Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2021 18(4): 3293-3303. **OPEN ACCESS**

Phenotypic and molecular identification and characterization of rhizobacteria isolated from the Rhizosphere of *Aloe vera*

Muhammad Ibrahim[1], Fatima Ashraf[1], Muhammad Wajid Khurshid Sipra[2], Adeel Hussain Chughtai[3], Muhammad Aslam[2], Sarfraz Ahmed[4], Sawera Asad[1], Sawaira Maqsood[1], Humaira Nawaz[5] and Muhammad Nawaz[6*]

[1]Department of Biochemistry; Bahauddin Zakariya University (60800) Multan, **Pakistan**
[2]Department of Pathology Quaid-e-Azam Medical College, Bahawalpur**, Pakistan**
[3]Institute of Chemical Sciences, Bahauddin Zakariya University (60800) Multan, **Pakistan**
[4]Department of Basic Sciences, KBCMA College of Veterinary and Animal Sciences, University of Veterinary and Animal Sciences, Lahore (51600) Narowal Campus, **Pakistan**
[5]College of Earth and Environmental Sciences, University of the Punjab, Lahore, **Pakistan**
[6]Department of Environmental Sciences, Bahauddin Zakariya University (60800) Multan, **Pakistan**

***Correspondence:** mnawaz@bzu.edu.pk **Received 17-09-2021, Revised: 21-12-2021, Accepted: 22-12-2021 e-Published: 23-12-2021**

Rhizosphere is a hub of bacterial populations. The current study was conducted to investigate the bacterial diversity in the rhizosphere of *Aloe vera* growing in Multan region. Bacteria were isolated and characterized by phenotypic and molecular techniques. For the identification and confirmation of 41 bacterial strains having distinct morphological and biochemical characteristics, 16S rRNA gene sequence analysis was performed. The dominant bacterial species were i.e. *Bacillus albus* (19.5%), *Rhizobium pusense* (19.5%), *Enterobacter cloacae* (12.19%), *Chryseobacterium gambrini* (9.7%), *Flavobacterium anhuiense* (4.8%) and *Stenotrophomonas maltophilia* (4.8%) while *Pseudomonas stutzeri, Tatumella terrea, Micrococcus aloeverae, Kocuria marina, Kocuria rosea, Bacillus paramycoides, Chryseobacterium daecheongense, Micrococcus endophyticus, Exiguobacterium mexicanum, Microbacterium zeae, Brachybacterium paraconglomeratum* and *Ochrobactrum pituitosum* were populated up to 2% each. *Bacillus albus* and *Rhizobium pusense* were found to be predominant spp. under the study areas. Bacterial 16S rRNA gene sequences having accession numbers MK993437 to MK993477 have been submitted in the database of Genbank. This study demonstrated that the divergent groups of rhizobacteria are present in the rhizosphere of *Aloe vera.*

Keywords: Cultivable bacteria, phylogenetic assay, plant growth promotion, Rhizosphere, *Rhizobium*, 16S rRNA gene sequencing

INTRODUCTION

Aloe vera is a medicinal plant as it is accompanied by multiple health advantages and surprising that rarely any part of human body remains unswayed by its therapeutic glance (Manvitha and Bidya, 2014). *Aloe vera* is a source of *Aloe vera* gel that is obtained from the interior of fleshy leaves of *Aloe vera* plant. A “latex” or the aloin component is found in the pericarpal layer below the rind of fleshy leaves (Pressman, Clemens, and Hayes, 2019). The gel extracted from *Aloe vera* leaves comprises of a variety of bioactive compounds, minerals and phenolic contents (Maan, Reiad Ahmed, Iqbal Khan, Riaz,

and Nazir, 2021). The beneficial therapeutic properties of *Aloe vera* plant have been employed for a number of commercial applications (Maan et al. 2018). Rhizosphere is absolutely the most complex microhabitat, comprised of integrated network of soil, plant roots and a diverse consortium of bacteria, fungi, eukaryotes, and archaea. Rhizosphere conditions have a direct impact on crop growth and production. The nutrient-rich rhizosphere environments stimulate the plant growth and production (Hakim et al. 2021). Various microbes have been identified to inhabit rhizosphere, some of which are nitrogen fixing bacteria, proteobacteria, plant growth promoting rhizobacteria, mycorrhizal and mycoparasitic fungi (Omotayo and Babalola, 2021). The rhizosphere supports a vital microbial community (Ahmad, Ahmad, and Khan, 2008). Numerous studies have revealed that rhizodeposition and root exudates render a conducive dwelling for microbial profuse and activity in the soil environment (Hartmann, Rothballer, and Schmid, 2008; Smalla, Sessitsch, and Hartmann, 2006). Soil microbes are inquisitive to guarding soil functions in both natural and sorted agricultural soils by contributing in crucial phenomena like; decaying of basic matter, expulsion of toxins and recycling of carbon, nitrogen, phosphorus and sulfur (Bloem, Ruiter, and Bouwman, 1997; du Preez, van Huyssteen, and Mnkeni, 2011). In *Aloe vera* rhizosphere, there is a range of microbes interacting with soil, root exudates and plant growth promoting bacteria (Meena et al. 2017).

