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Characterization, ScoT based diversity analysis and molecular identification of a novel rhizobacterial (*Streptococcus thermophilus*) isolates from dhaman (*Cenchrus setigerus*) in arid zone of Rajasthan

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Abstract

Novel solutions for plant growth enhancements are required to ease the burden imposed on our environment and other resources. Here we look at potential solutions to these issues by examining some of the research conducted regarding the biological applications of plant growth promoting rhizobacteria (PGPR) associated in Dhaman grass (*Cenchrus setigerus*). There is a lack of information regarding the rhizospheric microbial communities of *Cenchrus setigerus*, which can play a vital role in promoting plant root growth. The current research aimed at discovering plant growth-promoting rhizobacteria

(PGPR) in the rhizosphere of soil samples collected from around the root zone of *Cenchrus setigerus* growing Jodhpur; Jaisalmer and Bikaner districts of Rajasthan, without causing harm and their relevance in desert grass has grown due to nutritive used in feed and fodder for arid environmental conditions. The isolated bacterial strains were characterized based on different morphological, biochemical, and molecular markers, along with soil attributes such as nutrient status and soil microbiological activities. Among the isolates, eighteen produced indole acetic acid (IAA), ten solubilized phosphate, and fourteen showed hydrogen cyanide (HCN) production. In this study, the Start Codon Targeted (SCoT) marker system was found to be best effective technique to estimating valuable in the genetic diversity studies and detecting 100% polymorphism information content (PIC) among the bacterial communities. Population structure analysis (based on ΔK values) indicated the presence of genetically pure bacterial isolates. Based on molecular identification validation of sequences from selected pure isolates uncovered four that exhibited high similarity to *Streptococcus* strains found in public genomic databases, as established through partial 16S rRNA-ITS gene sequencing, these isolates were further analyzed for bioinformatics study sequences have been submitted to NCBI with accession numbers PV566939, PV566921, PV566757, and PV566932, and were classified as *Streptococcus thermophilus.* The findings of the present investigation showed that *thermophilles* bacteria isolated from the rhizosphere of Dahman grass in the arid zone of Jodhpur, Rajasthan, possess promising plant growth promoting characteristics. The discovery of these novel PGPR strains could probably assist *Cenchrus setigerus* in improving their mechanisms of adaptation to drought stress and may be potential application as biofertilizer, and biodecomposers, and in the future, as biocontrol agents for crop improvement under sustainable agriculture in arid regions.

Key words: Dhaman grass, PGPR, PIC, SCoT marker, SEM, *Streptococcus thermophilus*, 16S rRNA-ITS gene sequencing.

Introduction

Drought stress is a pervasive impediment to agricultural endeavors in many areas of the earth's. Previous studies have shown that abiotic stress conditions (drought or flooding), can adversely impact the root microbiome, root exudates and microbial diversity, thereby affecting plant growth, productivity, and soil fertility. Among the various strategies to mittigate drought stress, inoculation of the rhizosphere with plant growth-promoting rhizobacteria (PGPR) has shown significant potential such as produce siderophores, antibiotics and cyanides which can further enhance the availability of soil nutrients, atmospheric nitrogen, and antagonistic activity against phytopathogens and harmful microbes in the soil thereby providing multiple benefits for plant roots and plant growth for the supporting under dry environments.

The rhizosphere, soil region surrounding plant roots, is a hotspot of microbial activity[1] where a diverse community of bacteria and other microorganisms interacts with plant roots[2], influencing plant growth and soil health[3]. These microorganisms play vital roles in nutrient cycling, plant growth promotion, disease suppression, and soil fertility[4]. In arid and semi-arid zones, where soil conditions are often poor, and water availability is limited, the rhizosphere microbiota becomes even more crucial for plant survival and growth[5]. Understanding these microbial communities can provide valuable insights into how plants adapt to harsh conditions and how microbial functions can be leveraged for sustainable agricultural practices[6].

Almost 30% of their fixed carbon by terrestrial plants is come out in the form of root exudates, consequently changing the content of surrounding soil[7]. The root exudates composition reflects the contradictory-concomitantly attractive and repulsive behavior of the plant relation with microorganisms. The determination of microbial communities C, N, P and S contents by fumigation techniques has permitted in a better quantification of nutrient activity in soil[8].

The role of rhizospheric microorganisms in mitigating pesticide-induced stress, enhancing plant growth, improving soil fertility, and increasing stress tolerance in *Cenchrus setigerus* and *Pennisetum pedicellatum*[9]. Common PGPRs species that colonize plant roots and enhance the plants under drough condition, identified among bacterial genera such as *Azospirillum, Rhizobium, Pseudomonas, Bacillus, Azospirillum, Serratia, Xanthomonas, Burkholderia, Klebsiella, Acinetobacter, Azotobacter, Mesorhizobium, Alcaligenes, Enterobacter, Arthrobacter, Erwinia*, and *Flavobacterium*. In arid soils, rhizobacteria are possess various beneficial properties, including around 80% of them are diazotrophic, nitrogen fixation *e.g*., *Azotobacter* and *Rhizobium*, phosphorus-solubilizing bacteria *e.g., Pseudomonas* and *Bacillus*, and plant growth-promoting through production of substances like phytohormones (*e.g.,* IAA, gibberellic acid, cytokinins and ethylene), enzymes, HCN, antibiotics, siderophores (sequester iron), as well as by lowering ethylene concentration via ACC deaminase activity. Among these, IAA acts as an important signal molecule, regulating plant development by initiation growth, cell division, and cell enlargement. Several studies have highlighted the beneficial roles of rhizobacteria[6], and other plant growth-promoting bacteria like *Cynobacteria*[10] in triggering and enhancing plant growth as well as yield through various mechanisms, theirby reducing the use for frequently application of chemical fertilizers and traditional methods. The beneficial effects of PGPR have been exploited in many areas, including biofertilizers, biocontrol, microbial rhizoremediation and biopesticides for enhancing soil and crop improvement in sustainable agriculture.

Dhaman (*Cenchrus setigerus*) is a drought-tolerant perennial grass species native to the arid regions of Rajasthan, India, and is well-suited to surviving in dry, nutrient-poor soils. This species, which plays an important role in soil stabilization and erosion prevention, benefit from its diverse rhizosphere microbiota[11], which may aid in nutrient acquisition and resilience to abiotic stress, and

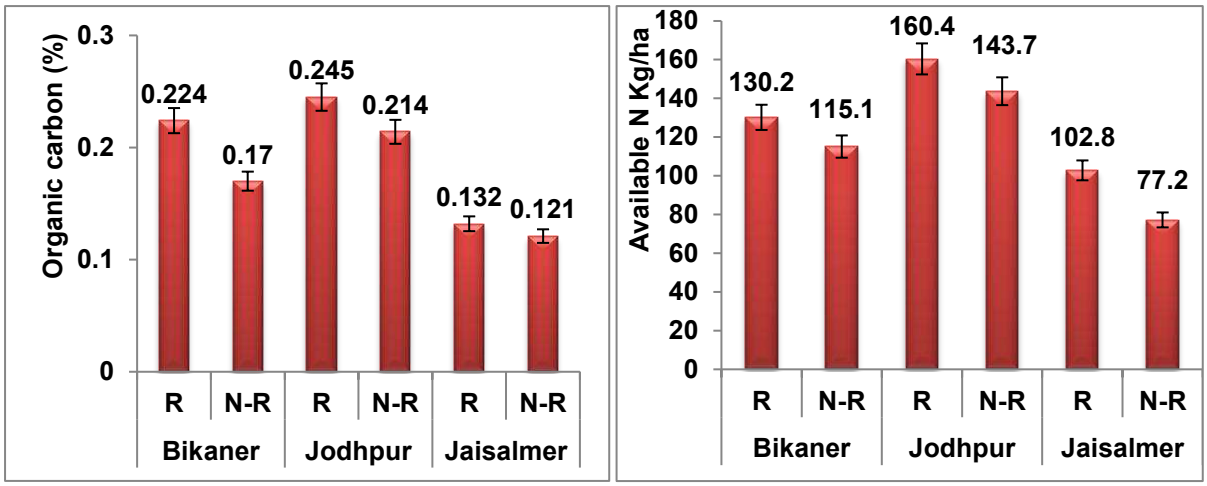
additionally serve as nutritious feed and fodder for animals. Rajasthan's arid zone, with its extreme climatic conditions, offers a unique opportunity to study plant-microbe interactions in water-scarce environments and to explore the potential of these microbial communities for enhancing soil fertility and supporting sustainable agriculture[12].

In Rajasthan, microbial communities in the rhizosphere of native plants have been found to exhibit unique adaptive features that help plants thrive under drought stress[13,14]. Incorporating a diversity analysis of bacterial isolates is crucial to uncovering ecological and mutualistic relationships within the rhizosphere of *Cenchrus setigerus*. This diversity supports the idea that microbial communities in plant roots are not only abundant but also highly specialized to meet the specific needs of plants in extreme environment[15]. Furthermore, high-throughput sequencing molecular technologies, such as 16S rRNA sequencing, have enabled more accurate identification of these bacterial communities, revealing a wealth of microbial diversity that can be harnessed for biotechnological applications, including bioremediation and biofertilizer development, which are increasingly importance in climate change Soni et al.[16,17]. In the case of *Cenchrus setigerus*, molecular techniques have revealed the presence of a diverse range of bacteria that may offer potential benefits in terms of plant growth promotion and stress alleviation[18].

