^a
CEC (mg.dm^{-3})	68.45^a	73.61b	72.73^b	67.78^a
SAR	10.24^b	13.51a	11.15^a	12.42^b

Note: EC (Electrical conductivity); OM (Organic matter); P (Phosphorous); K (Potassium); Ca (Calcium); Mg (Magnesium); NO^{-3} (Nitrate ion); H+Al (potential acidity); V (base saturation index); CEC (Cation exchange capacity) and SAR (Sodium adsorption ratio). Letters represent statistically significant values at 5% level.

Morphological and Biochemical Characterization of Haloalkaliphilic Bacterial Isolates

A total of 41 bacterial strains were isolated from the rhizosphere and roots of *D. annulatum* by using Hap medium with high salt concentration (3% NaCl) and pH (9.2). These isolates were identified on the basis of morphological and biochemical characterization. Out of 41, 40% bacterial isolates were identified as members of genus *Bacillus*, 16% isolates were related to *Kocuria,* 12% isolates were belonging to *Exiguobacterium*, 8% isolates were related to *Citricoccus*, 8% isolates were identified as Staphylococcus and 4% isolates were realted to Micrococcus (Fig. 1).

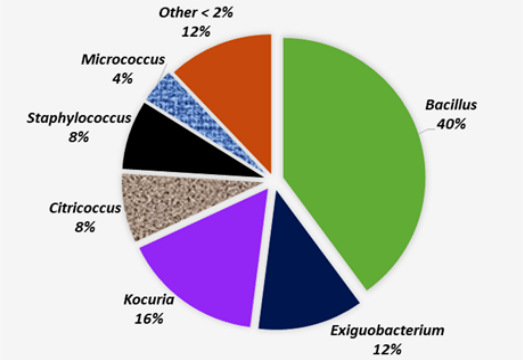

Figure 1: Relative abundance of haloalkaliphilic bacterial isolates from the rhizosphere, rhizoplane and root endosphere of *D. annulatum*

Phylogenetic Analysis of Haloalkaliphilic Bacterial Strains

On the basis of morphological and biochemical characterization, 22 bacterial isolates were selected for molecular characterization and phylogenetic analysis. Sequence analysis of 16S rRNA gene showed that 9 bacterial strains, PGRS2, PGRS7, PGRS9 and PGRS10 from the rhizosphere, PGRP3, PGRP6 and PGRP7 from the rhizoplane and PGHP2 and PGHP8 from the root endosphere of *D. annulatum* were identified as different species of Bacillus (Table 2 and Fig. 2). Three bacterial strains (PGRS1, PGRS3 and PGHP1) had 99% similarity with *Exiguobacterium mexicanum,* 3 bacterial strains (PGRS5, PGRP4 and PGHP9) were related to *Kocuria* (*K. rosea and K. polaris*), 2 bacterial strains (PGRP2 and PGHP4) showed 99% similarity with *Citricoccus alkalitolerans* and one strain (PGHP5) had 99% similarity with Staphylococcus equorum. Bacterial strains related to *Oceanobacillus, Enterococcus, Virgibacillus* and *Micrococcus* were also identified in this study (Table 2 and Fig. 2).

Table 2: Identification of haloalkaliphilic bacterial isolates from the rhizosphere, rhizoplane and root endosphere of D. annulatum

Isolate code	Organism identified	Accession No.	Closest type strain in NCBI data base	Sequence length(bp)	Sequence similarity (%)
PGRS1	Exiguobacterium	MH489029	E. mexicanum DSM 6208 (JF505980)	1306	99
PGRS2	Bacillus	MH489030	B. pseudofirmus ATCC 700159 (NR_026137)	1415	99
PGRS3	Exiguobacterium	MH489031	E. mexicanum DSM 16483 (JF505982)	1326	99
PGRS5	Kocuria	MH489032	K. rosea ATCC 186 (KM114943)	1212	99
PGRS6	Oceanobacillus	MH489033	O. oncorhynchi DSM 16557 (KJ145755)	1305	99
PGRS7	Bacillus	MH489034	B. cohnii DSM 6307 (JF689927)	1265	99
PGRS9	Bacillus	MH489035	B. alcalophilus JCM 5262 (NR_036894)	1345	99
PGRS10	Bacillus	MH489036	B. polygoni NCIMB 14282 (NR_041571)	1013	99
PGRS11	Enterococcus	MH489037	E. durans ATCC 19432 (NR_036922)	1332	99
PGRS12	Virgibacillus	MH489038	V. halodenitrificans DSM 10037 (HG931337)	1273	99
PGRP2	Citricoccus	MH489039	C. alkalitolerans KCTC 19012 (KF322100)	1034	98