Root exudates include a large variety of compounds released by plants into rhizosphere, containing low-molecular-weight primary metabolites like saccharides, organic acids and amino acids) and secondary metabolites like phenolic compounds, terpenoids and flavonoids. The changes in the exudate composition could have impacts on plant itself, on other plants, on soil properties i.e. amount of soil organic matter and on soil microbes (Gargallo-Garriga et al. 2018). Root exudation is an important process that determines the plant interactions with soil environment. Root exudates affect rhizosphere-dwelling microbes (Canarini, Kaiser, Merchant, Richter, and Wanek, 2019). Therefore, the study was aimed to identify and characterize the rhizobacterial flora of the rhizosphere of *Aloe vera* plant and to assess their enzymatic activities.

MATERIALS AND METHODS

Study area

Four diverse locations were selected for sampling i.e. i) Multan (30°18'14.6"N 71°49'21.6"E; alt.122 m), ii) Khanewal (30°28'64.1"N 71°93'20.3"E; alt.128 m), iii) Abdul Hakeem (32°87'39.0"N 70°65'07.1"E; alt.413 m) and iv) Kabirwala (30°46'74.1"N 71°75'98.0"E; alt.413 m).

Sampling

Rhizosphere samples were collected after uprooting the plant from a depth of 15 cm using the method of (Barea, 2005). Samples were taken from each location by selecting two plants on 15 November 2018 and 15 January 2019. Samples were collected in sterile plastic bags, packed in labeled plastic boxes and stored at 4°C. The samples were processed within 24 hours for bacterial isolation.

Isolation and growth of cultivable rhizobacteria

Culturable bacteria were isolated by serial dilution method (Ben-David and Davidson, 2014). Soil suspension was prepared by dissolving 10 g soil in 90 mL Normal saline in a sterile flask (Ukpaka, 2016). Soil suspension was taken on shaking incubator (LSI-3016A, Labtech, Korea) for 10 min at 300 rpm in order to get a homogenous soil suspension stock solution. The stock solution was diluted to get six serial dilutions (10^{-1} to 10^{-6}). A volume of 100 µL from each dilution was spread on nutrient agar culture plates in triplicate. Incubation period was extended up to 48 hours at 37°C. The viable colonies were counted to measure the survival efficiency of bacteria using the formula (Ibrahim, Ahmad, Nawaz, Aslam, and Shad, 2020).

Viable Cell Count (CFU/g soil) =Nc/Vi ×df

Where, Nc, Vi, and df are the number of colonies, the volume of inoculation, and dilution factor respectively

Purification and preservation of cultivable bacteria

By sequential streaking process pure bacterial colonies were obtained. Pure bacterial colonies were stored as LB glycerol stocks (LB: glycerol, 50:50) at -70°C. Overnight LB broth cultures were grown using LB glycerol stocks as seed cultures for further analysis (Ibrahim et al. 2020).

Morphological identification

Morphological characteristics of bacterial isolates like color, size, odor, margin, texture,

opacity, cell-shape, spore formation, cell morphology, elevation, gram staining (Table 1) were identified using the standard methods (G Cappuccino and Sherman, 2014).

Biochemical identification

To evaluative the bacterial enzyme activities and biochemical characteristics standard methods of (G Cappuccino and Sherman, 2014) and (Paul, 2019) were used.

Molecular characterization

Extraction of bacterial genomic DNA

Genomic DNA of bacterial isolates was extracted using commercial bacterial DNA kit (Vivantis: GF-BA-100, USA) (Pongsilp, Nimnoi, and Lumyong, 2012). Fresh bacterial culture was grown at 37°C for 18-24 h at 300 rpm in a shaking incubator (Lab-tech LSI-3016A, Korea). DNA was visualized by 1% pre-stained agarose gel with ethidium bromide in 1x TBE buffer. 1kb DNA ladder was used as standard.

Amplification of 16S rRNA gene

The amplification of 16S rRNA gene was performed by PCR (Agilent, Sure Cycler 8800, USA) with initial denaturation at 94°C for 30 s, annealing at 55°C for 30 s, followed by extension at 72°C for 1 min and final extension at 72°C for 10 min. Forward 5'CAGCAGCCGCGGTAATAC3' and reverse 5′ACGGGCGGTGTGTACAAG3′ primers capable of initiating PCR amplification on a wider range of bacteria derived from *Escherichia coli* (Porteous and Armstrong, 1993). The reaction mixture (50 µL) contained 5 µL of 10x PCR buffer, 3 µL of 25 mM $MgCl_2$, 0.4 µL of 100 mM of each dNTPs, 0.5 µL of 10 pmole forward primer, 0.5 µL of 10 pmole reverse primer, 0.25µL of 5 U/µL *Taq polymerase*, 2 µL of 20 ng template DNA and nuclease free water 38.35 µL) for 35 cycles. PCR amplified products were analyzed by1% agarose gel with pre-stained ethidium bromide in 1x TBE buffer. Amplified gene was observed under Gel DocTM EZ Imager (Bio-Rad, USA).

Sequencing of PCR products

The 16S rRNA gene sequencing of 41 PCR products was performed using respective primers by Macrogen Inc. (South Korea). Morphological and biochemical characteristics of bacterial isolates were taken into consideration in order to identify prominent differences between bacterial isolates. Percentage sequence similarity of bacteria was compared with their nearest relatives available on the database of GenBank using BLAST analysis. Partial sequences of 16S rRNA genes were deposited in the database of GenBank https://www.ncbi.nlm.nih.gov (Table 3).