This study aims to isolate and characterize of the bacterial isolates from the rhizosphere of Dhaman grass *(Cenchrus setigerus*) in the arid zone of Rajasthan, to analyse their diversity using molecular tools. There is a lack of information regarding the rhizospheric microbial communities of *Cenchrus setigerus*; therefore, these isolates were assessed for their plant growth-promoting abilities through traditional methods and validating the ITS region via partial 16S rRNA sequencing, the research will contribute to a better understanding of how rhizosphere bacteria can be utilized to improve soil fertility, enhance plant growth, and promote sustainable agricultural practices in arid regions.

Results

In this investigation, preliminary soil analysis are presented in Fig.1. Total 12 types of the soil samples with three replication were collected from rhizospheric and adjacent region soils of dhaman grass at three different sites of the ARS, Bikaner; nearby area CAZRI, Jodhpur; and Jaisalmer; analyzed for fertility status by estimating Organic carbon content and Available nitrogen content ranged from 0.170% to 0.245%; 77.2 to 160.4 kg/ha in bulk (non-rhizosphere) soil and rhizosphere soil respectively. When analyzed the samples location wise, the utmost value of organic carbon and available nitrogen found in Jodhpur soils (0.245%) and (160.4 kg/ha) with respect to host plant the organic carbon values in the soils found to be at par Fig.1. In this study, concluded that higher amount of carbon content and available nitrogen is considered valuable for presence and survival of microorganisms and bacterial population in soil.

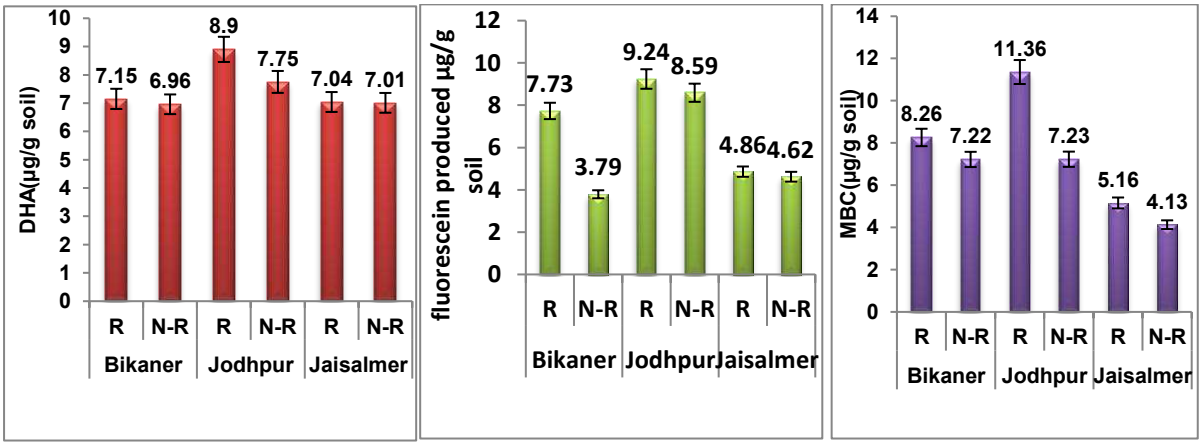


(a) **(b)**

Fig.1: Fertility status by estimating (a) Organic carbon and (b) Available nitrogen (R= rhizosphere soil, NR = non-rhizosphere soil)

Soil microbiological properties of soil samples

In this study, the results of the soil microbiological properties analysis- including Dehydrogenase enzyme activity (DHA), Fluorescein diacetate (FDA) hydrolysis and Soil microbial biomass carbon (MBC,) in these soils ranged 6.96 to 8.9 µg/g TPF released/g/day; 3.79 to 9.24 µg/g soil; 4.13 to 11.36µg/g in non- rhizosphere to rhizosphere soil respectively, presented in Fig. 2a,b,c. In this investigation, the highest soil microbiological properties were found in the Jodhpur soil, which were significantly higher than those of the Bikaner and Jaisalmer soils.



(a) **(b)** **(c)**

Fig. 2: Soil microbiological properties of soil samples (a) Dehydrogenase enzyme activity µg/g TPF (b) Fluorescein diacetate µg/g soil and (c) Soil microbial biomass carbon

Isolation of bacteria from dhaman grass rhizospheric and adjacent region of soil samples

The pure bacterial colonies were obtained by growing the isolated bacteria on nutrient agar media and different colonies were recovered after repetitive streaking to obtain pure culture plate. The purified bacterial isolates were then coded, and code AS was identified.

Quantification of bacterial population

In the current study, bacterial population ranged from 7×10^5cfu/g in non-rhizosphere soil to 8.5×10^6cfu/g in rhizosphere soil shown in Fig. 3. In conclusion, relative study of bacterial population of rhizosphere soil was higher than non-rhizosphere soil across all three districts of Rajasthan. In this investigation, the highest bacterial population denisity was recorded in Jodhpur dhaman rhizosphere soil (8.5×10^6cfu/g), which were significantly higher than those of the Bikaner and Jaisalmer soils.

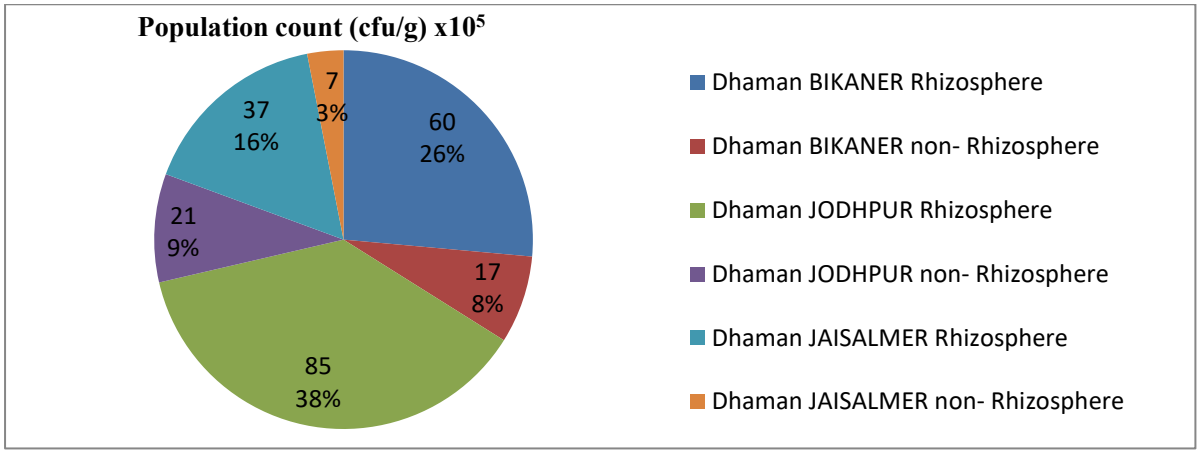


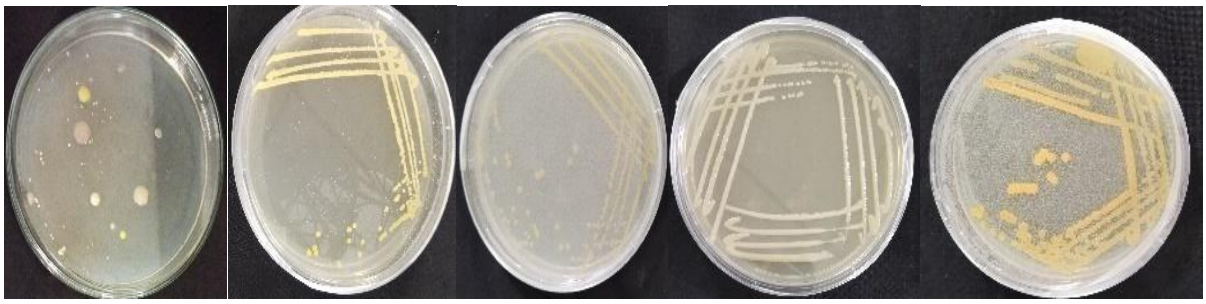
Fig. 3: Effect of sampling site and host plant in accord with proximity to plant roots on cultivable bacterial population of soil.

Cultural characterization of bacterial isolates

In the present study, the morphological characterization and *in vitro* multiplications of all the eighteen bacterial isolates were carried out on nutrient agar plates and colonial characteristics were recorded in terms of size, shape, elevation, margin, texture, opacity and pigment presented in Table 1 and Fig. 4. In this characterization, the pure bacterial isolates were seen to be light yellow, white and light orange in color.

