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Original Article

MOLECULAR DIVERISTY OF *NOCARDIOPSIS ALBA SP.* ISOLATED FROM THE COASTAL REGION OF GUJARAT, INDIA

DANGAR, K. G., KALASAVA, A. B., DAVE, A. V. AND SINGH, S. P.*

Department of Biosciences, Saurashtra University, Rajkot 360005, Gujarat (India).
E. mail: satyapsingh@yahoo.com, Cell: 098254 87556

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Abstract: ***Nocardiopsis species are in demand for finding biotechnological potential and its adaptation strategies in saline habitats. Here we have reported the characterization of cultivable actinomycetes isolated from the coastal regions of the Gujarat, India. The assortment of seven diverse strains was obtained based on polyphasic approaches. The 16S ribosomal RNA (rRNA) gene sequencing and analysis of each strain showed their affiliation to genus of Nocardiopsis belonging to family Nocardiopsaceae which covered gram positive bacteria. The classical approaches to screen the diversity and capability have been covered under the umbrella of morphologic, biochemical and antibacterial properties of each isolated Nocardiopsis strains. While the molecular tapping of isolated Nocardiopsis albastrains was based on 16S r RNA gene sequences, and detection on repetition of nucleotide and its thermodynamics of sequences along its average nucleotides identities was performed to justify the diversity. Thus, ongoing efforts to characterize soil Nocardiopsis alba capability will add to our understanding to mine the utility of these bacteria as a source of useful products for biotechnology.***

Key words: *Nocardiopsis alba,* 16s rRNA gene Sequence

INTRODUCTION

Actinomycetes are associated with the super kingdom of Gram positive bacteria with high G +C content. They belong to the Phylum of Actinobacteria, Subclass Actinobacteridae, and OrderActinomycetales [1], *Amycolatopsis halophile* [2], *Haloglycomyces albus*[3], *Kocuria halotolerans* [4], *Myceligenerans halotolerans,* [5], *Salinactinospora qingdaonensis* [6] *Nocardiopsis coralliicola* [7] *Prauserella marina* [8], *Streptomyces pharmamarensis* [9] and *Yuhushiella deserti* [10] are reported as halophilic and halo tolerant actinomycetes from the saline habitats . The discovery of actinomycetes from the saline habitats showed versatile genus, with abundant numbers of species. Novel antibiotics [11] enzymes [12] and bioactive compounds [13,14] have been reported from the actinomycetes of the saline habitats.

During the last two decades, the laboratory of Prof. Satya P. Singh at the Saurashtra University has explored halotolerant actinomycetes [15,16]. Halophilic actinomycetes; *Streptomyces sannnanensis* strain RJ-1 and *Streptomyces aburaviensis* strain Kut-8 have been reported for the production of antibiotics [11,17]. Besides antibiotics, the alkaline serine proteases with the ability to catalyze under multiple extreme conditions are also reported form many haloalkaliphilic actinomycetes, such as *Nocardiopsis alba* OK-5 [12,18]. *Nocardiopsis dassonvillei* strain OK-18 has been recently reported for the production of extracellular alkaline protease [19].

***Dr. S. P. Singh,** Prof. & Head, Department of Biosciences, Saurashtra University, Rajkot

The genus *Nocardiopsis* belongs to the phylum actinobacteria and family *Nocardiopsaceae* under the Gram positive bacteria. It's characterized with the high endemic branches residing in various habitats of soil, water, deep-sea, or extreme niches, such as arctic ice, saline and coastal region. [18,20-26].

The species of the *Nocardiopsis* genus are known to produce number of enzymes, like cold-adapted α-amylase and thermostable α-amylases [27,28]. *Nocardiopsis species SD5* produced extracellular keratinase and *Nocardiopsis dassonvillei subspecies prasina OPC210* produced alkaline serine protease [29,30].The members of this genus are capable to display multitudes of the adaptation strategies in saline habitats. In *Nocardiopsis halophile,* ectoines production was observed which works as compatible solutes and provides a strategy to survive under high salinity [31,32]. In this report, we describe the properties of salt tolerant actinomycetes from the selected saline habitats of Gujarat Coast.

MATERIALS AND METHODS

Sample collection and analysis: The saline soil samples were collected in sterilize plastic bags from two different sites located in Porbandar (21° 37"N69° 49"E) and Veraval (20° 53" N 73° 26" E) of the Coastline Gujarat, India. pH, and electrical conductivity (E.C.) were measured by the method described by Jackson [33].

Isolation, enrichment and preservation of *nocardiopsis alba sp.*strains: 1 g of soil was suspended in 5 ml sterile water and subjected to serial dilutions of 1:100, 1:1000, 1:10000, and 1:100000 [34]. From each dilution, 100 µl aliquot was spread with glass road on the Actinomycete isolation agar ((AIA) (Hi media, India). The medium contained 40.0 g (w/v) NaCl, 2.0 g sodium caseinate, 0.1 g asparagine, 4.0 g sodium propionate, 0.5 g of K_2HPO_4, 0.1 g of $MgSO_4 \cdot 7H_2O$, 0.1 g of $FeSO_4$, 5.0 g of glycerol and 15 g of agar (Hi-media) in 1 liter [35]. The plates were incubated for 3-4 weeks at 28ºC. Dry, powdery and chalky white colonies were obtained and further purified on AIA with containing additional 5% of NaCl (w/v) [36-40].