Molecular Evolutionary Genetics Analysis

Phylogenetic tree of bacterial gene sequences was performed to study the evolutionary history of bacteria using MEGA11 (Tamura, Stecher, Kumar, and evolution, 2021).

Data analysis

The 16S rRNA gene sequences were aligned by BLAST for finding analogy among the sequences of bacterial isolates already reported and available on NCBI database. MEGA 11 was used for phylogenetic tree construction and finding the evolutionary relationship between bacteria. The evolutionary history was inferred using the Neighbor-Joining method, that is a key tool for the creation of Phylogenetic tree.

RESULTS

Phenotypic attributes of rhizobacteria of *Aloe vera* plant

Morphological characteristics

Morphological characterization was performed on the basis of bacterial morphology. Bacterial isolates showed variations in size i.e. pin-point (22%), small (32%), medium (26%) and large sized (20%) (BAROLIA, 2016). Most of the bacteria were circular (83%) and irregular (17%). Bacterial isolates showed pigmentation like white (17%), off-white (39%), lemon-yellow (10%), yellow (22%) orange and orange yellow 5% each. 2% bacteria showed pink colouration. All the bacterial isolates were to be of foul smelling. Most of the bacteria showed opacity (76%), smoot appearance (95%) moist texture (98%), raised elevation (68%), entire margin (83%) Fifty-one percent bacteria gave Gram-staining test positive and 12% bacteria gave endospore formation test. Most of the bacteria were *Bacilli* and *Cocci* as depicted in Table 1.

Table 1: Morphological characteristics of rhizobacteria of *Aloe vera* plant

Sr. No.	Tested parameter	Characteristics	Total isolates (n)	Percentage (%)
1	Colony size	Pin-point	9	22
		Small	13	32
		Medium	11	26
		Large	8	20
2	Colony shape	Circular	34	83
		Irregular	7	17
3	Color	Off-white	16	39
		White	7	17
		Yellow	9	22
		Lemon-Yellow	4	10
		Orange	2	5
		Orange-Yellow	2	5
		Pink	1	2
4	Odor	Foul-smelling	41	100
5	Opacity	Transparent	10	24
		Opaque	31	76
6	Appearance	Smooth	39	95
		Dull	2	5
7	Texture	Moist	40	98
		Dry	1	2
8	Elevation	Flat	13	32
		Raised	28	68
9	Margin	Entire	34	83
		Irregular	3	7
		Undulate	4	10
10	Gram staining	+ve	21	51
		-ve	20	49
11	Spore formation	-ve	36	88
		+ve	5	12
12	Cell morphology	Cocci	19	46
		Bacilli	16	39
		Coco-Bacilli	6	15

Table 2: Biochemical characteristics and enzymatic activities of rhizobacteria of *Aloe vera* plant

Sr. No.	Test parameter	Enzymatic activity	Characteristics	Total isolates (n)	Percentage (%)
1	Triple sugar iron test	-	-ve	22	54
		-	Glucose fermentation	10	24
		-	lactose, sucrose fermentation	9	22
		-	H_2S production	-	-
2	Starch hydrolysis	α-amylase	+ve	10	24
			-ve	31	76
3	Urease test	Urease	+ve	9	22
			-ve	32	78
4	Catalase test	Catalase	+ve	41	100
5	Citrate utilization	Citrate permease	+ve	9	22
			-ve	32	78
6	Indole test	Tryptophanase	+ve	9	22
			-ve	32	78
7	Coagulase test	Coagulase	+ve	22	54