Table 1: Morphological features in terms of isolates from Rhizosphere and non-Rhizosphere

S. NO.	Isolates	Colony Shape	Colony Margin	Colony Elevation	Colony Texture	Colony opacity	Colony Size	Pigment Production
1.	AS-10	Circular	Entire	Convex	Smooth	Opaque	Small	Orange
2.	AS-11	Circular	Entire	Convex	Smooth	Translucent	Small	Light yellow
3.	AS-12	Circular	Entire	Convex	Smooth	Translucent	Medium	White
4.	AS-13	Irregular	Filiform	Convex	Smooth	Translucent	Medium	Light yellow
5.	AS-14	Circular	Entire	Flat	Rough	Opaque	Medium	Off white
6.	AS-15	Circular	Entire	Convex	Rough	Opaque	Large	White
7.	AS-16	Circular	Entire	Convex	Smooth	Translucent	Medium	Light yellow
8.	AS-17	Circular	Filiform	Raised	Rough	Opaque	Medium	Light yellow
9.	AS-18	Irregular	Undulate	Flat	Rough	Opaque	Large	White
10.	AS-19	Circular	Curled	Convex	Smooth	Translucent	Medium	White
11.	AS-20	Circular	Entire	Convex	Smooth	Translucent	Medium	White
12.	AS-21	Circular	Entire	Convex	Smooth	Translucent	Medium	White
13.	AS-22	Circular	Entire	Convex	Smooth	Opaque	Medium	Off white
14.	AS-23	Irregular	Filiform	Flat	Rough	Opaque	Large	Light orange
15.	AS-24	Irregular	Filiform	Raised	Rough	Translucent	Medium	White
16.	AS-25	Circular	Entire	Convex	Smooth	Translucent	Small	Orange
17.	AS-26	Circular	Entire	Convex	Smooth	Opaque	Large	Light yellow
18.	AS-27	Irregular	Curled	Convex	Smooth	Opaque	Medium	Pink



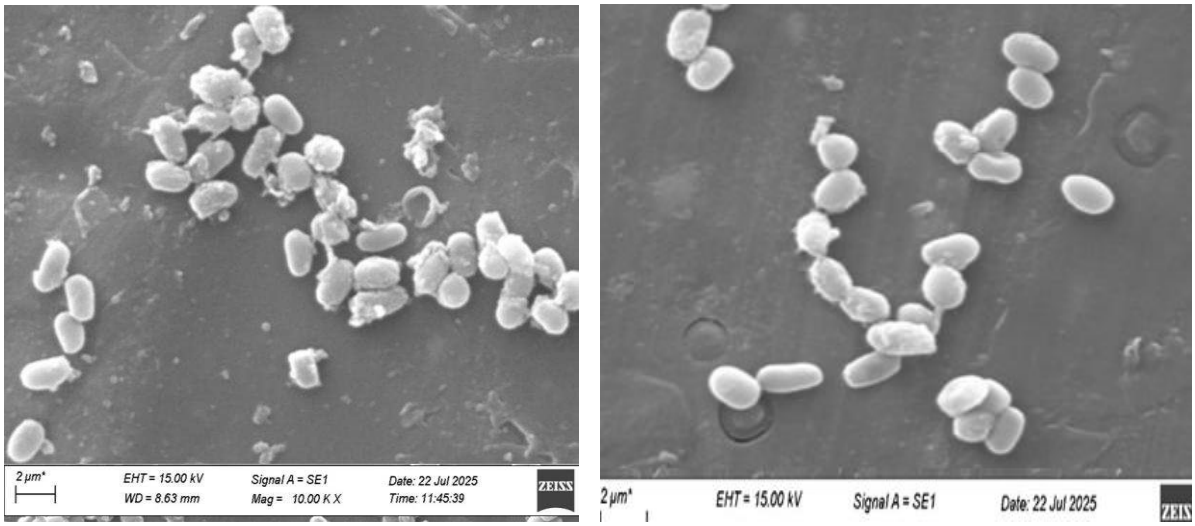
(a) **(b)** **(c)** **(d)** **(e)**

Fig. 4: (a) Culturable diversity of bacteria isolated from dhaman grass in terms of colony morphology as observed on nutrient agar plates, (b) AS-11, (c) AS-13, (d) AS-21, (e) AS-23 and SEM characterization of the isolates (f) AS-13 and (g) AS-21.

Microscopic analysis of all the isolates was carried out by Gram's staining for cellular morphological characterization Shown Table 2. Different isolates exhibited variations cell sizes and morphology when viewed under the microscope. In the study, the pure bacterial isolates AS-11, AS-13, AS-21 and AS-23 were found to be cocci shape and Gram positive in reaction further confirm through advanced microscopy SEM as presented in Fig. 4 e, f.

Table 2: Microscopic characteristics of isolates

S. NO.	Isolates	Shape	Gram's Reaction
1.	AS-10	Coccus	Gm –ve
2.	AS-11	Coccus	Gm +ve
3.	AS-12	Coccus	Gm –ve
4.	AS-13	Coccus	Gm +ve
5.	AS-14	Rod	Gm –ve
6.	AS-15	Rod	Gm –ve
7.	AS-16	Coccus	Gm –ve
8.	AS-17	Coccus	Gm –ve
9.	AS-18	Rod	Gm –ve
10.	AS-19	Coccus	Gm –ve
11.	AS-20	Coccus	Gm –ve
12.	AS-21	Coccus	Gm +ve
13.	AS-22	Coccus	Gm –ve
14.	AS-23	Coccus	Gm +ve
15.	AS-24	Rod	Gm –ve
16.	AS-25	Coccus	Gm –ve
17.	AS-26	Coccus	Gm –ve
18.	AS-27	Rod	Gm –ve



(f) AS-13 (g) AS-23

Fig. 4: Scaning electron microscopic analysis of rhizospheric bacterial isolates (e) AS-13 and (f) AS-23.

Biochemical characterization of bacterial isolates

In this characterization, total 18 bacterial isolates were studied for various biochemical studies and results are presented in Table 3 and Fig. 5. The biochemical tests were carried out in order to determine the different biochemical characteristics of the isolates as addressed in the discussion section.

Table 3- Biochemical characterization of isolates

S. No.	Isolates Name	Catalase Activity	Oxidase Activity	Starch Hydrolysis	Urease Activity
1.	AS-10	++	+	-	-
2.	AS-11	+	+	+	+
3.	AS-12	+++	++	-	-
4.	AS-13	+	+++	+++	-
5.	AS-14	+++	+++	+	+
6.	AS-15	+++	+++	+	+
7.	AS-16	+	++	+	-
8.	AS-17	+++	+++	+	+
9.	AS-18	++	+	+	+
10.	AS-19	+	+	-	-
11.	AS-20	+++	++	+	+
12.	AS-21	+	+++	-	+
13.	AS-22	++	+++	-	+
14.	AS-23	+++	++	+	+
15.	AS-24	+++	+++	+	+
16.	AS-25	+++	+++	-	+
17.	AS-26	+++	++	-	-
18.	AS-27	+	+	-	-

- = no production, + = less production, ++ = moderate production, and +++ = High production

(a) (b) (c) (d)

Fig. 5- Biochemical characterization of pure isolate (a) Catalase activity (AS-23), (b) Starch hydrolysis (AS-21), (c) Urease activity (AS- 11), and (d) Oxidase activity,

Characterization for plant growth promoting properties of the isolates

In the present study, the isolates were screened for plant growth promoting potential in terms of HCN production on 200 ml Luria broth in 25 ml tubes with 0.88 g glycine. Among these fourteen isolates were detected with high HCN production activity shown in Table 4. For estimation of the indole

acetic acid (IAA) production potential all of these isolates were grown on 300 ml peptone broth containing 0.75 mg tryptophan (precursor of IAA). Contrasting results were detected for IAA production. All the 18 isolates found capable of IAA production shown in Table 4 and Fig. 6a,e and ten for phosphate solubilizing prospective all the isolates were grown on Pikovskaya agar medium. Among these, total ten bacterial isolates showed phosphate solubilization activity shown in Table 4 and Fig. 6b,c,f. In conclusion, the highest IAA activity and TCP solubilization were found in isolates AS-21 and AS-13, respectively, the pure bacterial isolates AS-13 and AS-21 observed positive HCN production, as highlighted in Fig. 6d.

Table 4- Plant growth promoting properties of the isolates

			IAA Concentration		Tri -calcium Phosphate Solubilization			
S. No.	**Isolates**	**HCN production**	**Qualitative response pink coloration**	**Quanitative response (µg/ml)**	**Qualitative response**	**Colony diameter in (mm)**	**Hallow zone diameter in (mm)**	**Phosphate solubilizing index**
1.	AS-10	-	+	0.663	-	6	-	0
2.	AS-11	-	+	0.703	+	8	11	2.38
3.	AS-12	+	+	0.683	-	5	-	0
4.	AS-13	+ +	++	0.725	+++	30	38	2.27
5.	AS-14	+ + +	++	0.722	-	4	-	0
6.	AS-15	+ + +	+	0.654	++	15	18	2.2
7.	AS-16	+ + +	+	0.636	-	3	-	0
8.	AS-17	+ + +	++	0.717	+	4	6	2.4
9.	AS-18	+	+	0.648	+	11	14	2.27
10.	AS-19	+	+	0.648	-	2	-	0
11.	AS-20	+ +	++	0.735	+	10	14	2.4
12.	AS-21	+	+++	0.848	+	11	13	12.18
13.	AS-22	+	++	0.744	-	5	-	0
14.	AS-23	-	+	0.699	+	10	12	2.2
15.	AS-24	-	+++	0.806	+	11	13	12.18
16.	AS-25	+ +	+	0.658	-	6	-	0
17.	AS-26	+ + +	+	0.636	-	7	-	0
18.	AS-27	+	+	0.679	+	9	11	2.22
	S Em±			0.0047				
	CDat5%			0.0135				

- = no production, + = less production, ++ = moderate production. and +++ = High production

(a) (b) (c) (d)

Fig. 6- Plant growth promoting (PGP) Potentiales of the pure bacterial isolates (a) IAA production as generation of pink colour in tryptophane enriched peptone broth (AS-11, AS-13,

AS-21 and AS-23), (b) Phosphate solubilization as clear halo-zone surrounding the bacterium inoculums on pikovskaya agar plate (AS-11, AS-21, AS-23) and (c) AS-13, and (d) HCN production as indicated by yellow color of filter paper strips on glycine enriched nutrient media (AS-13 and AS-21).