Phenotypic analysis of the isolated actinomycetes: Each isolate was directly suspended in a drop of sterile distilled water and transferred onto a sterile glass slide surface. The colonies were characterized on the actinomycetes isolation agar (AIA) after 4 days of the incubation at 28°C [40,41]. For further differentiation of the isolated *Nocardiopsis* strains, they were studied for the biochemical properties. The biochemical tests included production of urease and indole production, Oxidase test, Nitrate reduction, Catalase test, oxidase, citrate utilization test , TSI test Slant. The individual isolate was inoculated into the respective biochemical medium with 5% NaCl, pH 8 and incubated at 28°C for 72 h [42], followed by the periodic monitoring and observation of the results

Salt and pH profile: The growth media for the isolates was supplemented with 5% NaCl and pH 8 and the organisms were incubated at 28°C for 72h and followed by further monitoring. Each strains was incubated in 5 mL YEME medium (Yeast peptone malt glucose broth, 5 g peptone, 3 g yeast extract, 3 g Malt extract, 50 g NaCl, and 10 g glucose in 1 liter distilled water) and at varying pH of 8-12 and salt 5-15% and incubated at 28°C for 72h. The growth was monitored at 540 nm [42, 43].

Antibiotics resistance and sensitivity of the Actinomycetes: 1 ml of 48 - 96 h old activated cultures were spread over the Actinomycetes isolation agar plates (5% w/v NaCl, pH 8) followed by putting an antibiotic impregnated discs Universal disc 1, 2, and 3 (HI media) on the agar. The growth was observed after 4 days of incubation at 28 °C based on the Bauer- Kirby test [42]. The appearance of the zone of inhibition around the each antibiotic disc demonstrated the resistance and sensitivity of the organisms towards the specific antibiotics. The antibiotics used were (number shown in the brackets indicate the concentration of the antibiotics in mg: Cefpodoxime (10), Chloramphenicol (30), Vancomycin (30), Streptomycin (10), Rifampicin (5), Levofloxacin (5), Ceftriaxone (30), Clindamycin (2), Augmentin (30), Amikacin (30), Cefixime (5), Tetracycline (30), Co-Trimoxazole (25), Colistin (10), Netillin (30),Norfloxacin (10), Ciprofloxacin (5), Cephotaxime 30 Gentamicin (10), Furazolidone (50), Amoxycillin (10), Ampicillin (10), Cefuroxime (30), Cefadroxil (30), Penicillin (10), units Cefaclor (30), Azithromycin (15), Erythromycin (15), Cefaperazone (75), Clarithromycin (15).

GENOTYPIC ANALYSIS OF THE HALOALKALIPHILIC ACTINOMYCETES:

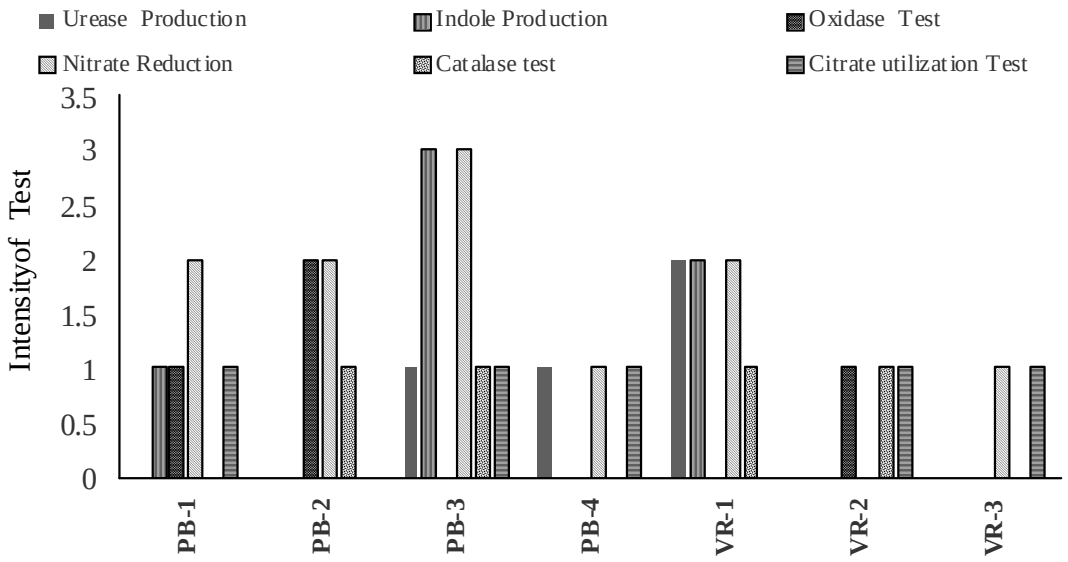

Fig. 1: The detail projection of the biochemical reactions of all the isolates

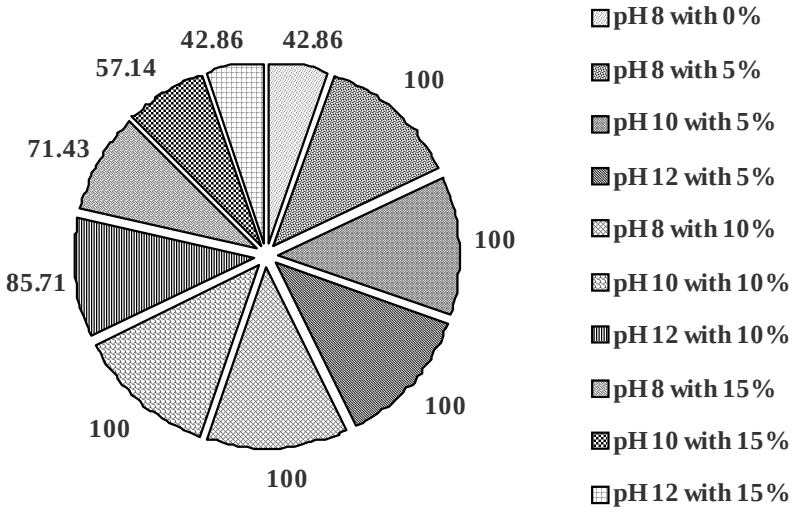

Fig. 2: Salt and pH profile of isolates

Table 1: Culture characteristics of each isolate

Isolate	Size (mm)	Shape	Margin	Texture	Elevation	Consistency	Opacity	Earthy Aroma
PB-1	4	Round	Irregular	granular	Slightly raised	Rigid	Opaque	-
PB-2	7	Round	Entire	Rough	Raised	Rigid	Opaque	+
PB-3	6	Round	Entire	granular	Raised	Smooth	Opaque	+
PB-4	8	Irregular	Irregular	Smooth	Slightly raised	Powdery	Opaque	-
VA-1	6	Irregular	Irregular	granular	Slightly raised	Powdery	Opaque	-
VA-2	3	Round	Entire	Rough	Raised	Smooth	Opaque	+
VA-3	4	Irregular	Irregular	Rough	Slightly raised	Smooth	Opaque	-