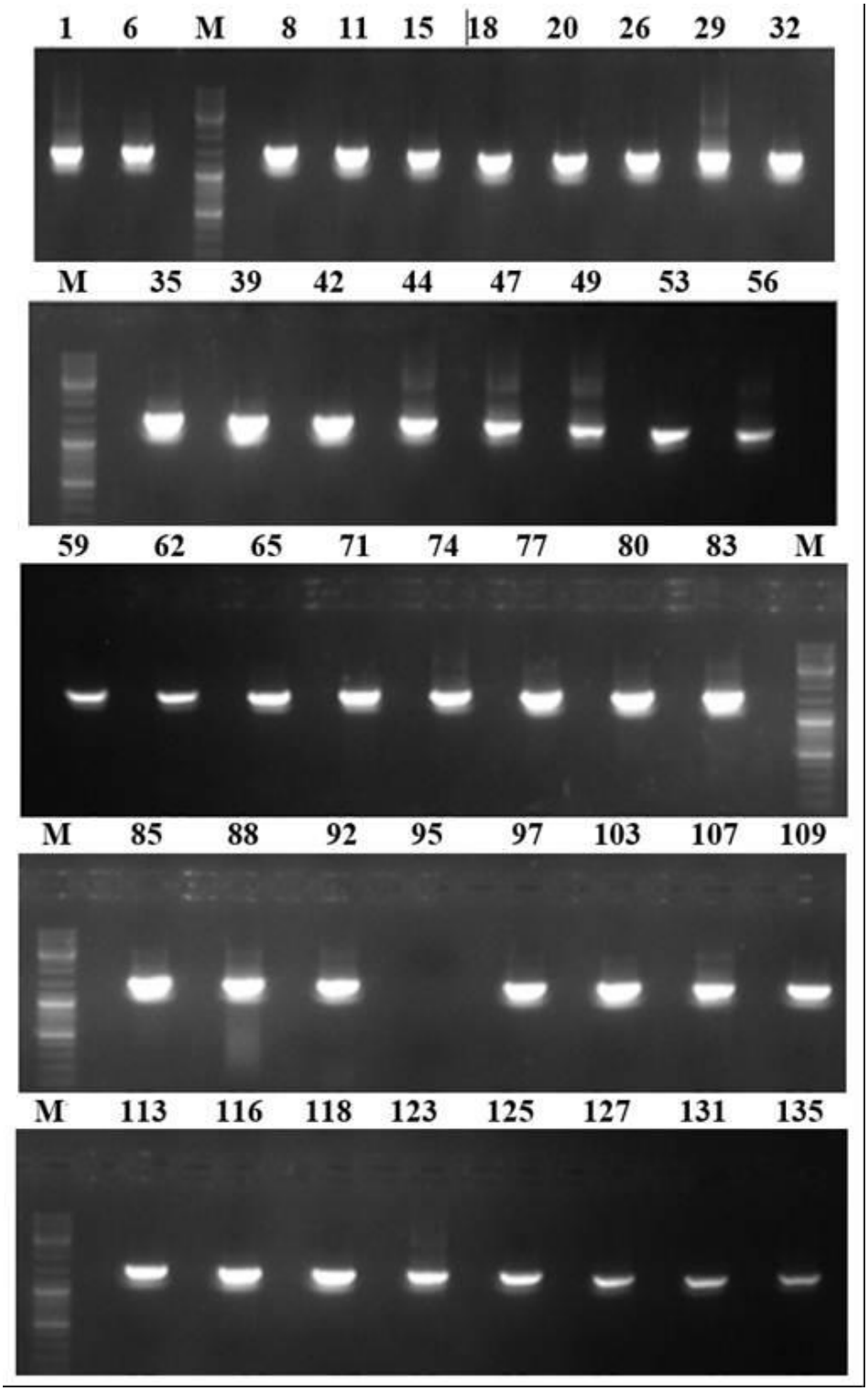

Figure 1: Electrophoresis patterns of 16S rRNA gene
(M = 1kb DNA marker)