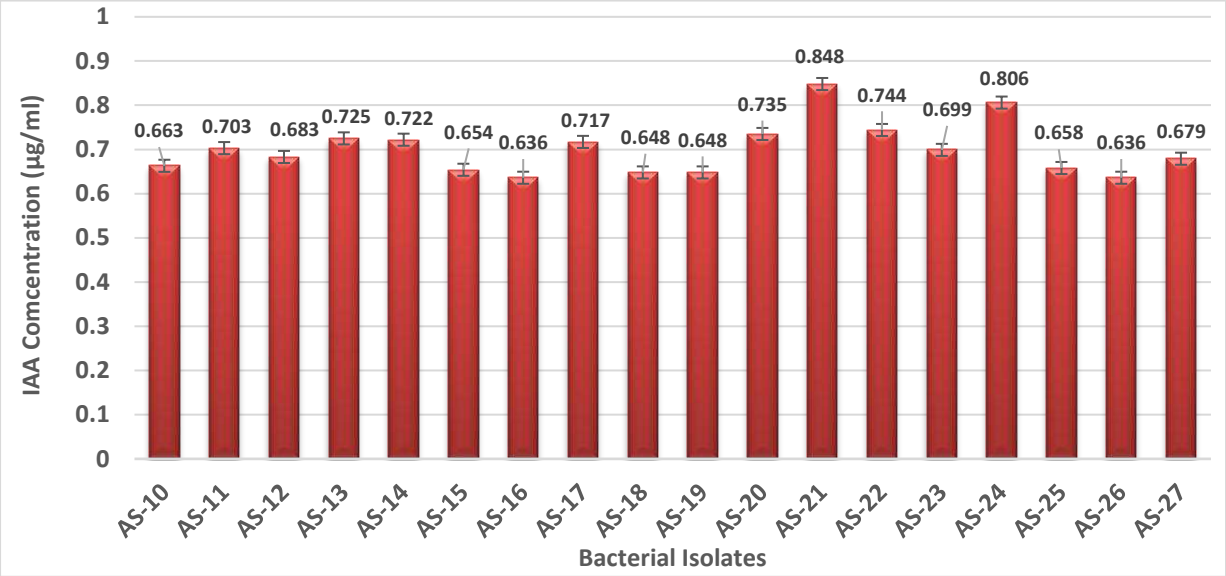


Fig. 6: (e) IAA Concentration (µg/ml) of bacterial isolates

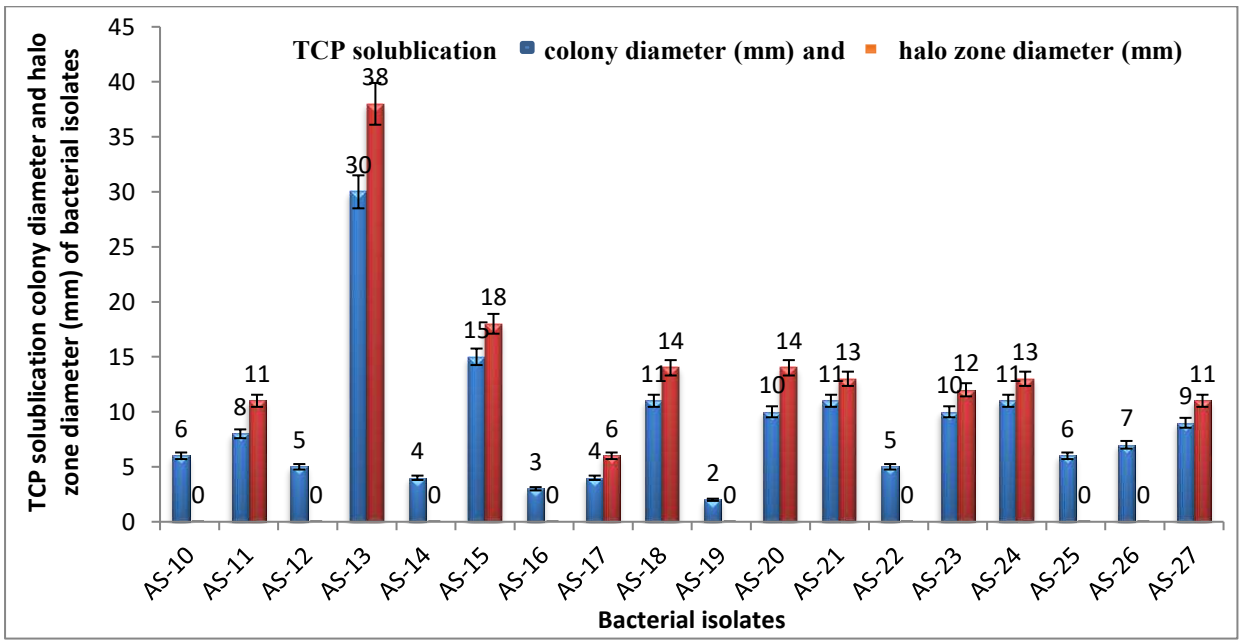


Fig. 6: (f) TCP solublication colony diameter and halo zone diameter (mm) of bacterial isolates

Molecular characterization of bacterial Isolates from rhizosphere and adjacent region of dhaman grass

Isolation and purity check of bacterial genomic DNA

In this investigation, genomic DNA isolated from 10 bacterial isolates (AS-11, AS-12, AS-13, AS-14, AS-17, AS-18, AS-21, AS-23, AS-25 and AS-27) out of 18 bacterial isolates, based on physico-morpho-biochemical, and plant growth promoting characterization, using modified freeze and thaw method and quantified by UV-VIS spectrophotometer. The quality of DNA was measured at A_{260} nm absorbance and A_{260}/A_{280} was recorded in between 1.75 to 1.80. The observed quality parameters of genomic DNA were considerably very well.

Amplification of SCoT markers

For molecular characterization of bacterial isolates, 13SCoT markers were selected from public literature Collard and Mackill[28], shown in Table 5. For profiling in 10 bacterial isolates, the selected primers were custom synthesized. All the 13SCoT markers were produced significant number of polymorphic bands, as highlighted in Fig. 7. These 13SCoT markers were analysed for further study.

Table 5- SCoT primer used for profiling in among 13 isolates in the present study

S. No.	Primer ID*	Sequence (5' - 3')	Tm (°C)
1.	SCoT02	CAACA**ATG**GCTACCACCC	45
2.	SCoT13	ACGAC**ATG**GCGACCATCG	45
3.	SCoT14	ACGAC**ATG**GCGACCACGC	45
4.	SCoT15	ACGAC**ATG**GCGACCGCGA	45
5.	SCoT16	ACC**ATG**GCTACCACCGAC	45
6.	SCoT18	ACC**ATG**GCTACCACCGCC	45
7.	SCoT19	ACC**ATG**GCTACCACCGGC	45
8.	SCoT20	ACC**ATG**GCTACCACCGCG	45
9.	SCoT21	ACGAC**ATG**GCGACCCACA	45
10.	SCoT22	AACC**ATG**GCTACCACCAC	45
11.	SCoT23	CACC**ATG**GCTACCACCAG	45
12.	SCoT25	ACC**ATG**GCTACCACCGGG	45
13	SCoT26	ACC**ATG**GCTACCACCGTC	45

Assessment of polymorphism and marker efficiency

Polymorphism of SCoT markers and their efficiency was calculated using iMEC marker efficiency calculator (Amiryousefi)**.** The binary matrix (0 and 1) were scored from the electrophoretic gel image of all the markers and used for detecting polymorphism. The results detected by analysis of 13SCoT markers were ranged from 50bp to 3000bp. All the 13SCoT primers were amplified a total of 133 scorable bands of which all were polymorphic presented in Table 6 with an average of 10.23

polymorphic bands per primer. All the SCoT markers showed 100% polymorphism. The polymorphism Information Content (PIC) value was 57%. The average Heterozygosity index (H) and Marker Index (MI)) value was 0.63. The discriminating power (D) an average of 0.60, and the effective multiplex ratio (E) was obtained as one indicating by 100% efficiency of all 13 markers used in this study. The further was study of all the 13SCoT markers in the shown in Table 6.