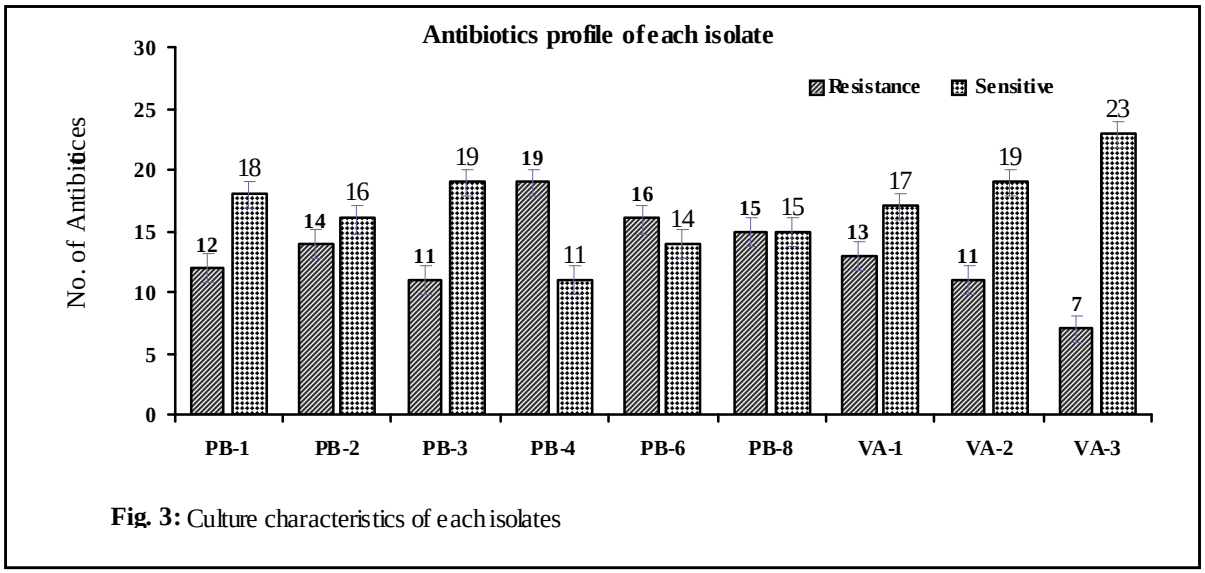

Fig. 3: Culture characteristics of each isolates

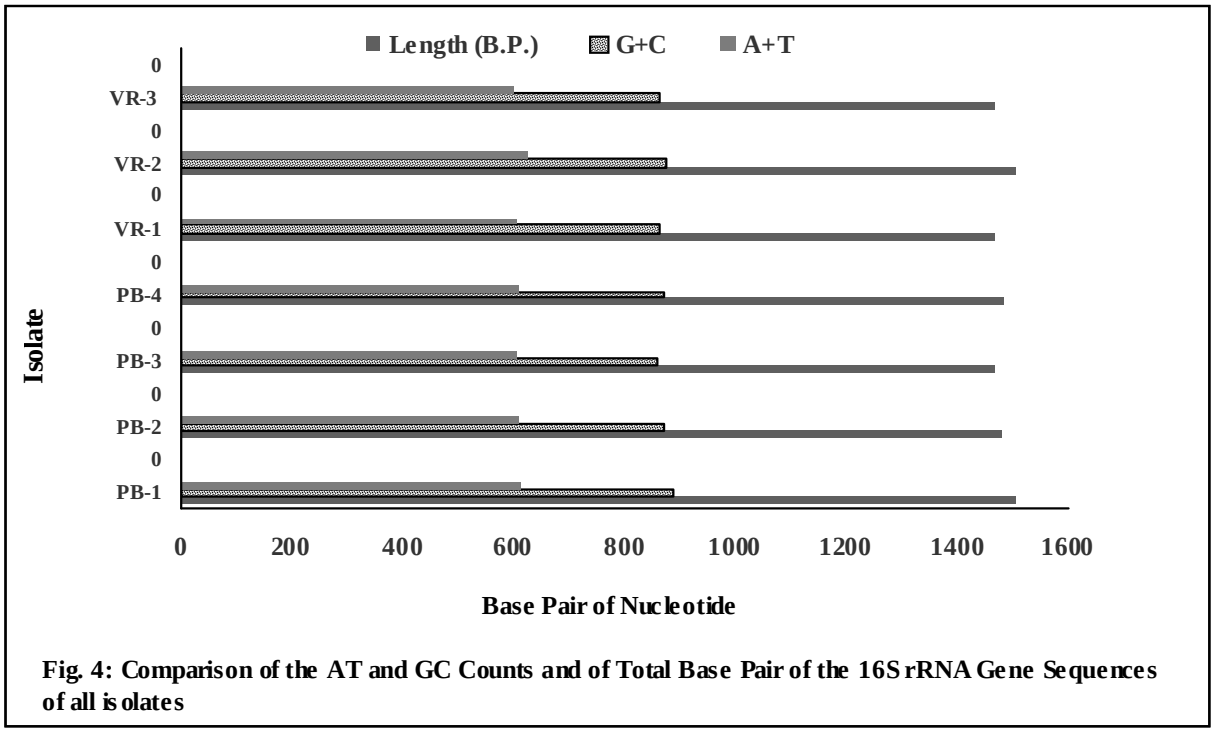

Fig. 4: Comparison of the AT and GC Counts and of Total Base Pair of the 16S rRNA Gene Sequences of all isolates

Table 2: Biochemical reactions of all the isolates

Name of Isolates	Urease Production	Indole Production	Oxidase Test	Nitrate Reduction	Catalase test	Citrate utilization Test
PB-1	-	+	+	++	-	+
PB-2	-	-	++	++	+	-
PB-3	+	+++	-	+++	+	+
PB-4	+	-	-	+	-	+
VR-1	++	++	-	++	+	-
VR-2	-	-	+	-	+	+
VR-3	-	-	-	+	-	+

16S rRNA gene amplification and sequencing of the haloalkaliphilic Actinomycetes: For the extraction of the genomic DNA, the selected haloalkaliphilic actinomycete were grown on the Yeast Peptone Malt Glucose broth (5 g peptone, 3 g yeast extract, 3 g Malt extract, 50 g NaCl, and 10 g glucose in 1 liter distilled water), pH 8. The pH was adjusted by adding separately autoclaved 20% Na_2CO_3 for 72 hrs. at 28°C. The total genomic DNA was prepared as per the method described in the Hi PurA Streptomyces DNA purification kit -MB 527- 50p, HI-media [44]. The 16SrRNA gene was amplified and sequenced using primers: 27F (AGA GTT TGA TCM TGG CTC AG) ,1492 R (TAC GGY TAC

CTT GTT ACG ACT T) and 518F (CCA GCA GCC GCG GTA ATA CG), with 35 amplification cycles at 94°C for 45 sec, 55°C for 60 sec, and 72°C for 60 second. About 1,400 BP of DNA fragments were amplified and sequenced by using Big Dye terminator cycle sequencing kit (Applied Biosystems, USA). The sequencing products were resolved on an Applied Biosystems model 3730XL automated DNA sequencing system (Applied Biosystems, USA) [45,46].

Phylogenetic analysis of the 16 S rRNA sequences: The 16 S rRNA sequences were compared with the available sequences in the NCBI database (http://www/ ncbi.nlm.nih.gov/) using Basic Local Alignment Search Tool (BLAST) [47]. Multiple sequence alignment was carried out using CLUSTALW [48] and phylogenetic tree was constructed using MEGA 6 by Neighbor Joining method with 1,000 bootstrap replicates. Nucleotide compositions of A, T, G, C, A+T % and G+C % frequency of nucleotide content and molecular weight of the obtained sequences were determined by CLC Work Bench (http://www.clcbio.com/products/ clcgenomicsworkbench) [49].

The 16S rRNA gene sequence data were deposited in Genbank. The 16 S rRNA sequences generated in this study added values to the unexplored world of the actinomycetes of the saline habitats.

Intra species diversities based on the comparative studies of the 16S rRNA gene sequences similarities and ANI calculator: The identification of the intra species diversity by using BLAST (**B**asic **L**ocal **A**lignment **S**earch **T**ool) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) [47], comparing the one 16S rRNA gene sequences as the query sequences of same species from same site and BLAST against another same species of same site. The calculation of the ANI Average Nucleotide Identity (ANI) (enve-http://enve-omics.ce.gatech.edu/ani//) between two species isolated from the same sites based on the one way ANI [50]. Comparative statements indicated the intra species diversity base on the most similar species on the basis of the 16S rRNA gene similarities and ANI calculator.

Analysis of the oligo nucleotide sequences andopen reading frame: Most similar species sequences of 16S rRNA genes were subjected to finding the open reading frame by using CLC Work Bench *(http://www. clcbio. com/products/ clcgenomicsworkbench)* [49]. The online available, OligoCalc (http://basic.northwestern.edu/biotools/ OligoCalc.html) [51] Software was used for the detection of the physical and thermodynamic constraints of the sequences.