PGRP3	Bacillus	MH489040	B. alcalophilus ATCC 27647 (NR_036889)	1421	99
PGRP4	Kocuria	MH489041	K. polaris CIP 107764 (KF876845)	1244	99
PGRP6	Bacillus	MH489042	B. halodurans NRRL B-3881 (HQ446864)	1354	99.45
PGRP7	Bacillus	MH489043	B. alkalinitrilicus DSM 22532 (NR_044204)	1298	99.12
PGHP1	Exiguobacterium	MH489044	E. mexicanum DSM 16483 (JF505982)	1469	99.24
PGHP2	Bacillus	MH489045	B. clarkii DSM 8720 (KY849416)	1405	99.63
PGHP4	Citricoccus	MH489046	C. alkalitolerans DSM 15665 (KF322104)	1438	99.87
PGHP5	Staphylococcus	MH489047	S. equorum ATCC 43958 (AB975354)	1354	99.42
PGHP6	Micrococcus	MH489048	M. luteus CCM 169 (KJ843153)	1343	99.74
PGHP8	Bacillus	MH489049	B. pseudofirmus DSM 8715 (NR_026139)	1256	99.25

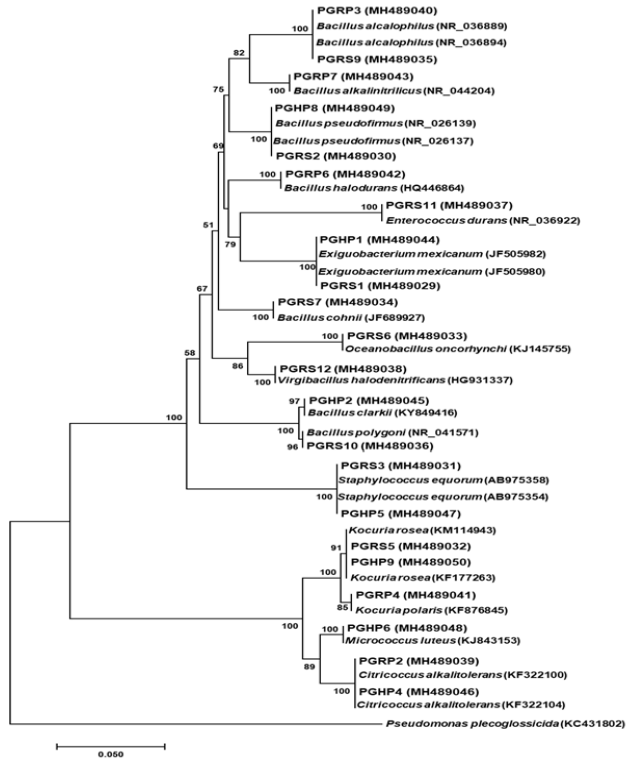

Figure 2: Phylogenetic tree based on16S rRNA gene sequences of haloalkaliphilic bacterial strains from the rhizosphere, rhizosplane and root endosphere of *D. annulatum*. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches.

Phenotypic characterization of haloalkaliphilic bacterial strains

All the strains had ability to grow at pH range from 8 to 12, but only few strains were able to survive at pH 4 and 6 (Fig. 3A). Mostly strains were able to grow at salt concentrations of 3-10% NaCl but only few strains (28%) especially members of *Bacillus* had ability to grow at 12% NaCl concentration (Fig. 3B). All the strains could grow well at temperature 28 and 37^{0}C but only 38 % bacterial strains could tolerate at 4 and 62% strains were able to grow at 42^{0}C (Fig. 3C).