Table 6- Characteristics of SCoT markers analyzed in this study

Markers	Primers (5-3)	Allele Size (bp)	NTB	NPB	% P	PIC	H	E	MI	D
ScoT2	CAACA**ATG**GCTACCACCC	50-2500	10	10	100	0.686848	0.735976	1	0.735976	0.480342
ScoT13	ACGAC**ATG**GCGACCATCG	50-3000	17	17	100	0.534707	0.603391	1	0.603391	0.633987
ScoT14	ACGAC**ATG**GCGACCACGC	50-2700	14	14	100	0.553243	0.626633	1	0.626633	0.585714
ScoT15	ACGAC**ATG**GCGACCGCGA	50-2500	9	9	100	0.451286	0.517778	1	0.517778	0.516049
ScoT16	ACC**ATG**GCTACCACCGAC	50-2500	9	9	100	0.550524	0.621975	1	0.621975	0.634568
ScoT18	ACC**ATG**GCTACCACCGCC	250-3000	10	10	100	0.493779	0.5832	1	0.5832	0.546667
ScoT19	ACC**ATG**GCTACCACCGGC	50-3000	9	9	100	0.530032	0.572628	1	0.572628	0.275325
ScoT20	ACC**ATG**GCTACCACCGCG	50-3000	9	9	100	0.684571	0.73402	1	0.73402	0.742165
ScoT21	ACGAC**ATG**GCGACCCACA	100-2500	10	10	100	0.589203	0.6632	1	0.6632	0.693333
ScoT22	AACC**ATG**GCTACCACCAC	100-2500	9	9	100	0.56019	0.636049	1	0.636049	0.622222
ScoT23	CACC**ATG**GCTACCACCAG	100-2500	9	9	100	0.585918	0.659753	1	0.659753	0.637037
ScoT25	ACC**ATG**GCTACCACCGGG	50-2000	10	10	100	0.5478	0.62	1	0.62	0.675556
ScoT26	ACC**ATG**GCTACCACCGTC	250-1500	7	7	100	0.574515	0.649796	1	0.649796	0.704762
Mean			10.23	10.23	100	0.5648	0.6326	1	0.6326	0.5960

NTB = No. of total band, NPB = No. of polymorphic band, %P = percent of polymorphism, PIC = Polymorphism information content, H = Heterozygosity index, D = Discriminating power, E = Effective multiplex ratio, MI = marker index, bp = base pair.

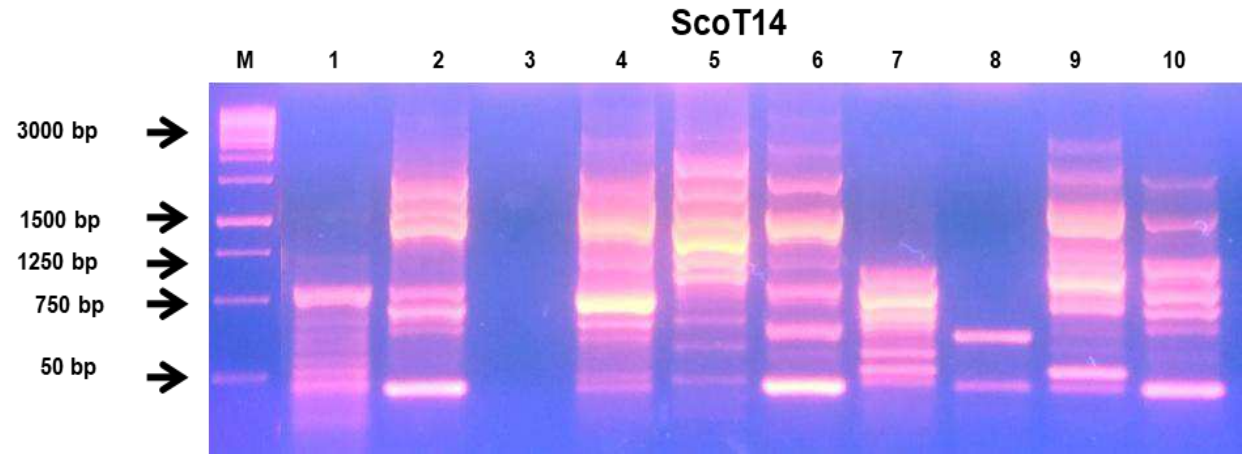


Fig. 7- Representing photograph of SCoT markers profiling in bacterial isolates SCoT14; M= 1kb ladder and the no. of 1 to 3 depict PCR products from bacterial isolates.

Genetic diversity analysis of bacterial isolates

To detect the genetic variability among the 10 bacterial isolates, the marker data were analysed in NTSys software using binary matrix (0 and 1). Based on SCoT markers analysis, the high genetic variability within rhizospheric bacterial populations was revealed in the present study. The neighbour-joining (NJ) dendrogram generated based on SCoT markers showed a clear clustering pattern of genetically closer individual in Fig. 8. The 10 bacterial isolates were clustered in to two major groups. Among them, Group-I was consisted with three isolates (AS-11, AS-13, AS-21 and AS-23) and Group-II was consisted with seven isolates (AS-12, AS-14, AS-17, AS-18, AS-25 and AS-27). Group-I was differentiated at coefficient 0.34 similarly coefficient. The Group-II was bifurcated at 0.48 similarly coefficient in the UPGMA dendrogram.

Among the 10 bacterial isolates, in Group-I; first two isolates (AS-11 and AS-13) showed similarity at 0.95 coefficient and another isolates (AS-21 and AS-23) showed similarity at 0.98 coefficients. Remaining isolates belong to Group-II, showed low similarity among the bacterial isolates indicated low relationship, whereas high similarity indicates showed high relationship. In the investigation our finding iasolates AS-11, AS-13, AS-21 and AS-23 were more similar above 95% to each other based on genetic diversity analysis dendrogram, it may be possibility to same species, further confirm through next generation 16s rRNA-ITS sequencing.

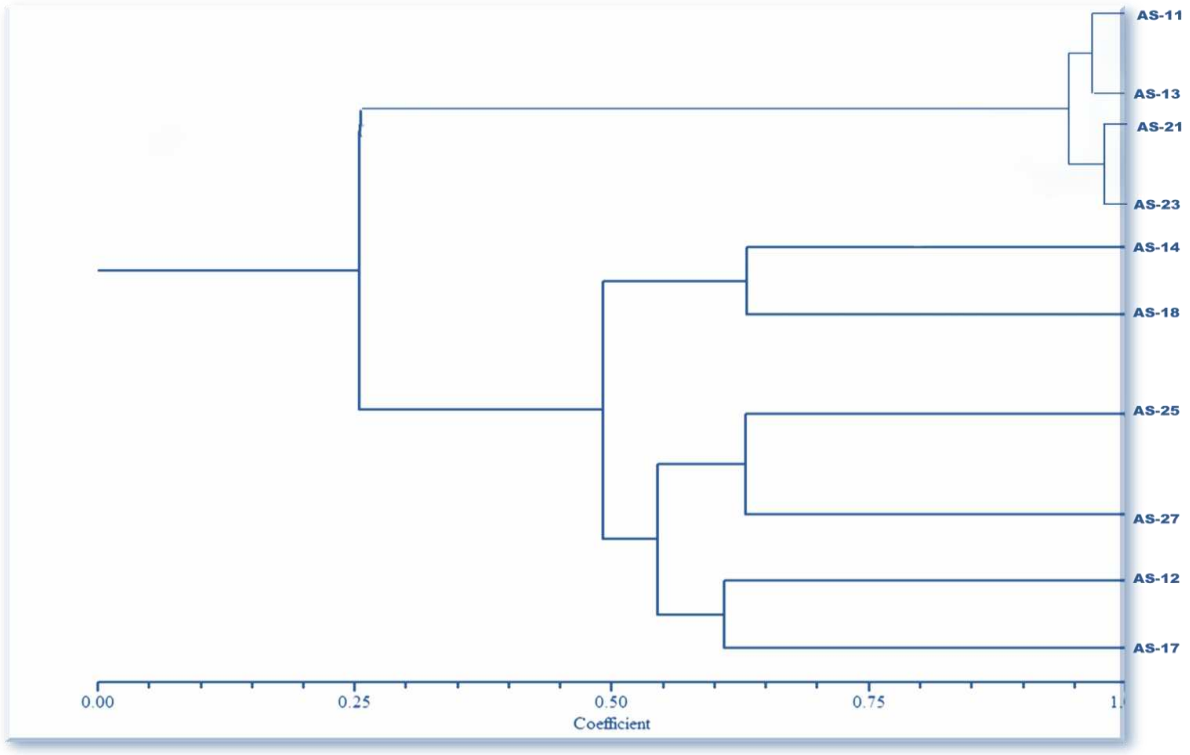


Fig. 8- Genetic diversity analysis of bacterial isolates by the neighbour-joining (NJ) dendrogram generated based on SCoT markers

Bacterial population structure analysis

The population structure analysis of the 10 bacterial isolates was carried out using STRUCTURE 2.0 software by putting delta K value from 1 to 10 with 9 iterative replicates. The K value from 0 to 10 indicated the possible number of population, whereas the highest value of delta K indicating the possible structural population.