Identification of the nucleotides repeats in 16S rRNA gene sequences: FAIR (http://bioserver1.-physics.iisc.ernet.in/fair/) program was used to find the clustered and exact repeats in the nucleotide sequences. For each sequences, the minimum number of the residues in a repeat was 10 and number of the occurrence was kept 2 [52].

RESULTS

Sample collection and analysis: The sampling sites of Porbandar and Veraval displayed high salinity and variable. The alkaline range of pH was 7.8-8.3, while the salinity was in the range of 8.31 and 9.29EC (ms/Cm^2) respectively.

Isolation and preservation of the haloalkaliphilic Actinomycetes: Seven different haloalkaliphilic actinomycetes mostly belonging to the *Nocardiopsis* were isolated from the coastal region of Gujarat. Majority of the isolated *Nocardiopsis* strains were able to grow at 5% (w/v NaCl), pH 8.0 on the actinomycetes isolation agar and hence were referred as halo-tolerant and alkaliphilic in nature. The isolated *Nocardiopsis* strains were abbreviated on the basis of the area of their origin. Porbandar and Veraval were abbreviation and the isolates were designated as *Nocardiopsis* strains PB and VA, 4 and 3, respectively.

Phenotypic analysis of the actinomycetes: The

isolated actinomycetes strains were gram positive, long filamentous morph types. While studying cultural characteristics of the actinomycetes on the actinomycetes isolation agar, good growth and aerobic nature of the isolates were revealed. They showed sporulation with compact, chalk like dry colonies of white to dark brown, substrate mycelia and colored pigmentation in a few isolates. Table no.1 showed the culture characteristics of each isolate. The identification of the biochemical and metabolic activities were based on the biochemical properties used to obtain the map of the diversity. The detail projection of the biochemical reactions of all the

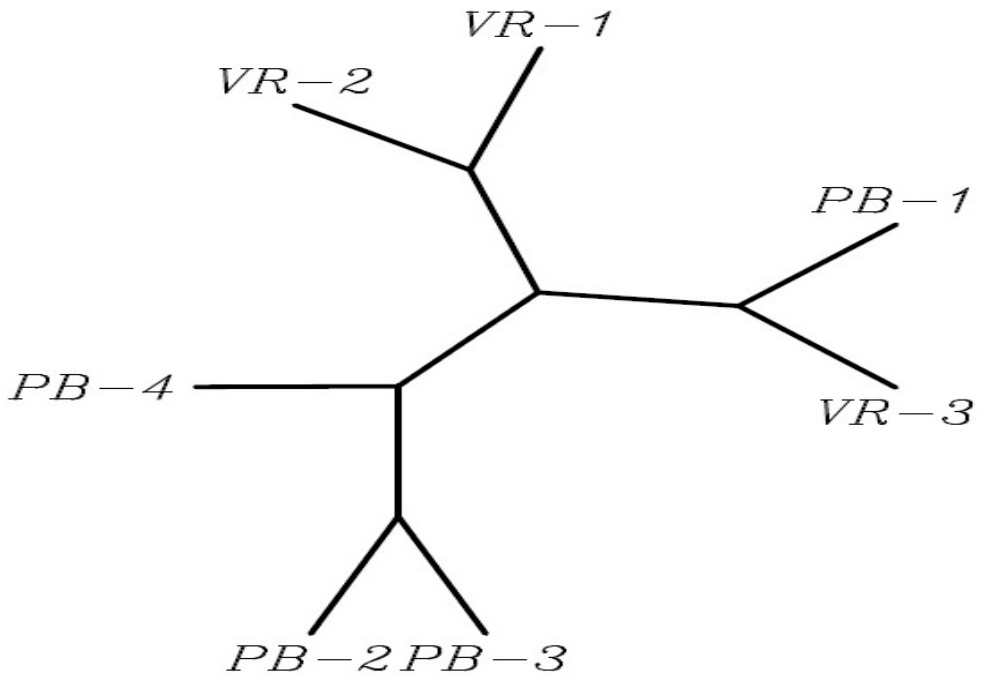

Fig. 5: Phylogenetic Tree of all Isolates.

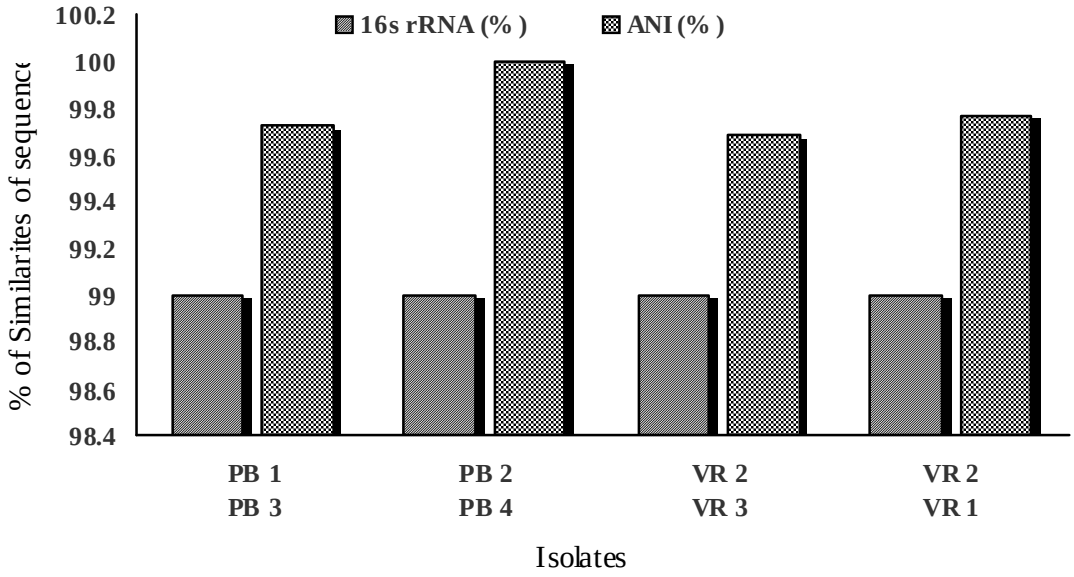

Fig. 6: Comparative analysis of the Similarity of 16S rRNA and ANI Gene Sequences of all Isolates

