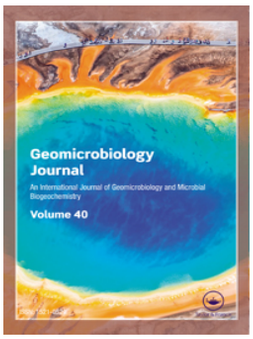

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# Genetic and Physiological Diversity of Marine Actinobacteria from the Okha Coast, Gujarat, India

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**ABSTRACT**

Saline and alkaline habitats of the Okha coastline, Gujarat, India, were explored for the diversity of actinobacteria. Based on the phenotypic and genotypic heterogeneity, nine haloalkaliphilic actinobacterial strains were characterized and identified. The PCR amplification of 16S rDNA using universal and genus-specific primers corroborated the trends that emerged through cultural and physiological characterization. *Streptomyces* was the most abundant genus amplified with the *Streptomyces*-specific primers (StreptB/E, StreptB/F), whereas other actinobacterial strains were amplified by the universal primers U1 and U2. Further molecular diversity was investigated by the gradient PCR-DGGE as a fingerprinting tool that generated group-specific DGGE patterns. Based on the nucleotide homology and phylogenetic analysis, strains OK-1 and OK-2 were identified as *Streptomyces somaliensis*. At the same time, OK-3 and OK-7 were detected as *Streptomyces* sp., while OK-5, OK-6, OK-8, OK-9, and OK-10 belonged to *Nocardiopsis alba*. Further, the cluster analysis using the UPGMA method generated 3 clusters based on biochemical characterization, sugar utilization, and enzyme production. The dendrogram based on the DGGE band pattern created with Jaccard-distance revealed two major clades with 33.33% similarity. Further, the study of alpha diversity calculation using phenotypic characteristics discloses highly diverse sugar utilization abilities. Moreover, a stress value of 0.1236 was obtained based on the NMDS analysis of the plots using Bary-Curties dissimilarity. Overall, the distinct phenotypic, metabolic, and molecular profiling illustrated the diversity among marine actinobacteria.

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**KEYWORDS**
DGGE; haloalkhaliphilic actinobacteria; heterogeneity; NMDS; 16S rDNA; *Streptomyces* specific primers

## Introduction

The actinobacterial genera, especially *Streptomyces,* and *Nocardiopsis* are among the richest sources of commercially significant novel bioactive metabolites (Leutou et al. 2018; Ryu et al. 2021; Zhang X et al. 2021). However, the nobility of the selected and screened microbial agents remains a significant concern. Thus, diverse habitats should be explored to isolate novel microorganisms, followed by improved pretreatments of the samples. The Okha Coast, Gujarat, in the Western is the central point of the Gulf of Kutch and the Arabian Sea. The intertidal region of the Okha coastline is 0.7–0.9 Km long consisting of stones and sand (Pankaj 2022; Thakur et al. 2008). Till today, diverse floral and faunal species are reported from the coastal areas of Gujarat, India. However, only limited research has focused on the microbial diversity of coastal Gujarat (Majithiya and Gohel 2022c; Raiyani and Singh 2023; Sharma et al. 2021). Therefore, the Okha coastline was selected in this study to investigate actinobacterial diversity. Cultivating actinobacteria from varied saline habitats plays a pivotal role in investigating the physicochemical properties of the organisms and their ecological relevance. Similarly, to investigate molecular characteristics of the cultivable actinobacteria, a variety of species-specific and universal primers can be used to amplify the 16S rDNA (Gohel and Singh 2018; Majithiya and Gohel 2022a; Rathore et al. 2021). Therefore, the focus of the present work was identifying molecular and physicochemical characteristics of haloalkaliphilic actinobacteria that are useful to project the taxonomic and phylogenetic diversity of extreme actinobacteria.