The delta K value was identified by interpreting structure software result using STRUCTURE HARVESTOR TOOL (http://taylor0.biology.ucla.edu/structureHarvester). The peak at K2 value was seen very high followed by K3, which indicating there are two populations among the ten isolates represented in Fig. 9 a.

The population structure analyses data represented in Evanno Table output file, as highlighted in Fig. 9 b. The Evanno Table indicates delta K value ranged from 0.066431 to 4.830496 it indicates the 9 and 2 numbers of populations, respectively. It suggests that the K2 has the highest delta K value. Thus, the population structure analysis of the 10 bacterial isolates using 13SCoT markers was indicating the 2 major populations with admixture in each population shown in Fig10. Among the 2 population observed three populations indicates pure form of bacterial isolates.

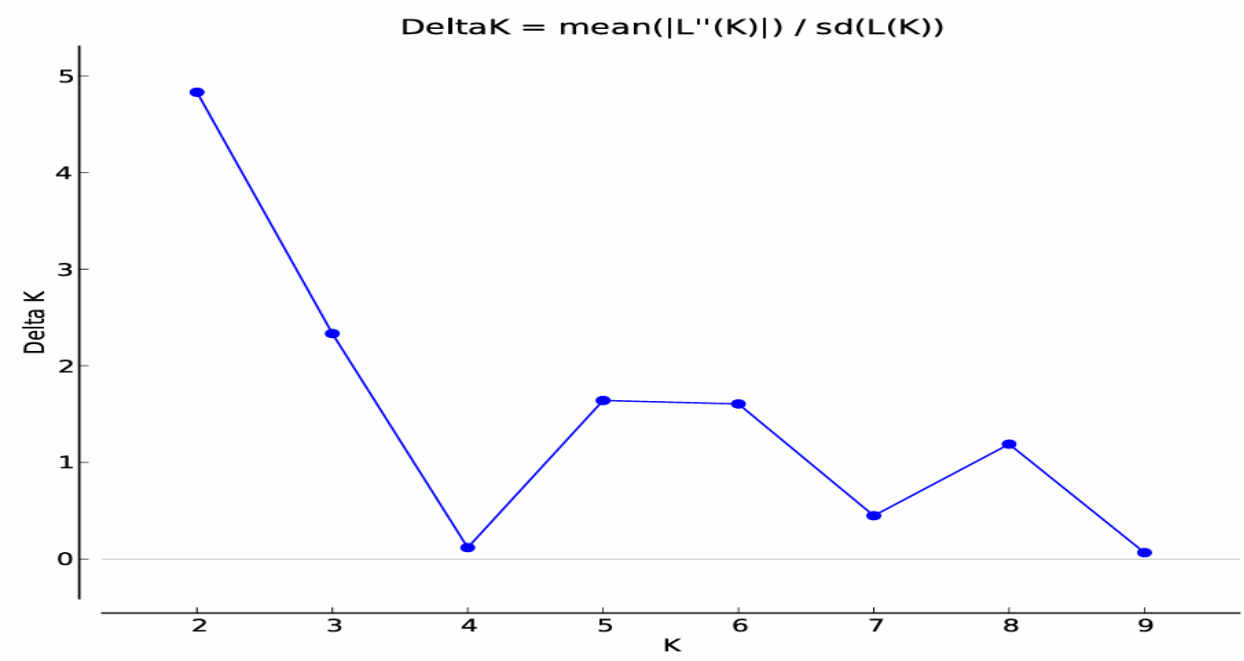


Fig. 9- (a) Bacterial population structure analysis by delta K Value

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	6	-1120.550000	3.651712	—	—	—
2	6	-4678.266667	829.849301	-3557.716667	4008.583333	4.830496
3	6	-12244.566667	3264.327031	-7566.300000	7616.916667	2.333380
4	6	-12193.950000	6643.387003	50.616667	786.000000	0.118313
5	6	-11357.333333	6565.845928	836.616667	10775.333333	1.641119
6	6	-21296.050000	6270.048189	-9938.716667	10066.566667	1.605501
7	6	-21168.200000	15793.867447	127.850000	7082.916667	0.448460
8	6	-13957.433333	10826.019881	7210.766667	12874.116667	1.189183
9	6	-19620.783333	11168.229481	-5663.350000	741.916667	0.066431
10	6	-26026.050000	19379.099439	-6405.266667	—	—

Fig. 9- (b) The population structure analyses data represented in Evanno Table

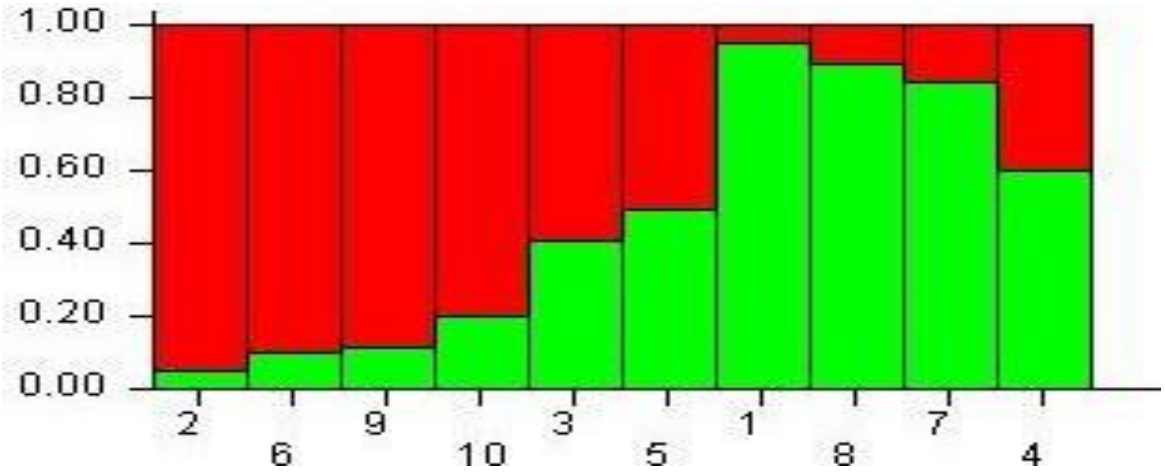


Fig. 10- The population structure analyses of bacterial isolates

Sequences validation and Molecular identification by partial 16s rRNA gene amplification of pure form of isolates

Based on morphological, biochemical and biological assessments, while considering PGPR activities in high temperature, four promising strains were identified AS-11, AS-13, AS-21 and AS-23, which screened high response of plant growth promoting activities. Thus, all these strains were selected for further genetic identification by molecular markers. For sequence validation of the bacterial isolates, the *Streptococcus* specific ITS1 and ITS4 primer set as (forward- 5′TCCGTAGGTGAACCTGCGG-3′) and (reverse - 5′-TCCTCCGCTTATTGATATGC-3′) primers respectively was profiled on the genomic DNA of 10 bacterial isolates. As a result, around 200 bp size fragment was amplified in AS-11, AS-13, AS-21 and AS-23 each. Further, the sequence of PCR products were validated through Sanger sequencing by providing ITS1-ITS4 primer set. The four bacterial isolates under study were

identified using partial 16S rRNA gene sequencing and after using the BLAST-N program (NCBI), a complete identification showed 100, 99 98 > 99% similarity of their partial sequence with the available sequences from the NCBI database. The obtained sequences were submitted to NCBI and, together with other relevant information, their accession numbers were provided (Table 7). According to molecular assessments, AS-11, AS-13, AS-21 and AS-23, strains were identified as members of *Streptococcus* strains, respectively (Table 7). The current research identified these four bacterial species, through DNA molecular analysis, in Dhaman rhizospheres for the first time.

Table 7- Sequences validation and Molecular identification of four selected bacterial isolates based on partial 16S rRNA gene sequencing.

Pure bacterial isolates	Accession number at NCBI	No. of amplified base pairs	Isolates identification	Forward and reverse primers
AS-11	PV566932	194	*Streptococcus thermophilus* AS-11	*Streptococcus* specific ITS1 and (forward- 5′TCCGTAGGTGAACCTGCGG-3′) and ITS (reverse - 5′TCCTCCGCTTATTGATATGC3′)
AS-13	PV566939	192	*Streptococcus thermophilus* AS-13	*Streptococcus* specific ITS1 and (forward- 5′TCCGTAGGTGAACCTGCGG-3′) and ITS (reverse - 5′TCCTCCGCTTATTGATATGC3′)
AS-21 100%	PV566757	181	*Streptococcus thermophilus* AS-21	*Streptococcus* specific ITS1 and (forward- 5′TCCGTAGGTGAACCTGCGG-3′) and ITS (reverse - 5′TCCTCCGCTTATTGATATGC3′)
AS-23	PV566921	196	*Streptococcus thermophilus* AS-23	*Streptococcus* specific ITS1 and (forward- 5′TCCGTAGGTGAACCTGCGG-3′) and ITS (reverse - 5′TCCTCCGCTTATTGATATGC3′)

Discussion

Today, In the course of emergent emphasis on sustainable and low input agricultural practices; there is also a growing concern to investigate soil microbial populations to facilitate enhanced growth and fitness of crop plants in the era of climate change. In the present investigation the effect of various sites, plant types and management practices is clearly pragmatic on bacterial population structure, functions and qualitative traits.