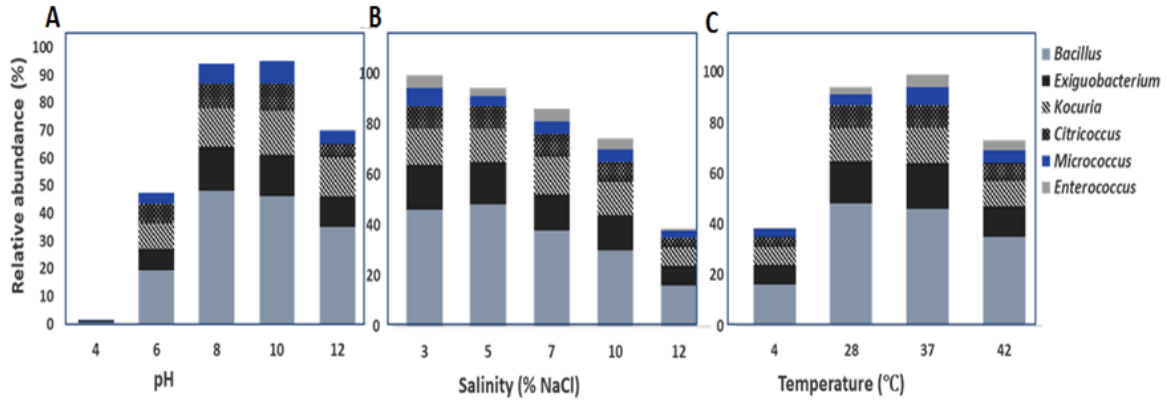

Figure 3: Phenotypic characterization of alkaliphilic bacterial isolates from the rhizosphere, rhizoplane and root endosphere of *D. annulatum*; **(A)** pH **(B)** salinity and **(C)** Temperature tolerance profile

Enzyme producing ability of haloalkaliphilic bacterial strains

Mostly haloalkaliphilic bacterial strains showed ability to degrade carbohydrates, lipids, proteins and gelatin at high salinity and pH (Table 3 and Fig. 4). Out of 22, sixteen bacterial strains showed positive results for protease activity, 20 strains had ability to degrade lipids, and 16 strains showed positive activity for amylase enzyme, 16 strains showed positive results for gelatinase, 14 strains were positive for cellulase activity and 14 strains showed positive results for xylanase activity (Table 3 and Fig. 4).

Table 3: Screening of hydrolytic enzymes produced by haloalkaliphilic bacterial strains from the rhizosphere, rhizoplane and root endosphere of D. annulatum

Bacterial strains	Protease	Lipase	Amylase	Cellulase	Gelatinase	Xylanase
PGRS1	-	++	-	-	+	++
PGRS2	+++	-	++	+	++	-
PGRS3	++	+	-	-	+	++
PGRS5	-	+	+++	-	++	+
PGRS6	++	++	++	++	++	-
PGRS7	+++	+	-	++	-	++
PGRS9	-	++	++	+	+++	-
PGRS10	++	+	++	++	++	+
PGRS11	++	-	-	-	+++	-
PGRS12	+++	+	-	++	+	++
PGRP2	-	+	++	++	-	+
PGRP3	++	++	++	+	+++	+
PGRP4	+	+	+	-	+	-
PGRP6	++	++	++	++	-	+
PGRP7	+++	++	+	+	+++	-

PGHP1	+	+	-	-	+	++
PGHP2	++	++	+	++	++	++
PGHP4	-	+	++	+	-	-
PGHP5	+	+	+	+	-	+
PGHP6	++	++	++	-	-	+++
PGHP8	++	++	+	++	++	-

Note: -, no activity; +, low activity; ++, medium activity; +++, high activity

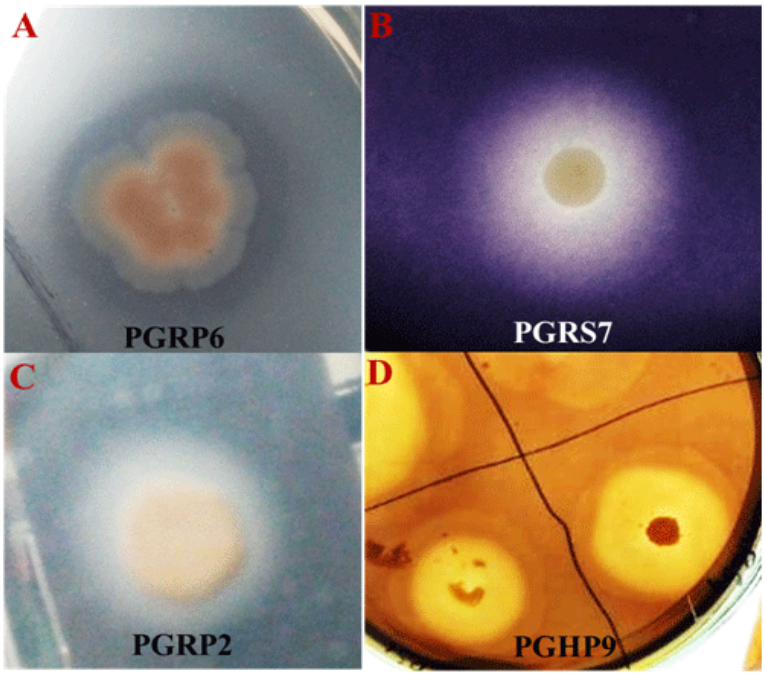

Figure 4: Enzyme assays for haloalkaliphilic bacterial strains from the rhizosphere, rhizoplane and root endosphere of D. annulatum using a drop spot technique; **(A)** Protease **(B)** Amylase **(C)** Lipase and **(D)** Cellulase

Discussion

High pH and salinity present a multifold challenge to all organisms in terms of ionic disequilibria and perturbed osmotic balance. Microorganisms that isolated and characterized from highly saline and saline-sodic soils have adapted special genetic and morphological modifications to survive under such extreme conditions [35, 36]. Here, we reported haloalkaliphilic bacterial diversity from the rhizosphere, rhizoplane and root endosphere of a halophyte (*D. annulatum*). The isolated bacterial strains were also screened for production of industrially important enzymes such as amylases, proteases, lipases, cellulases and gelatinase.