isolates is depicted in table 2 and figure 1 showed the biochemical characterization of each isolate.

Salt and pH profile: Each , 3 (42.85%) were able to grow up to 5% NaCl with pH 8, 7 isolates (100%) were able grow up to 5% NaCl with pH 8,10, 12 as well as pH 8, 10 and 10% salt. 6 isolates (85.77%) grew at 12% NaCl; pH 12, while 5 isolates (71.42%) were able grow at 15% NaCl with pH 8, 10, as well as 15 % NaCl pH 10,12 isolates 4 (57.42) and 3 (42.58%) were able grow up to on YEME medium. The overall profile of salt and pH is presented in figure 2.

Antibiotic sensitivity of the isolates: Antibiotic sensitivity/resistance profile of the isolates was generated by using Dodeca disc. After 48-96 h of growth, the zones of clearance for the isolates were measured and documented in mm. Among the total 7 isolates, majority were resistant against 30 antibiotics PB-1,PB-2,PB-3, PB-4, VR-1, VR-2 and VR-3 showed the sensitivity 18,16,19,11,14,15,17,19 and 23 and while the resistance were 12,14,11,19,16,15,13,11 and 7 respectively. Figure 6 represents the antibiotic resistant and sensitivity profile. The graphical representation of each isolate antibiotic profile showed in figure 3.

GENOTYPIC ANALYSIS OF THE ISOLATES

Phylogenetic analysis based on the 16S rRNA sequences: Table No. 3: Phylogenetic analysis based on the 16s rRNA sequences. The 16S rRNA gene sequences are widely used and reliable technique in the bacterial taxonomy. The 16S rRNA gene sequencing was carried out followed by the construction of the phylogenetic tree to compare the evolutionary distances among the studied actinomycetes of the saline habitats. Characterization of the actinomycetes on the basis of the molecular signature of 16S rRNA genes is illustrated in table 3. Figure 4 showed the comparison of the AT and GC counts and of total base pair of the 16S rRNA Gene Sequences of all isolates. Figure 5 of phylogenetic affiliation by tree repetition showed each isolate represented on the different branch. Actinomycetes have high guanine and cytosine content in their DNA, some times as high as 70%. Each isolate in this study contained higher G+C content compared to A+T.

Intra species diversities based on the comparative studies of the 16s rRNA gene sequences similarities and ANI calculator: Comparative statement of the 16S rRNA gene sequence similarities and ANI is provided in table 4. There are significant differences in the percentage values obtained by ANI as compared to the 16S r RNA gene similarities percentage value between the same species. Figure 6 showed comparative analysis of the Similarity of 16S rRNA and ANI gene sequences of 7 isolates.

Analysis of the oligo nucleotide sequences and open reading frame: The open reading frame was created by CLC Workbench. It showed the positions of differences along with the locations and start of the code (Table 5). The screening of the intra species diversity of *Nocardiopsis alba* isolated from the Porbandar and Veraval showed variation in melting temperatures. While the thermodynamics constant displayed variations in ΔG, ΔH and ΔS showed in table 6. The conserved sequences showed dissimilarity in the structure of rRNA. Table 7 represented the position of the open reading frame as along with start and stop codon.

Identification of the nucleotides repeats in 16S rRNA gene sequences: FAIR (http://bioserver1.physics.iisc.ernet.in/fair/) program was used to find clustered and exact repeats in the nucleotide sequences. For each sequence, the minimum number of the residues in a repeat was 10 and number of occurrence was 2. The results showed several nucleotides patterns as well as clusters among the sequences of different isolates. The details sequence analysis along with the repeats of the nucleotides is depicted in the table 8.

DISCUSSION

The study is based on the phenotypic and genotypic characteristics of seven *Nocardiopsis* strains isolated from the saline habitats of Coastal Gujarat, India. The physiochemical properties of the collected soil samples from the Gujarat coast line showed EC values in range of 7-9 (mS/Cm^2), while the organic carbon content was low at <0.5(g/Kg) [53] have reported higher EC values, which was linked with the lower organic carbon in soil samples. In our study in this report, all actinomycete strains were identified to belong to the *Nocardiopsis* genus. Apart from the Coastal Gujarat, other coastlines of India, such as Goa and Andaman and Nicobarare reported to harbor the actinomycetes belonging to *Nocardiopsis*