Preliminary analysis in our study we isolated 18 bacterial strain, rhizosphere and bulk soils of dhaman (*Cenchrus setigerus*) grasse collected from Bikaner; Jodhpur and Jaisalmer districts of

Rajasthan, showed that low to medium with respect to availability of organic carbon and nitrogen. These results agree with the findings of[19] who reported that lower level of N may be attributed due to numerous climatic and edaphic factors, such as lower amount of organic carbon, emanating from minimal vegetation cover along with raised pH, favoring higher volatilization losses. Carbon content plays an imperative role in regulating the diversity and structure of the soil microbiome[20]. Furthermore, [21] reported enhanced N absorption by crop as well as increased crop yield resulting due to additional soil microbial biomass.

Soil microbial biomass is both the source as well as sink of nutrients and also mineralization of organic substrates. In this investigation, we concluded significant variation among the samples in terms of all three soil biological attributes when analyzed with respect to place, proximity from plant roots and the species of host plant. We found samples from rhizosphere and Jodhpur sites favored higher activities of both the soil enzymes DHA, FDA as well as amount of MBC in contrast to counterpart soils. Overall, the low microbial activities rhizosphere and bulk soils may be ascribed to higher pH and very low OC status of these soils. [22]also described higher dehydrogenase and β-glucosidase enzyme activities in rhizosphere soils than in non-rhizosphere soils.

The morphological characteristics of isolates deduced from the observed, colony characteristics recorded in terms of size, shape, elevation, margin, texture, opacity, pigment. The colonies were in color appearance such as orange, light yellow, white or off-white etc. and microscopic features showed the fourteen isolates were shown negative and four positive grams staining reaction. These findings are similar to the findings of[23]. Bacterial enumeration revealed multifold increase in population count as well as morphological diversity from dhaman rhizosphere than the bulk soils which showing clear rhizospheric effect as a result of volatile compounds (root exudates) such as organic acids, sugars, proteins and amino acids etc. secreted by root cells and root associated microflora[24]. Our findings are supported by the work of[25] who reported greater microbial populations in rhizosphere soils.

Results from various biochemical tests conducted on all the 18 isolates indicated that they were tested positive for oxidase and catalase activity, additionally, 10 isolates were able to hydrolyse starch and 11 isolates were found positive for urease enzyme activity. [26]shown that root associated bacteria depicting catalase activity must be strongy resistant against chemical, mechanical and environmental stress. Our data support the results of[27], who studied the wheat rhizosphere and found all nine bacterial isolates positive for catalase activity.

In the present investigation, ten isolates out of the eighteen were found to be efficient phosphate solubilizers were also able to grow on Pikovskaya agar plates and hence able utilize insoluble tricalcium phosphate. The solubilization of phosphates was confirmed from the formation of clear zones which is concerned with the P-solubilization by the isolates. Phosphorous solubilization results are reported to

vary depending on kinds of the metabolin, how quickly it is released, and also the degree of its spread on the medium. Therefore, observational method of P solubilizing zone can only be used for qualitative assays [28] In the present study, the expansions of diameter of ZOS of the isolates after 3 DAI were found all 10 PSB isolates belong to rhizosphere. [29]reported high population of phosphate-solubilizing bacteria (PSB) in the rhizosphere in contrary to bulk soil.

In this study, 14 bacterial isolates found positive for HCN production among which five were high and three were medium producer, over 70% belong to rhizosphere. Various other studies have also reported function of HCN in disease suppression in different crops[30,31] suggested a potential and environmentally friendly method for bio-control of weeds through HCN produced in the rhizospheres of seedlings by scrutinized PGPR.

The amounts of IAA produced by the isolates were found to be between the range 1 to 23 μg ml^{-1} reported by[32] and [33]. The results of mean comparison related to different isolates indicated that concentrations of IAA produced by the isolates under study are in agreement with the results of former studies reported by[34] and[35]. In their reports, our findings it is revealed that all the bacterial isolates were able to produce IAA in the varying from 0.636 to 0.848μg/ml; indicating a substantial inevitability among these isolates for IAA production. It was suggested that rhizobacteria synthesize auxins consecutively to agitate physiological processes of the host plant for their personal advantage[36]. The IAA production by rhizospheric bacterial isolates from diverse oilseed and cereal crops has been reported in various studies, including[37] and[38]**.** Although all the isolates were able to produce IAA and supported the amounts reported in earlier investigations, where rhizobacteria isolated from *Brassica campestris* and produced IAA in the range of 6.02–29.75 μg/ml[39]. However, the IAA production range of the bacterial isolates under our study is much lower as compared to the range 78 to 101 μg ml^{-1} reported by[40]. The production of IAA is supported these isolates optimized for IAA production potential quantitatively, as well as qualitatively all of these have fitness for this particular attribute.

Molecular characterization and phylogenetic analysis

Genetic Diversity Analyses

In the present investigation, 13SCoT markers were used for molecular diversity analyses among 10 selected isolates and the dendrogram constructed which confirmed two major groups. Here these two main groups owing to three different sites, dhaman grass and also the samples belong to rhizosphere[41] reported that diversity in root exudates sustains a collection of diverse microorganisms, contributing to a host's adaptability and recruitment of rhizospheric and endophytic microbial populations according to its requirements. [42]also observed that rhizospheric microbial diversity and

carbon allocation were influenced by plant species. Our results are also coherent with those of Ladygina and[43], who proposed that tree species influenced microbial diversity and nitrogen availability in rhizosphere soil. [44]reported the top ten bacterial phyla in rhizosphere soil and found the greatest relative abundance of *Proteobacteria* and *Actinobacteria* among them. [45]analyzed the diversity of *Fusarium oxysporum* f. sp. *lentis* using eight SSR markers and found more molecular variability of this fungal species within regions. However, the profile of bacterial micro biota is more influenced by the root fraction than by soil or plant species.

Polymorphism and SCoT markers efficiency detection

The various environmental factors may have an effect on variability in the performance of PGPR. A large number of studies applied morpho-physiological and biochemical methods aimed on the isolation and identification of microbes. Currently, polyphasic approaches are being exploited, involving techniques to determine both phenotypic and genotypic characteristics[46]**.** The phenotypic profiling in this study demonstrated notable metabolic adaptability in three bacterial species, namely *Ochrobactrum* spp., *Pseudomonas* spp., and *Klebsiella* spp. Further results of BOX-PCR and partial sequencing of 16S rRNA genes showed better discriminatory power, favoring the fingerprinting of root-associated bacterial isolates through a prominent scale of polymorphism. [47]characterized the diversity and genetic structure in *Dalbergia nigra* mini-garden where eleven primers were selected, which generated 180 fragments (70.76% polymorphism) and the polymorphic information content (PIC) for the markers used averaged 0.34, considering them as moderately informative. Similarly, the high number of polymorphic bands and huge polymorphic fragment achieved in our investigation show prospective of SCoT marker analyses. We obtained polymorphic bands from 7 to 17 in these markers showing 100 % polymorphism. The polymorphism Information content (PIC) was detected as 0.45 to 0.70 and also the average value of this index 57%.

Population Structure Analysis

In the present study, the population structural analyses of 10 selected bacterial isolates using 13SCoT markers signifying the 2 main populations amid admixture in each population. Additionally, we also found four isolates are there as pure culture of population, may serve as prominent resources for further detailed studies in terms of functional and structural characterization. The findings of diversity analyses of our study show superiority in contrast to results of[48] who analyzed cultivable diversity of 17 isolates from field grown crops using RAPD markers and reported 12 isolates represents similar banding pattern. Our investigation also agrees with these findings.

Identification of bacterial isolates

BLAST result indicated that AS-11 strain was >98% similar to *Streptococcus thermophilus* (PV566932); AS-13 strain was >99% similar to *Streptococcus thermophilus* (PV566939.1); AS-21 strain was 100% similar to *Streptococcus thermophilus* (PV566757.1) and AS-23 strain was 99% similar to *Streptococcus thermophilus* (PV566921.1). The fist occurrence of lactic acid bacteria (LAB) in soil has been reported by[49], isolated 24 lactic acid bacteria, including *Streptococcus* spp., from soil using culture dependent method and 16S rDNA sequencing, confirming that LAB can inhabit soil environments. Similarly[50], reported three *Streptococcus* strain among 21 LAB from rhizosphere soils of mango, banana and guava trees. A recent study by[51], isolated rhizospheric bacteria showing bacteriocin-type antimicrobial activity and identified strains with high similarity to *Streptococcus* spp., *Enterococcus* spp. and *Gemella bergeri*. These studies collectively illustrate that soil and rhizosphere environments can inhibits *streptococcus* spp. In agreement with these findings, our molecular identification revealed four strains from rhizosphere region of Dhaman with high sequence identity to *Streptococcus thermophilus* (NCBI accession numbers PV566939, PV566921, PV566757, and PV566932). In current findings support our discovery of *Streptococcus thermophilus* in rhizosphere region of Dhaman grass, indicating that *Streptococcus* species may exhibit ecological adaptability and contribute biofrtilizer and biocontrol functions outsisde its typical dairy niche.