Actinobacteria are Gram-positive bacteria with remarkable adaptation to saline and alkaline environments that make them an important group within the microbial community of saline habitats (Majithiya and Gohel 2022b, 2020; Sharma et al. 2021). *Streptomyces* and *Nocardiopsis* genera are well known for their applications in the medicine, textile, detergent, food, and leather industries (Al-Dhabi, Esmail, Ghilan, Arasu, Duraipandiyan, et al. 2019; Gohel and Singh, 2015; Sharma et al. 2020). The 16S rDNA amplification using species-specific PCR offers an alternative approach for the rapid identification of many strains. Earlier,16S rDNA of *Streptomycetes* (Gohel and Singh 2018; Rintala et al. 2001), *Nocardiopsis* (Rathore et al. 2021; Salazar et al. 2002; Sunish et al. 2020), *Streptomonospora* (Zhi et al. 2006) and *Nesterenkonia* (Gohel and Singh 2018; Zhi et al. 2008) were amplified using genus-specific primers. Following the 16S rDNA amplification with species-specific primers, the denaturing gradient gel electrophoresis (DGGE) can be a significant tool for fingerprinting to evaluate

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microbial diversity (Wolińska et al. 2019). The DGGE is carried out using chemical denaturant, urea, and formamide, while in the temperature gradient gel electrophoresis (TGGE), a temperature gradient is employed (Myers et al. 1985). Both these techniques provide comparable fingerprints of the microbial communities (Zhao et al. 2018). In DGGE, the number, precise position, and intensity of the bands in a gel track estimate the relative abundance of the numerically dominant ribotypes in the studied habitat. Nevertheless, the distinct band patterns generated by the pure cultures of the actinobacteria in the present study indicated heterogeneity of the 16S rDNA in the genome. Besides, this study also explored the phylogenetic status of the actinobacteria using species-specific primers. Overall, applying the fingerprinting techniques, such as DGGE evaluated diversity among the actinobacterial species of the saline and alkaline habitats of Coastal Gujarat in Western India.

## Materials and methods

### Isolation strategies

Marine samples, including soil, water, and rocks, were collected from the Okha study site, Coastal Gujarat, India (Latitude 22.20 N, Longitude 70.05 E) during winter season. The samples were collected from a depth of 10–15 cm. The pH, temperature, and salinity of the water samples were determined as 8.5 ± 0.2, 27 °C ± 2.0 and 32.40 ds/m ± 0.34, respectively. The collected samples were stored in sterile plastic containers at −20 °C until further use. For the cultivation of actinobacteria, desiccation ($CaCO_3$ treatment) and heat shock (incubation at 60 °C for 2 h) treatments were applied to enhance the growth of the actinobacterial species (Gohel and Singh 2018). The serial dilutions and plating were carried out at the $10^{-4}$ dilution on ISP-3 and actinomycetes isolation agar (Himedia Laboratories Pvt. Ltd., India) supplemented with 0–20% NaCl (w/v). The pH was adjusted to 9 using separately autoclaved 20% $Na_2CO_3$. After incubation at 28 °C ± 2.0 in static conditions for 15–20 days, tough, leathery, and chalky white colonies adhered to the agar surface were selected and sub-cultured on the respective agar plates. All the isolated and purified colonies of different strains were preserved in 30% glycerol at −20 °C till further use. To identify the actinobacterial strains, the 16S rDNA were amplified using universal primers followed by Sanger's sequencing. Further, for the sequence similarity and phylogenetic analysis, the dendrogram trees were constructed by the MEGA 11 software using the CLUSTER W algorithm. The constructed phylogenetic trees were further edited using the iTOL interactive tree of life (https://itol.embl.de). The actinobacterial 16S rDNA sequences are deposited in NCBI GenBank.