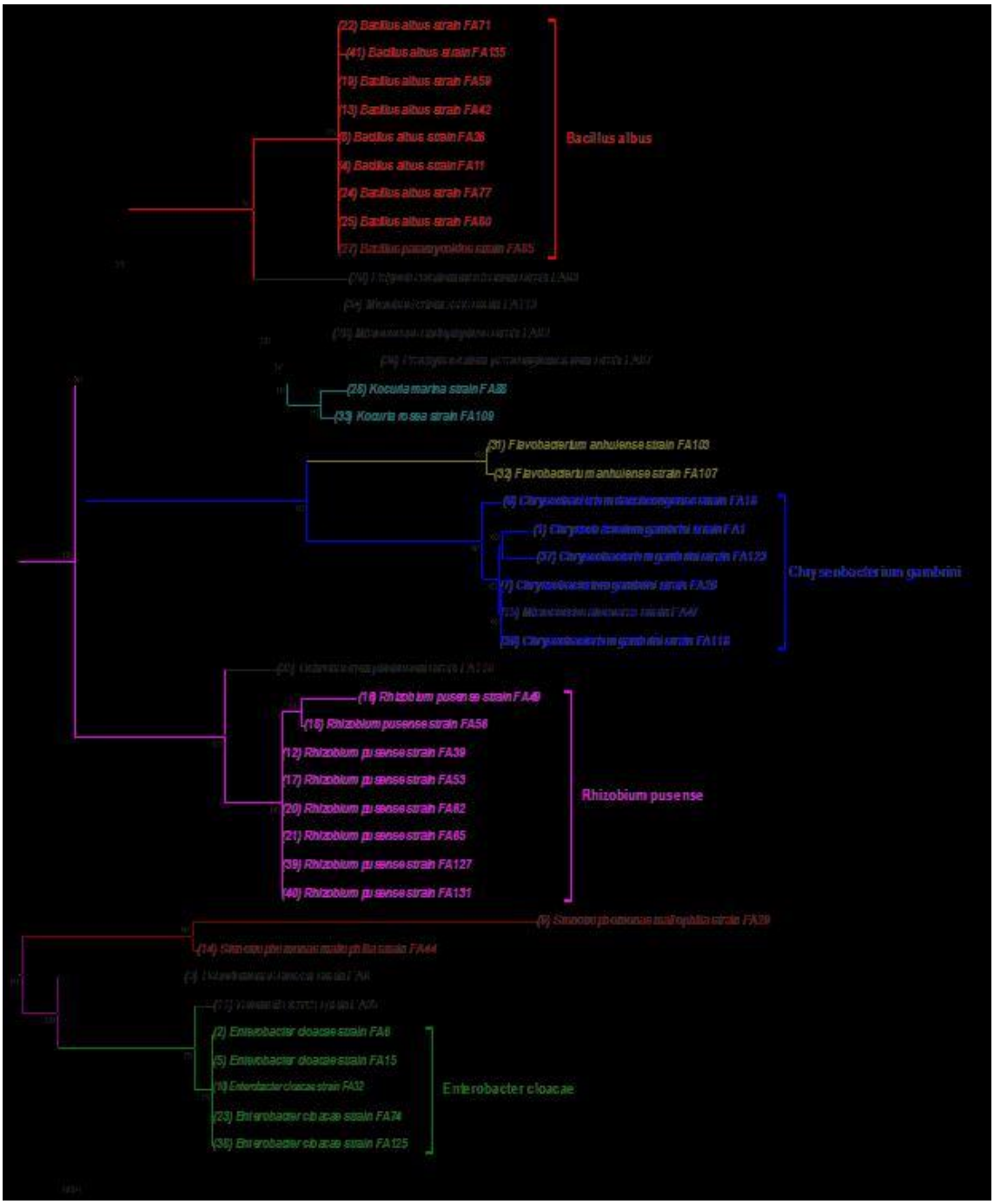

Figure 2: Molecular phylogenetic relationship of rhizobacteria of *Aloe vera* plant

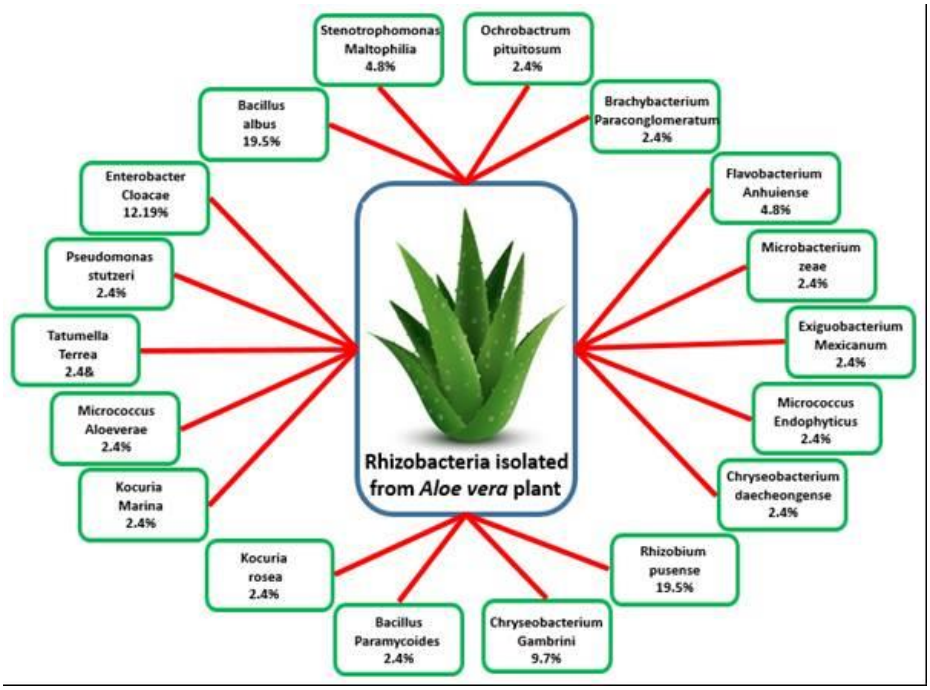

Figure 3: Percentile summary of various rhizobacteria isolated from the rhizosphere of *Aloe vera* plant

Biochemical and enzymatic activities

Twenty-four percent bacteria gave glucose fermentation reaction. Twenty-two percent bacteria gave lactose and sucrose fermentation reaction. 24% bacteria were observed showing α-amylase and 22% urease enzyme activities. All the bacteria showed catalase enzyme activity. Most of the bacteria (54%) gave coagulase activity. 22% bacteria were observed having citrate permease and tryptophanase activities (Table 2)

Molecular characterization

Amplification of 16S rRNA gene

The length of 16S ribosomal RNA gene is 1540 bp. The PCR products of 16S ribosomal gene visualized by Gel DocTM EZ Imager (Bio-Rad, USA) are depicted in Figure 1. Electrophoretic patterns of DNA show the clear gel bands having maximum yield of gene.

Phylogenetic relationship

From the BLAST analysis, forty-one rhizobacteria from rhizosphere of healthy *Aloe vera* plant were identified. The phylogenetic tree was created to study metamorphic relationships of 16S rRNA sequences of described bacterial species of *Aloe vera*. Forty-one sequences were captured to access phylogenetic tree of *Aloe vera* plant bacterial diversity. A quite distinct phylogenetic tree was obtained (Figure 2). The bacteria on the basis of 16S rRNA gene sequences have been branched into four clades (i.e. *Bacillus albus*, *Chryseobacterium gambrini*, *Rhizobium pusense* and *Enterobacter cloacae*)). *Bacillus albus* was the larger clade with nine species. Eight strains of *Bacillus albus* were arosed in *Bacillus albus* clade. Single strain of *Bacillus paramycoides* specie was also observed in *Bacillus albus* clade showing close evolutionary relationship with taxa of this clade. Five strains of *Chryseobacterium gambrini* and one strain of *Micrococcus aloeverae* appeared in *Chryseobacterium gambrini* clade. The bacterial species (i.e. *Exiguobacterium mexicanum*, *Microbacterium zeae*, *Micrococcus endophyticus*, *Brachybacterium paraconglomeratum*, *Kocuria rosea, Kocuria marina and Flavobacterium anhuiense*) were materialized in between *Bacillus albus* clade and *Chryseobacterium gambrini* clade showing an evolutionary divergence from the rest of taxa present in the phylogram. Eight strains of *Rhizobium pusense* species and one strain of *Ochrobactrum pituitosum* species appeared in *Rhizobium pusense* clade showing evolutionary relationship with this clade. Five strains of *Enterobacter cloacae* also lied in *Enterobacter cloacae* clade. Bacterial species of *Tatumella terrea, Stenotrophomonas maltophilia and*