A total of 22 haloalkaliphilic bacterial strains have been identified from the rhizosphere and roots of *D. annulatum.* Phylogenetic analysis showed that these isolates were related to nine different bacterial genera *Bacillus, Exiguobacterium, Kocuria, Citricoccus, Staphylococcus, Enterococcus, Oceanobacillus, Virgibacillus* and *Micrococcus* (Table 2). Previous studies on the isolation of haloalkaliphilic bacterial strains from Soda Lake Magadi (Kenya) showed that *Bacillus, Exiguobacterium* and *Halomonas* were the dominant genera identified from these environments [5, 37]. The abundance of Gram positive bacteria (*Bacillus, Exiguobacterium, Kocuria, and Staphylococcus*) is attributed to their cell wall and endospore formation in Bacillus enable them to survive in hypersaline and saline-sodic environments. Members of Actinobacteria *Kocuria, Citricoccus* and *Micrococcus* identified in this study have been previously reported from hypersaline soil of halophytes and Texcoco Lake [38, 39].

Bacterial isolates characterized in this study were alkaliphilic and moderately halophilic in nature. More than 87% strains were able to grow at pH more 10, salt concentrations 5-10% and temperature 28-42^{0}C. Previous studies also reported that alkaliphiles, moderately halophiles and mesophiles are more abundant as compare to extremely halophilic and thermophilic

bacteria in different soils [38, 40]. Halophilic strains from the groups, *Virgibacillus* and *Oceanobacillus* show optimum growth at salt concentration 5-10% NaCl and 28-37 ^{0}C [41].

Most of the bacterial strains showed ability to degrade different organic compounds such as carbohydrates, lipids, proteins and gelatin. More than 90% bacterial strains showed lipase activity, 73% bacterial strains showed proteolytic activity, 72% strains had ability to degrade carbohydrates, 73% strains showed positive results for gelatinase activity, 64% strains had ability to degrade cellulose and 63% strains showed xylanase activity (Table 3). Alkaliphilic, halophilic and mesophilic bacteria isolated from different saline and saline-sodic environments showed their ability to produce different industrially important enzymes such as amylases, proteases, lipases, gelatinase and xylanase [9, 11]. Enzymes produced by haloalkaliphilic bacteria have structural and catalytic properties to function properly even at high salinity, pH and temperature [42]. Lipase and protease producing alkaliphilic and halophilic bacteria have been previously isolated marine environment and food sources such as fish sauce [37, 43]. Halophilic bacterial strains related to *Bacillus and Oceanobacillus* are known to be a good source of different hydrolytic enzymes such as α-amylases, lipase, protease and xylanases [14, 42]. Members of Actinobacteria *Citricoccus, Kocuria* and *Micrococcus* have been well known for production of lipases, cellulases, amylases and gelatinase [39, 44]. Haloalkaliphilic cellulases and xylanases have been produced by different alkaliphilic and halophilic bacteria such as *Kocuria, Bacillus* and *Staphylococcus* [45]. Members of *Exiguobacterium* have been isolated from hypersaline tropical soils. These bacteria are able to grow at high pH and considered as good source of alkaliphilic enzymes such as proteases, lipases, cellulases and gelatinase [42, 46].

Conclusion

This study was the first report of its kind that deals with characterization of haloalkaliphilic bacteria from the rhizosphere and roots of *D. annulatum*. Twenty two haloalkaliphilic bacterial strains were identified on the basis of 16S rRNA gene analysis from the rhizosphere, rhizoplane and root endosphere. Nine strains showed more than 99% similarity with different species of Bacillus. Other dominant bacterial genera included *Kocuria, Exiguobacterium, Citricoccus, Oceanobacillus* and *Staphylococcus* was identified in this study. Most of the bacterial strains showed positive results for industrially important enzymes such as amylases, cellulases, proteases, lipases, gelatinase and xylanases. The ability of these bacterial strains to survive at high salinity, pH and temperature showed their potential biotechnological applications especially as a source of various enzymes.

Acknowledgement

We are highly thankful to **Higher Education Commission** [Project # HEC (FD/2012/1843)] for research grant.

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