### Morphological, cultural, and physiological characteristics

The physiological features of the actinobacteria were investigated on ISP-5 agar medium (Himedia Laboratories Pvt. Ltd., India) commonly used for the characterization of *Streptomyces* species prepared with 5% NaCl, pH 9. The pH was adjusted using separately autoclaved 20% $Na_2CO_3$ (Shirling and Gottlieb 1966). The phenotypic characteristic, microscopic examinations by Gram's staining, mycelial development, and pigmentation were observed after a growth of 4 days at 28 °C ± 2.0. The biochemical characterization included ammonia production (AP), nitrate reduction (NR), methyl red (MR), catalase (Cat), triple sugar iron (TSI), $H_2S$ production ($H_2S$), and oxidase (Oxi) performed in the presence of 5% NaCl (w/v) as previously described by Gohel and Singh (2018). To determine the hydrolytic properties of the actinobacteria, the solid medium containing 0.5% yeast extract, 0.5% bacteriological peptone, 10% NaCl, and pH 9 (adjusted using separately autoclaved 20% $Na_2CO_3$) was supplemented with various substrates, including 03% gelatin, 01% soluble starch, 03% casein, 02% cellulose powder, and 01% tributyrin for hydrolysis of gelatin (Gel), starch (St), casein (Cas), cellulose (Cell), and lipid (Lip), respectively. The inoculated plates were then incubated at 28 °C ± 2.0 for 4 days. Similarly, fermentation of sugars, arabinose (Ara), raffinose (Raf), lactose (Lac), galactose (Gal), mannose (Man), xylose (Xyl), sucrose (Suc), fructose (Fru), glucose (Glu) and maltose (Mal) were studied. Further, a phenogram based on the molecular diversity and phenotypic characteristics was created using the unweighted pair group method with arithmetic averages (UPGMA) using PAST 4 software. The halophilic nature of the actinobacteria was determined with starch agar supplemented with 0–20% NaCl (w/v) at pH 9 (adjusted using separately autoclaved 20% $Na_2CO_3$). Similarly, the alkaliphilic nature of the organisms was investigated in the pH 7–11, adjusted by the previously autoclaved 20% $Na_2CO_3$ (w/v). A Scatter plot of the Principal Component Analysis (PCA) was generated using PAST 4 software using the pH and NaCl concentrations as the principal components.

### Diversity indices and analysis of the phenogram

The diversity of the haloalkaliphilic actinobacteria was further analyzed statistically using Rv3.6.1package vegan v 2.5–6 (Hinsu et al. 2021; Travlos et al. 2018). Briefly, alpha diversity was calculated in terms of various diversity indices, including Pielous evenness index J, Species richness S, Simpson 1-D, Shannon H. While the beta diversity was analyzed by NMDS (Non-Matric Dimensional Scaling) on Bary-Curtis distance calculated by the Phyloseq package.

### DNA extraction and amplification of 16S rDNA

To extract the genomic DNA, the actinobacterial isolates were grown on YEME broth (Yeast Extract-Malt Extract medium). The procedure for DNA extraction was based on the method described by Sambrook (1989) with few modifications (Gohel and Singh 2018). Briefly, cell pellets were collected from the actively growing culture by centrifugation at 6708×*g* for 15 min. The pellets were then suspended in sodium chloride Tris EDTA buffer and re-centrifuged at

Table 1. PCR condition for amplification of specific gene.

| Primer | Number of cycles, initial denaturation | Denaturation | Annealing temperature | Extension and final extension |
|---|---|---|---|---|
| U1F 5′-RDTTGWCYGBCR-3′ U1R 5′-ARGRKGWCCVCGCA-3′ | 30 cycles, 94 °C for 10 min | 94 °C for 30s | 52.3, 55.3, and 59.4 °C for 30s | 72 °C for 1 min and 72 °C for 10 min |
| U2F 5′-CCVVCGCGKAWMG-3′ U2R 5′-WCGBMYTKTMGAYTC-3′ | 30 cycles, 94 °C for 10 min | 94 °C for 01 min | 52.3, 55.3, and 59.4 °C for 1 min | 72 °C for 1 min and 72 °C for 10 min |
| Strep B 5′MAVCYGGAAMGGGK-3′ StrepE 5′-CMCRGAWTCCGWY-3′ | 30 cycles, 94 °C for 8 min | 94 °C for 01 min | 50.7, 53.9, and 56.7 °C for 47s | 72 °C for 1 min and 72 °C for 10 min |
| Strep B 5′-MAVCYGGAAMGGGK-3′ StrepF 5′-MKKGCVCCARMA-3′ | 30 cycles, 95 °C for 8 min | 94 °C for 01 min | 54.1, 58.1, and 60.0 °C for 47s | 72 °C for 1 min and 72 °C for 10 min |
| NF 5′-CGCWRGKBGKGGAAR-3′ NR 5′-GRKCGGKTGCRHTCG-3′ | 30 cycles, 94 °C for 4 min | 94 °C for 30s | 53.3, 56.4 and 60.0 °C for 30s | 72 °C for 1 min and 72 °C for 10 min |