Pseudomonas stutzeri were located in between *Rhizobium pusense* and *Enterobacter cloacae*. These clades showed an evolutionary discrepancy from rest of taxa present in the phylogram. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the Phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura, Nei, and Kumar, 2004) and are in the units of the number of base substitutions per site. The analysis involved 41 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 788 positions in the final dataset. Evolutionary analyses was conducted in MEGA11 (Tamura et al. 2021).

Table 3: Taxonomic affiliation and percentage sequence similarities of bacteria of *Aloe vera* plant with their nearest relatives from GenBank database

Sequence ID	Allotted Accession number	Nearest relative	Accession number	Identity (%)
FA1	MK993437	Chryseobacterium gambrini 5-1St1a	NR042505.1	97
FA6	MK993438	Enterobacter cloacae DSM 30054	NR117679.1	98
FA8	MK993439	Pseudomonas stutzeri ATCC 17588	NR041715.1	99
FA11	MK993440	Bacillus albus MCCC 1A02146	NR157729.1	96
FA15	MK993441	Enterobacter cloacae DSM 30054	NR117679.1	98
FA18	MK993442	Chryseobacterium daecheongense NBRC	NR114019.1	99
FA20	MK993443	Chryseobacterium gambrini 5-1St1a	NR042505.1	98
FA26	MK993444	Bacillus albus MCCC 1A02146	NR157729.1	98
FA29	MK993445	Stenotrophomonas maltophilia IAM 12423	NR112030.1	86
FA32	MK993446	Enterobacter cloacae DSM 30054	NR117679.1	97
FA35	MK993447	Tatumella terrea DSM 13701	NR116800.1	97
FA39	MK993448	Rhizobium pusense NRCPB10	NR116874.1	96
FA42	MK993449	Bacillus albus MCCC 1A02146	NR157729.1	97
FA44	MK993450	Stenotrophomonas maltophilia IAM 12423	NR041577.1	99
FA47	MK993451	Micrococcus aloeverae AE-6	NR134088.1	99
FA49	MK993452	Rhizobium pusense NRCPB10	NR116874.1	92
FA53	MK993453	Rhizobium pusense NRCPB10	NR116874.1	97
FA56	MK993454	Rhizobium pusense NRCPB10	NR116874.1	96
FA59	MK993455	Bacillus albus MCCC 1A02146	NR157729.1	97
FA62	MK993456	Rhizobium pusense NRCPB10	NR116874.1	97
FA65	MK993457	Rhizobium pusense NRCPB10	NR116874.1	98
FA71	MK993458	Bacillus albus MCCC 1A02146	NR157729.1	99
FA74	MK993459	Enterobacter cloacae DSM 30054	NR117679.1	98
FA77	MK993460	Bacillus albus MCCC 1A02146	NR157729.1	96
FA80	MK993461	Bacillus albus MCCC 1A02146	NR157729.1	96
FA83	MK993462	Exiguobacterium mexicanum 8N 1245	NR042424.1	99
FA85	MK993463	Bacillus paramycoides MCCC 1A04098	NR157734.1	99
FA88	MK993464	Kocuria marina KMM	NR025723.1	99
FA92	MK993465	Micrococcus endophyticus YIM 56238	NR044365.1	99
FA97	MK993466	Brachybacterium paraconglomeratum LMG 19861	NR025502.1	99
FA103	MK993467	Flavobacterium anhuiense D3	NR044388.1	99
FA107	MK993468	Flavobacterium anhuiense D3	NR044388.1	99
FA109	MK993469	Kocuria rosea DSM 20447	NR044871.1	99
FA113	MK993470	Microbacterium zeae 1204	NR149816.1	98
FA116	MK993471	Ochrobactrum pituitosum CCUG 50899	NR115043.1	98
FA118	MK993472	Chryseobacterium gambrini 5-1St1a	NR042505.1	99
FA123	MK993473	Chryseobacterium gambrini 5-1St1a	NR042505.1	96
FA125	MK993474	Enterobacter cloacae DSM 30054	NR117679.1	99
FA127	MK993475	Rhizobium pusense NRCPB10	NR116874.1	96
FA131	MK993476	Rhizobium pusense NRCPB10	NR116874.1	95
FA135	MK993477	Bacillus albus MCCC 1A02146	NR157729.1	96

Taxonomic affiliation of rhizobacteria

All rhizobacteria showed from 86% to 99% similarity with their nearest relatives available on GenBank database. 15 bacteria showed 99%, 8 bacteria showed 98%, 7 bacteria showed 97% and 8 bacteria showed 96 % similarity. 2.4% bacteria showed 92%, 95% and 86% similarity as depicted in Table 3.