Material and methods

The study was carried out at "Plant Biotechnology Centre, Directorate of Research, College of Agriculture, S. K. Rajasthan Agricultural University, Bikaner" during the academic year 2021–2022. A total of eighteen isolates was used for studying the various plant growth promoting activities with triplet each for each experiment using a completely randomized design (CRD).

Soil samples collection site

The experimental material in research study consisting of the soil samples were collected from different sites rhizosphere and adjacent region of dhaman grass growing from Bikaner ARS (28.10° N latitude and 73.18° E longitude), Jodhpur near by area CAZRI (26.24° N latitude and 73.00° E longitude) and Jaisalmer (27.52° N latitude and 70.18° E longitude) District of Rajasthan in India. Intact root system was dug out and the rhizospheric and adjacent region. A total 12 Soil samples (approximately 100 g each) were collected from 10-25 cm depth soil samples were carefully taken with the help of an agricultural soil sampler in aseptic bags and immediately transported laboratory and allowed to pass through 2.0 mm sieve separately and stored under cold condition at 4°C for further processing. The names of the sites within the respective districts from which, the soil samples were collected with due consultation and permissions.

Preliminary soil analysis

Soil fertility stus parameters

Determination of Physical properties, soil samples were collected from different sites; rhizosphere and bulk soil of dhaman grass. Organic matter was calculated by Rapid titration method[52]. Available Nitrogen in soil sample was determined by distillating soil in alkaline permanganate method[53].

Soil microbiological parameters

Dehydrogenase activity (DHA) was assayed by triphenyl tetrazolium chloride method[54]**.** The hydrolysis of Fluorescein diacetate (FDA) was assayed by[55]. Soil microbial biomass carbon was determined by chloroform fumigation method[56].

Isolation of rhizobacteria from soil samples

Bacteria were isolated from stored soil samples. Samples were kept at ambient temperature during the expedition and at 4°C upon return to the laboratory. Sample (about 1 g) of air-dried soil was mixed with sterilized distilled water (9 ml) and considered as 10^{-1} dilution. The soil samples suspensions were subjected to vortex at 200 rpm for 30 minutes. 100 µl of soil mixtures was transferred to 9 ml of sterile distilled water to get 10^{-2} dilution and similarly stepwise final dilution of 10^{-4} to 10^{-6} was attained. About 100 µl of each suspension was spread on basic nutrient agar plates and incubated at 30°C temperature for 24 hrs. These colonies were plated separately in fresh Petri plates containing nutrient agar medium and purified by streak plate method, code number were allocated accordingly and maintained. Bacterial isolates having different morphological characteristics on agar plates were selected and preserved on nutrient agar slants. NA plates were observed for morphological characters and number of bacterial colonies (cfu/g).

Final count = Number of well-isolated colonies X Dilution factor/ Aliquot taken

Microscopic and Biochemical characterization of bacteria

For microscopic analysis, colony morphology of rhizospheric bacterial isolates through scanning electron microscope (SEM) was carried out by gram's staining or differential staining is used for categorizing the bacteria into two distinct groups either gram positive or gram negative. Hi Media gram's staining kit was used for Gram staining. Many biochemical tests Catalase activity,Oxidase test, Starch hydrolysis and Urease activity were carried out for characterization of rhizosphericand bulk soil of dhaman grasses[57]. Individual bacterial

suspension (100 µl having 10^6 CFU/ml) was inoculated and allowed to incubate for 24 h at 30˚C. After incubation, observations were recorded.

Screening of the bacteria with respect of their plant growth promoting traits

Primarily, bacterial isolates were grown in LB enriched with glycine for 72 h at 37 °C in a shaker. Bacterial isolates were screened for the HCN production by adapting the method of Lorck[58]**.** IAA production was detected by the modified using Salkowaski method[59]. Phosphate solubilizing abiitywas detected by Pikovskaya agar with insoluble tricalcium phosphate (TCP) method[60].

Molecular characterization and phylogenetic analysis of bacteria

Ten strains were selected for further molecular characterization and phylogenetic analysis. For this purpose, bacterial genomic DNA was isolated by freeze and thaw method with some modifications. DNA will be quantified using spectrophotometer (LabMate Asia Pvt Ltd, India). [61]**,** using ScoT markers PCRprofiling of assessing molecular diversity among the bacterial strains. The standard PCR amplification process was adopted in the present study. The PCR reaction mixture was prepared in 10µl reaction volume was prepared consisting of 5µl Go Taq reaction PCR master mixture (2X) 0.4µl of MgCl 2 (20mM) 1.5 µl Primers (10mM) 2µl DNA (50 ng/ml) and, nuclease-free water for volume make-up. The PCR reaction was performed in thermal cycler machine (Bio-Rad, USA) with following thermal cycling conditions: first initial denaturation was at 95°C for 5 min followed by 35 cycles of 95°C for 0.30 min, primer annealing 45°C for 0.30 min, primer extension 72°C for 2 min and final extension 72°C for 10 min. The tubes were placed in the Thermal Cycler (Eppendorf, Germany) for amplification. The PCR product was subjected to gel electrophoresis on 1.5% agarose gel.Gels were visualized under UV gel reader and photographed using Gel Documentation system (SynGene, UK). The polymorphism information of profiled ScoT markers was assessed by using iMEC online software by feeding of binary matrix data. The value inferred by iMEC software was recorded and interpretation was inferred[62]. The phylogenetic analysis assessed by using binary matrix in NTSys 2.0 software[63]. The pair-wise association coefficients were calculated from qualitative data matrix using jaccard's similarity coefficient. Cluster analysis for the genetic distance was carried out using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clustering method. The genetic distances obtained from cluster analysis through UPGMA were used to construct the dendrogram, depicting the relationships among the clones. Population structure analysis, the scored binary matrix data from molecular analysis was used to determine the number of populations present in bacterial strains of the Dhaman rhizosphere using STRUCTURE 2.0 software (www.stanford.edu).

Molecular identification by 16S rRNA gene amplification

Four strains were then selected for further molecular identification. For this purpose, amplification occurred through PCR reaction, followed by subsequent sequencing of the 16S rRNA gene (ITS1 and ITS4 primer set). The cultures were grown in NB for DNA extraction at 28 °C on a shaker (250 rpm). The DNA for 16S rRNA gene amplification were isolated using Qiagen's DNA extraction DNeasy® Blood and Tissue kit (250) and the 16S rRNA gene amplification were carried out using sanger'sequencing method with with the help of a pair of universal oligonucleotide primers ITS1 and ITS4 primer set as forward and reverse primers respectively. The partial 16S rRNA gene sequence obtained from studied bacteria were analyzed and identified with nucleotide BLAST search in Gene Bank of NCBI. The sequences of representative strains were submitted to the GenBank database NCBI and accession numbers were obtained.

Statistical Analysis

Statistical analysis the above experiments were carried out in triplet. The data obtained from their mean values were used for statistical analysis of variance (ANOVA) using a Completely Randomized Design (CRD) for the interpretation of results at p-value <0.05 and within the isolates.

Conclusion

The present study deals with investigating to explore the bacterial diversity in rizosphere region of dhaman grass where are inhabit of highly diversified bacteria for possessing plant growth promoting (PGP) activities such as production of HCN, IAA and phosphate solubilizatioin under drought conditions in arid zone of Rajasthan, India. In this research, a significant diversity of PGPRs was confirmed in Dhaman rhizospheres. We used to culture depended traditional methods for obtained pure bacterial isolates namely AS-11, AS-13, AS-21 and AS-23. Our finding shows the existance of more genetic variability in the rhizospheric bacterial populations. Notably, their diversity analysis based on the molecular study, SCoT technique, delta k value, phylogenetic analysis, and identified by partial 16S rRNA- ITS gene sequencing gives us an edge to have more cultured microorganisms with their taxonomy from indigenous environments. The microbial diversity can prove to be a valuable future resource in various agricultural and biotechnological processes. Such isolates may be used as a bio-fertilizer and bio-decomposers which help in nutrient transformation and stimulate plant growth by production of various substances in drough condition. The study can also be used in bioinformatics studies for further analysis of microbial population by means of diverse high-throughput techniques.

Author Contributors

Ms. A. Kumari is the first and main author of the manuscript who has conducted the research as a part of her partial fulfilment for obtaining her master's degree. Dr. V. Sharma was the

major guide and corresponding author under whose guidance the entire research work was conducted. Dr. Chet Ram was a Senior Scientist (Agricultural Biotechnology), ICAR- CIAH, Bikaner mainly molecular characterization including DNA isolation, gel electrophoresis, PCR amplification, ScoT marker analysis, gel elution and purification of isolates, *etc*., Dr. A. K. Sharma was a Professor and Head (Ret.), advisory committee member, who helped in arrangement of chemicals required for during research work.

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Data availability The data sets used in research are available in online repositories. The repositories name and accession number are as follows: NCBI Gene Bank database, accession numbers PV566939, PV566921, PV566757 and PV566932.

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