6708×*g*. After centrifugation, the cells were lysed by adding lysozyme solution (10 mg/ml prepared in Tris-Cl buffer pH 8) and 20% sodium dodecyl sulfate (w/v) for 2 h at 40 °C. It was then followed by the treatment of the Phenol: Chloroform: Isoamyl alcohol (25:24:1) and Chloroform: Isoamyl alcohol (24:1). The DNA was pooled by adding 3 M potassium acetate and chilled ethanol. The DNA was then suspended in an appropriate volume of molecular-grade water and preserved at −20 °C. The extracted genomic DNA was analyzed by spectrophotometer and agarose gel electrophoresis.

### Primers

The sequences of the 16S rDNA from a large number of isolates were amplified using two different universal primers U1 and U2, two *Streptomycetes* specific primers Strep B/E, Strep B/F, and one *Nesterenkonia* specific primer-N F/R (Table 1). The quality and amount of the extracted DNA were suitable for successful PCR amplification. Primer pair U1F/U1R (Edwards et al. 1989) and primer pair U2F/U2R (Lu et al. 2000) were used to amplify the actinobacterial 16S rDNA as a control. To ensure that the extracted DNA was of actinobacterial origin and suitable for successful PCR amplification, the *Streptomyces*-specific PCR primer Strep B (forward), StrepE (reverse), and StrepF (reverse) (Rintala et al. 2001; Suutari et al. 2002) were used for the amplification of the species-specific 16S rDNA fragments. The primer pairs StrepB/StrepE and StrepB/StrepF amplified 520 bp and 1070 bp gene fragments, with nucleotides position 139–657 and 139–1212, respectively (Pernodet et al. 1989). The *Nesterenkonia*-specific primer pair N F/R was used for the selective amplification of the 16S rDNA, corresponding to the region 194 − 215 and 1308 − 1328, respectively, with a 1120 bp fragment of the 16S rDNA (Zhi et al. 2008). The primers used in this study were synthesized by Sigma Aldrich USA.

### PCR amplification

The gradient PCR method was developed to specifically amplify 16S rDNA sequences from the nine actinobacterial strains using universal primers and genus-specific primers. The PCR amplification was done using a Gradient Master Cycler (Eppendorf, Germany). The conditions for PCR amplification using each primer set are described in Table 1.

The 16S rDNA amplification was confirmed and analyzed on 1.0% agarose gel with 5 µg/ml Et-Br and 6x gel loading buffer. The electrophoresis was carried out with 1x TAE buffer at 55 volts for 90 min, followed by observing the DNA bands under the Gel Doc System (Biorad, USA).

### Denaturing gradient gel electrophoresis

The denaturing gradient gel electrophoresis (DGGE) was carried out as described earlier (Gohel and Singh 2018). Briefly, amplified 16S rDNA region using specific primer pairs containing GC-clamp were separated using 08% (w/v) polyacrylamide gel in Tris-acetate-EDTA buffer (40 mM Tris, 20 mM acetate, 1 mM EDTA, pH 8.0). DGGE separates DNA sequences based on their denaturation ability achieved by 7 M urea and 40% formamide. Further, a dendrogram with Jaccard distance was constructed based on the DGGE band patterns using PAST 4 software.