Table 3: Phylogenetic analysis based on the 16s rRNA sequences

Name of Species	Gi No. / Accession No.	Length (B.P.)	A	T	G	C	G+C	A+T	AT%	GC%
Nocardiopsis alba strain PB-1	1012161369 / KU291207.1	1506	328	288	514	376	890	616	40.90305	59.09695
Nocardiopsis alba strain PB-2	1012161360 / KU291198.1	1481	322	289	508	362	870	611	41.25591	58.74409
Nocardiopsis alba strain PB-3	1012161371 / KU291209.1	1469	319	287	504	359	863	606	41.25255	58.74745
Nocardiopsis alba strain PB-4	1012161368 / KU291206.1	1484	323	288	510	363	873	611	41.17251	58.82749
Nocardiopsis alba strain VR-1	1012161363 / KU291201.1	1471	319	287	505	360	865	606	41.19646	58.80354
Nocardiopsis alba strain VR-2	1012161364 / KU291202.1	1506	339	289	505	373	878	628	41.69987	58.30013
Nocardiopsis alba strain VR-3	1012161367 / KU291205.1	1468	316	286	506	360	866	602	41.00817	58.99183

Table 4: Intra species diversities based on 16S rRNA gene sequence similarities

ISOLATES		MAX SCORE	TOTAL SCORE	QUERY COVER (%)	16srRNA (%)	ANI (%)
PB 3	PB 1	2565	2565	99	99	99.73
PB 4	PB 2	2650	2650	99	99	100
VR 3	VR 2	2567	2567	99	99	99.69
VR 1	VR 2	2567	2567	99	99	99.77

Table 5: Analysis of the Open Reading Frame

No.	Isolates	Length in Base Pair	Melting Temperature Calculation	Absorbance at 260 nm	Micromolar Contain	Micrograms of DNA
1	*Nocardiopsis alba* PB-1	1506	88.7° C	1	0.052	24.3
2	*Nocardiopsis alba* PB-2	1481	88.5° C	1	0.063	29
3	*Nocardiopsis alba* PB-3	1469	88.5° C	1	0.063	28.8
4	*Nocardiopsis alba* PB-4	1484	88.6° C	1	0.062	28.6
5	*Nocardiopsis alba* VR-1	1471	88.6° C	1	0.063	28.8
6	*Nocardiopsis alba* VR-2	1506	88.4° C	1	0.061	28.6
7	*Nocardiopsis alba* VR-3	1468	88.6° C	1	0.063	28.8

Table 6: Thermodynamic analysis of the 16S rRNA sequences

No.	Isolates	Thermodynamics Constant Conditions: 1 M NaCl of 25°C at pH 7			
		Length in sBase Pair	deltaG (Kcal/mol)	deltaH (Kcal/mol)	deltas cal/(°K*mol)
1	*Nocardiopsis alba* PB-1	1506	2609.7	13656.9	35603.4
2	*Nocardiopsis alba* PB-2	1481	2560.2	13407.5	34958.9
3	*Nocardiopsis alba* PB-3	1469	2540.8	13301	34678.1
4	*Nocardiopsis alba* PB-4	1484	2567.7	13441.5	35044.3
5	*Nocardiopsis alba* VR-1	1471	2544.4	13318.5	34722.9
6	*Nocardiopsis alba* VR-2	1506	2598.1	13628.7	35549.7
7	*Nocardiopsis alba* VR-3	1468	2542.7	13306.8	34690.7

genus [54,55].

The wide occurrence of the *Nocardiopsis* genus is also reported form some other saline habitats [56, 57]. The species of the *Nocardiopsis genus* include, *Nocardiopsis alba*, Nocardiopsis salina sp., and *Nocardiopsis arvandica* [18,58,59]. The growth patterns and phenotypic characterization of the isolates revealed chalky white colonies and variability in size, shape and elevation. Earlier, growth of *Nocardiopsis salina sp. Nov.* YIM 90010^T has been reported with white aerial mycelium on yeast extract/ malt extract (ISP 2) [58]. The organisms were aerobic, gram positive possessing high GC content with the ability to grow at 5% NaCl, pH 8 at 28 ^{0}C. A similar pattern is reported for *Nocardiopsis arvandica, HM7^T* obtained from Arvand River, Iran [59].

Table. 7: Position of the open reading frame of each isolates

Sequence	Start	End	Length	Found at strand	Start codon
Nocardiopsis alba PB-2	1106	1417	312	positive	TGG
Nocardiopsis alba PB-2	29	673	645	negative	TAT
Nocardiopsis alba VR-1	1106	1417	312	positive	TGG
Nocardiopsis alba VR-1	29	673	645	negative	TAT
Nocardiopsis alba VR-2	1107	1445	339	positive	TGG
Nocardiopsis alba VR-1	30	674	645	negative	TAT
Nocardiopsis alba VR-3	1104	1442	339	positive	TGG
Nocardiopsis alba VR-3	27	671	645	negative	TAT
Nocardiopsis alba PB-4	1109	1420	312	positive	TGG
Nocardiopsis alba PB-4	32	676	645	negative	TAT
Nocardiopsis alba PB-1	1108	1446	339	positive	TGG
Nocardiopsis alba PB-1	31	675	645	negative	TAT
Nocardiopsis alba PB-3	1102	1413	312	positive	TGG
Nocardiopsis alba PB-3	25	669	645	negative	TAT