DISCUSSION

The current study was conducted at four different locations; Multan, Khanewal, Kabirwala and Abdul Hakeem, analysis of rhizosphere of *Aloe vera* was carried out to determine the distribution, diversity and availability of microorganisms. According to different parameters i.e. morphological, biochemical and molecular analysis, characteristics of bacterial isolates were identified and described separately. Study of rhizosphere gave a detailed idea about microbial species, bacterial habitats and their interaction with environment. The rhizosphere is an active and highly compressed area of soil that is populated with multiple inter and intra species interactions and food web communications that crucially influence the flow of carbon and different modifications (Ho, 2016). Morphological attributes; colony size, shape, color, margin, elevation etc. were determined and series of biochemical tests including starch hydrolysis, triple sugar iron, urease, catalase, citrate utilization, indole, motility etc. were conducted accordingly. Variation in enzymatic activities of bacterial isolates also highlights the diversity of bacterial population in the study areas. It is obvious from our data that there exists a potential population of bacteria to produce enzymes. According to the previous studies, the rhizosphere soil bacterial flora of *Aloe vera* have been divided into 13 different genera; *Pseudomonas*, *Bacillus*, *Enterobacter*, *Pantoea*, *Cronobacter*, *Pseudomonas* (20.7%) and *Enterobacter* (13.8%) (Singh, 2019). In the current study, eighteen bacterial species were identified as *Bacillus albus* (19.5%), *Rhizobium pusense* (19.5%), *Enterobacter cloacae* (12.19 %), *Chryseobacterium gambrini* (9.7%), Flavobacterium anhuiense (4.8%) and *Stenotrophomonas maltophilia* (4.8%). Other bacterial species, *Pseudomonas stutzeri, Tatumella terrea, Micrococcus aloeverae, Kocuria marina, Kocuria rosea, Bacillus paramycoides, Chryseobacterium daecheongense, Micrococcus endophyticus, Exiguobacterium mexicanum, Microbacterium zeae, Brachybacterium paraconglomeratum, Ochrobactrum pituitosum* were populated up to 2%. *Bacillus albus* and *Rhizobium pusense* were dominant groups among all these bacterial species. All the rhizobacteria of *Aloe vera* plant showed their similarity from 86% to 99% with their nearest relatives available on nucleotide database. There were a total of 788 positions in the final dataset. Evolutionary analyses revealed that the percentage analogy of bacteria of *Aloe vera* plant was related with nearby relatives feasible on the database of Genbank using Basic Local alignment Search Tool (BLAST) (Altschul et al. 1997) . The diversity among the 18 identified bacterial strains is obvious and clearly reveals the authenticity of results. *Enterobacter, Pseudomonas and Rhizobium* belongs to the plant growth promoting that act as biocontrol agents. The rhizosphere microbiome controls the biochemical activities in rhizosphere and make associations between plant and soil microbes (Majeed, 2018).

Online availability of 16S rRNA gene sequence data

16S ribosomal RNA gene sequences of this study are available online in the database of GenBank (USA) having the accession numbers MK993437- MK993477.

CONCLUSION

Aloe vera plant has been the subject of much scientific study over the last few years due to its medicinal importance. From the rhizosphere of *Aloe vera* eighteen bacterial species were identified and characterized on the basis of phenotypic and molecular techniques. Among them *Enterobacter cloacae* (12.19%), *Bacillus albus* (19.5%), *Rhizobium pusense* (19.5%), *Chryseobacterium gambrini* (9.7%), *Stenotrophomonas maltophilia* (4.8%). *Flavobacterium anhuiense* (4.8%) were in abundance while *Pseudomonas stutzeri, Tatumella terrea, Micrococcus aloeverae, Kocuria marina, Kocuria rosea, Bacillus paramycoides, Chryseobacterium daecheongense, Micrococcus endophyticus, Exiguobacterium mexicanum, Microbacterium zeae, Brachybacterium paraconglomeratum* and *Ochrobactrum pituitosum* were populated up to 2% each. *Bacillus albus* and *Rhizobium pusense* were found to be predominant spp. under the study areas.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

All authors are thankful to Department of Biochemistry Bahauddin Zakariya University and Nishtar Medical University, Multan, Pakistan for providing research facilities.

AUTHOR CONTRIBUTIONS

FA, SA, SM and HN designed research project and performed the experimental works. AHC drew figures. MWKS, MA and SA revised the manuscript. MN checked the structure of manuscript. MI performed the Bioinformatics analysis. All authors read and approved final manuscript.

REFERENCES

Ahmad, F., Ahmad, I., and Khan, M. (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiol Res, 163*(2), 173-181.

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res, 25*(17), 3389-3402.

Barea, J. (2005). *Agua: la calidad de las aguas en España: un estudio por cuencas*: Greenpeace España.

BAROLIA, S. K. (2016). To study the presence of gut microbial parasites from fecal contents of pteropus giganteus from udaipur region.

Ben-David, A., and Davidson, C. E. (2014). Estimation method for serial dilution experiments. *J Microbiol Methods, 107*, 214-221.

Bloem, J., Ruiter, P. C. d., and Bouwman, L. A. (1997). Soil food webs and nutrient cycling in agroecosystems. In J. D. van Elsas, J. T. Trevors, and E. Willington (Eds.), *Modern soil microbiology* (pp. 245-278). New York: Marcel Dekker.