## Results

### Isolation strategies and identification of haloalkaliphilic actinobacteria

The isolation strategies of the haloalkaliphilic actinobacteria from the Okha coastal region are described in Table 2. The isolated actinobacteria were gram-positive with high G + C content having a filamentous structure with tough leathery colonies (Figure 1). Among the nine isolated actinobacterial strains, OK-5, OK-6, and OK-9 produced pigments when grown on ISP-5 agar. Based on the nucleotide homology of the 16S rDNA and phylogenetic analysis, actinobacterial strains OK-1, OK-2, OK-3, and OK-7 belonged to the group I identified as species belonging to the genera *Streptomyces*. Among them, strains OK-1 and OK-2 were identified as *S. somaliensis,* whereas strains OK-7 and OK-3 shared similarities with *Streptomyces* sp. The actinobacterial strains OK-5, OK-6, OK-8, OK-9, and OK-10 belong to group II and were identified as *Nocardiopsis alba* species. Among them, strains OK-6, OK-8, OK-9, and OK-10 shared the same cluster, whereas strain OK-5 belonged to a different cluster (Figure 2). The details of the GenBank accession number and G + C content are described in Table 3.

**Table 2.** Isolation parameters and phenotypic features of the haloalkaliphilic actinomycetes grown on the ISP-5 agar medium.

| | Isolation parameters | | | | | Phenotypic characteristics | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Site description | Treatment | Dilution | pH | % NaCl (w/v) | Growth | Aerial mycelia | Reverse side | Pigmentation |
| OK-1 | Brownish Sandy soil | $CaCO_3$ treated | 100.00 | 09 | 00 | Moderate | Creamish | Creamish | – |
| OK-2 | Brownish Sandy soil | $CaCO_3$ treated | 100.00 | 09 | 00 | Poor | Creamish light bluish | Light ceramish | – |
| OK-3 | Brownish Sandy soil | $CaCO_3$ treated | 100.00 | 09 | 00 | Moderate | White | Light ceramish | – |
| OK-5 | Brownish Sandy soil | $CaCO_3$ treated | – | 09 | 05 | Moderate | Creamish white | Creamish | Light brown |
| OK-6 | Crystaline sand | without $CaCO_3$ | 100.00 | 09 | 05 | Good | Creamish white | Dark brown | Green |
| OK-7 | Crystaline sand | without $CaCO_3$ | 100.00 | 09 | 05 | Good | Dark gray | Dark green | – |
| OK-8 | Crystaline sand | $CaCO_3$ treated | 10000 | 09 | 05 | Poor | Creamish white | Light ceramish | – |
| OK-9 | Stone sample | $CaCO_3$ treated | – | 09 | 10 | Good | White | Creamish | Yellow |
| OK-10 | Stone sample | $CaCO_3$ treated | 10000 | 09 | 10 | Good | Creamish white | Creamish brown | – |