Table 8: Identification of the nucleotides repeats in 16S rRNA gene sequences

No	Isolates	Types of Residues Repeats	Position of Repeats Residues in Nucleotide Sequences
1	*Nocardiopsis alba* PB-1	GTGGGGAATA	[332 to 341] [458 to 467]
2	*Nocardiopsis alba* PB-2	CCCTTCGGGG	[56 to 65] [1418 to 1427]
	Nocardiopsis alba PB-2	GAAGGTGGGG	[1146 to 1155] [1439 to 1448]
	Nocardiopsis alba PB-2	GTGGGGAATA	[330 to 339] [456 to 465]
3	*Nocardiopsis alba* PB-3	CCCTTCGGGG	[52 to 61] [1414 to 1423]
	Nocardiopsis alba PB-3	GAAGGTGGGG	[1142 to 1151] [1435 to 1444]
	Nocardiopsis alba PB-3	GTGGGGAATA	[326 to 335] [452 to 461]
4	*Nocardiopsis alba* PB-4	CCCTTCGGGG	[59 to 68] [1421 to 1430]
	Nocardiopsis alba PB-4	GAAGGTGGGG	[1149 to 1158] [1442 to 1451]
	Nocardiopsis alba PB-4	GTGGGGAATA	[333 to 342] [459 to 468]
5	*Nocardiopsis alba* VR-1	CCCTTCGGGG	[56 to 65] [1418 to 1427]
	Nocardiopsis alba VR-1	GAAGGTGGGG	[1146 to 1155] [1439 to 1448]
	Nocardiopsis alba VR-1	GTGGGGAATA	[330 to 339] [456 to 465]
6	*Nocardiopsis alba* VR-2	AAAAAAAAAAAAA	[1493 to 1505] [1494 to 1506]
	Nocardiopsis alba VR-2	AAAAAAAAAAAA	[1493 to 1504] [1495 to 1506]
	Nocardiopsis alba VR-2	AAAAAAAAAAA	[1493 to 1503] [1496 to 1506]
	Nocardiopsis alba VR-2	AAAAAAAAAA	[1493 to 1502] [1497 to 1506]
	Nocardiopsis alba VR-2	GTGGGGAATA	[331 to 340] [457 to 466]
7	*Nocardiopsis alba* VR-3	CCCTTCGGGG	[54 to 63] [1420 to 1429]
	Nocardiopsis alba VR-3	GAAGGTGGGG	[1144 to 1153] [1441 to 1450]
	Nocardiopsis alba VR-3	GTGGGGAATA	[328 to 337] [454 to 463]

The isolates displayed variability on the basis of various biochemical properties and hence were diversifiable based on these features. Earlier, an actinomycete, *Nocardiopsis kunsanensis* was shown to possess catalase positive reaction and lack of H_2S production and nitrate reduction [60]. Similarly, Li et al. [58] reported that *Nocardiopsis salina sp.*nov. was not able to produce urea and H_2S. On the other hand, many other actinomycetes possess the ability to reduce nitrate to nitrite [61-64].

All the isolates were able to grow at alkaline pH in the range of 8-12. A larger variability in the patterns of the pH dependence has been reported for the actinomycetes, have its place to the genus *Nocardiopsis*, isolated from the sandy soil of different parts of the world, noticeably Iran, Persian Gulf and Menellapraelonga [59,65,66].The studies suggest a wider range of pH in acidic and alkaline range for *Nocardiopsis*.

Nocardiopsis xinjiangensis OM-6 and *Nocardiopsis flavescens* are reported for their ability to grow at a wider range of salt concentrations; 0-20 % (w/v) [64,67]. The *Nocardiopsis* strains in the present study were able to grow at 28-32 ^{0}C.

Nocardiopsis sp. belonging to the family of *Nocardiopsaceae* display different adaptation strategies to grow and survive at different temperatures. *Nocardiopsis terrae YIM 90022* isolated from the saline soil of Qaidam Basin, China displayed the growth over a much wider temperature range of 10-45 ^{0}C, suggesting their unique adaptive feature from being psychrotolerant to thermotolerant [68]. The mesophilic *Nocardiopsis* strains; *Nocardiopsis litoralis JSM073097T and Nocardiopsis algeriensisB32* are reported to grow at temperatures in the range of 20-37 ^{0}C [69,70].

The phylogenetic analysis on the basis of the 16S rRNA gene sequences established the taxonomic affiliation of 7 isolates in two groups, *Nocardiopsis alba* and *Nocardiopsis sp.* The result demonstrated that the 16S rRNA gene sequences effectively act as fingerprinting tool for analyzing the actinomycetes of the saline habitats at species level. The comparison of the 16S rRNA gene sequence similarity of *Nocardiopsis synnemataformans sp. Nov* was belong to the genus *Nocardiopsis* [71].

16S rRNA gene sequences of *Nocardiopsis arvandica sp. nov.,* isolated from the sandy soil showed 99.8% and 99.3% similarity with *Nocardiopsis sinuspersici* DSM 45277^T and *Nocardiopsis quinghaiensis YIM 28A4^{T,}* respectively [65,72]. Each strain showed 99-100% similarities to the strains belonging to genus *Nocardiopsis* depicting the monophyletic clade in dendrogram.

CONCLUSION

The seven actinomycete strains isolated from the saline habitats of the coastal region of Gujarat, India are described in this study for their cultural and morphological characteristics, biochemical and metabolic diversity and phylogenetic affiliation. The ecological role of the dominant *Nocardiopsis* in the saline ecosystem is largely unknown. Therefore, exploration of massive polyphasic characteristics of the actinomycetes study reflects that although the actinomycetes belong to the same genera of *Nocardiopsis*, there exit heterogeneity in terms of phenotypic, genotypic and metabolic features of the isolates generated the map of diversity.

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