Canarini, A., Kaiser, C., Merchant, A., Richter, A., and Wanek, W. (2019). Root Exudation of Primary Metabolites: Mechanisms and Their Roles in Plant Responses to Environmental Stimuli. *10*(157). doi:10.3389/fpls.2019.00157

du Preez, C. C., van Huyssteen, C. W., and Mnkeni, P. N. S. (2011). Land use and soil organic matter in South Africa 1: a review on spatial variability and the influence of rangeland stock production. *South African Journal of Science, 107*, 27-34.

G Cappuccino, J., and Sherman, N. (2014). Microbiology: A Laboratory Manual TENTH EDITION. In: Pearson Education.

Gargallo-Garriga, A., Preece, C., Sardans, J., Oravec, M., Urban, O., and Peñuelas, J. (2018). Root exudate metabolomes change under drought and show limited capacity for recovery. *Scientific Reports, 8*(1), 12696. doi:10.1038/s41598-018-30150-0

Hakim, S., Naqqash, T., Nawaz, M. S., Laraib, I., Siddique, M. J., Zia, R., . . . Imran, A. (2021). Rhizosphere Engineering With Plant Growth-Promoting Microorganisms for Agriculture and Ecological Sustainability. *5*(16). doi:10.3389/fsufs.2021.617157

Hartmann, A., Rothballer, M., and Schmid, M. (2008). Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. *Plant and Soil, 312*(1-2), 7-14.

Ho, D. (2016). FY2015 LBNL LDRD Annual Report.

Ibrahim, M., Ahmad, F., Nawaz, H., Aslam, M., and Shad, M. A. J. J. o. T. U. f. S. (2020). Studies on correlations between soil chemistry and bacterial population in rhizosphere of Bt and non-Bt cotton and characterization of rhizobacteria. *14*(1), 1463-1474.

Maan, A. A., Nazir, A., Khan, M. K. I., Ahmad, T., Zia, R., Murid, M., and Abrar, M. (2018). The therapeutic properties and applications of Aloe vera: A review. *Journal of Herbal Medicine, 12*, 1-10. doi:https://doi.org/10.1016/j.hermed.2018.01.002

Maan, A. A., Reiad Ahmed, Z. F., Iqbal Khan, M. K., Riaz, A., and Nazir, A. (2021). Aloe vera gel, an excellent base material for edible films and coatings. *Trends in Food Science and Technology, 116*, 329-341. doi:https://doi.org/10.1016/j.tifs.2021.07.035

Majeed, A. (2018). *Genetic diversity and beneficial role of plant growth promoting rhizobacteria in oil seed producing Sunflower (Helianthus annuus L.) crop of Azad Jammu and Kashmir.* University of Azad Jammu and Kashmir Muzaffarabad,

Manvitha, K., and Bidya, B. (2014). Aloe vera: a wonder plant its history, cultivation and medicinal uses. *Journal of Pharmacognosy and Phytochemistry, 2*(5), 85-88.

Meena, V. S., Meena, S. K., Verma, J. P., Kumar, A., Aeron, A., Mishra, P. K., . . . Dotaniya, M. (2017). Plant beneficial rhizospheric microorganism (PBRM) strategies to improve nutrients use efficiency: a review. *Ecological Engineering, 107*, 8-32.

Omotayo, O. P., and Babalola, O. O. (2021). Resident rhizosphere microbiome's ecological dynamics and conservation: Towards achieving the envisioned Sustainable Development Goals, a review. *International Soil and Water Conservation Research, 9*(1), 127-142. doi:https://doi.org/10.1016/j.iswcr.2020.08.002

Paul, A. G. (2019). Lab Manual and Workbook in Microbiology 12th Edition. In: McGraw-Hill Education.

Pongsilp, N., Nimnoi, P., and Lumyong, S. (2012). Genotypic diversity among rhizospheric bacteria of three legumes assessed by cultivation-dependent and cultivation-independent techniques. *World Journal of Microbiology and Biotechnology, 28*(2), 615-626.

Porteous, L. A., and Armstrong, J. L. (1993). A simple mini-method to extract DNA directly from soil for use with polymerase chain reaction amplification. *Current microbiology, 27*(2), 115-118.

Pressman, P., Clemens, R., and Hayes, A. W. (2019). Aloe vera at the frontier of glycobiology and integrative medicine: Health implications of an ancient plant. *7*, 2050312119875921. doi:10.1177/2050312119875921

Saitou, N., and Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular biology and evolution, 4*(4), 406-425.

Singh, J. S. (2019). *New and Future Developments in Microbial Biotechnology and Bioengineering: Microbes in Soil, Crop and Environmental Sustainability*: Elsevier.

Smalla, K., Sessitsch, A., and Hartmann, A. (2006). The Rhizosphere:'soil compartment influenced by the root'. In: Blackwell Publishing Ltd Oxford, UK.

Tamura, K., Nei, M., and Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences, 101*(30), 11030-11035.

Tamura, K., Stecher, G., Kumar, S. J. M. b., and evolution. (2021). MEGA11: molecular evolutionary genetics analysis version 11. *38*(7), 3022-3027.

Ukpaka, C. (2016). BTX Degradation: The concept of microbial integration. *Chem. Int, 3*(8).