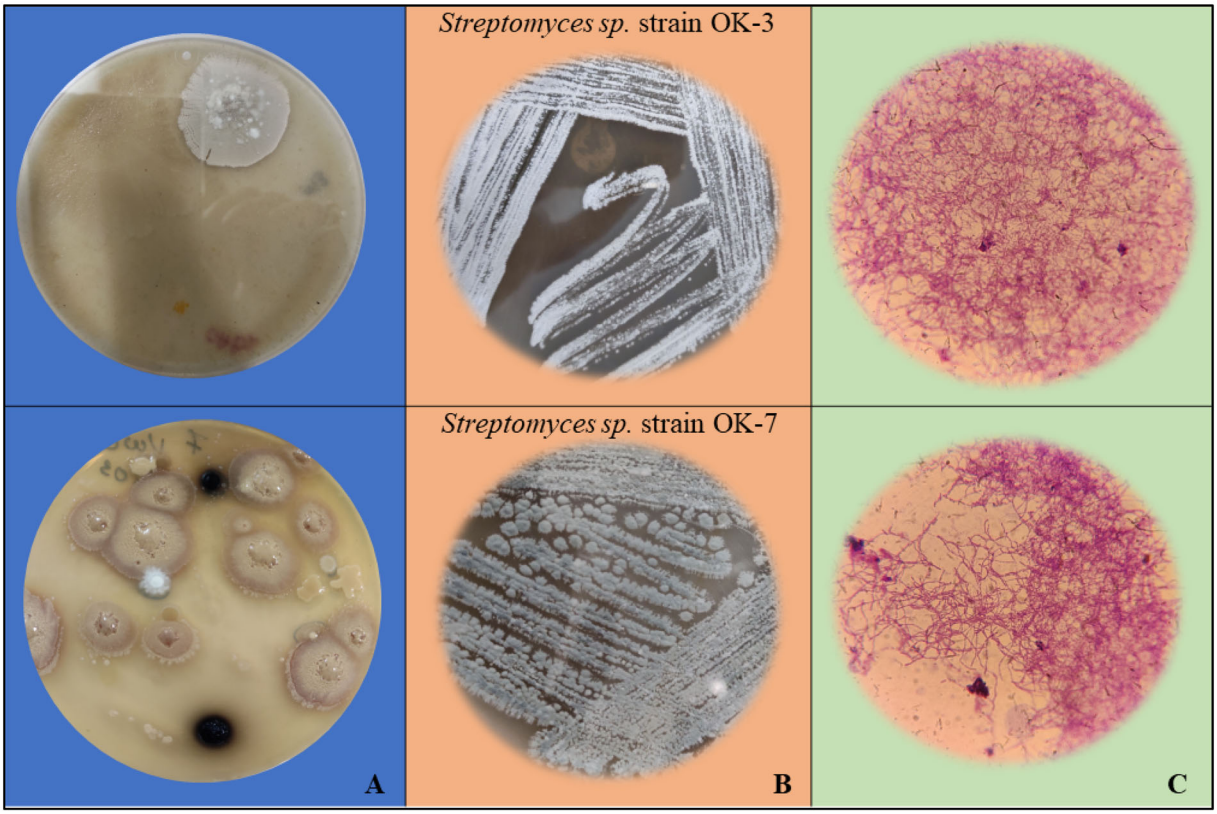


**Figure 1.** (A) Colonies of actinobacteria on the master plate. (B) Pure actinobacterial strains. (C) 100× Microscopic observation of the actinobacteria.

### *Morphological, cultural, and physiological characterization*

The morphological patterns of haloalkaliphilic actinobacteria were studied by growing them in actinomycetes-specific media. The colony size of each actinobacterial strain varied according to its ability to utilize organic and synthetic substances. In general, hard, chalky white, leathery, and pigmented colonies were observed. Most actinobacterial strains developed yellow, green, and brown pigmentation when grown on ISP-5 medium (Table 2), whereas grayish, bluish, whitish, and brownish aerial mycelial growth was observed on ISP-3 medium (Figure 1(A,B)). The microscopic observation of actinobacteria revealed filamentous growth of the vegetive hyphae and aerial mycelia (Figure 1(C)).

For further differentiation and characterization, the biochemical and metabolic activities of the isolates were studied. However, the extent of the result varied among the isolates. Besides, the maximum positive reactions were obtained for ammonia production (AP), nitrate reduction (NR), catalase (Cat), oxidase (Oxi), and triple sugar iron (TSI) fermentation (100%) followed by methyl red (MR) (66.66%) and $H_2S$ production (22.22%) as reflected in Figure 3. Among the nine cultures tested for hydrolytic activities, all displayed strong hydrolytic properties against gelatin (Gel), casein (Cas), starch (St), and cellulose (Cell). At the same time, only 33.33% of the isolates had moderate to weak action to hydrolyze lipids (Lip) (Figure 3). The sugar utilization ability of all nine actinobacterial strains was also distinct. The majority of the isolates fermented glucose (Glu), maltose (Mal), and lactose (Lac) (55.55%), followed by galactose (Gal) and mannitol (Man) (44.44%), fructose (Fru) (33.33%), sucrose (Suc) and xylose (Xyl) (22.22%), and raffinose (Raf) (11.11%), while none of the isolates utilized Arabinose (Ara) (Figure 3). The diversity of the isolates

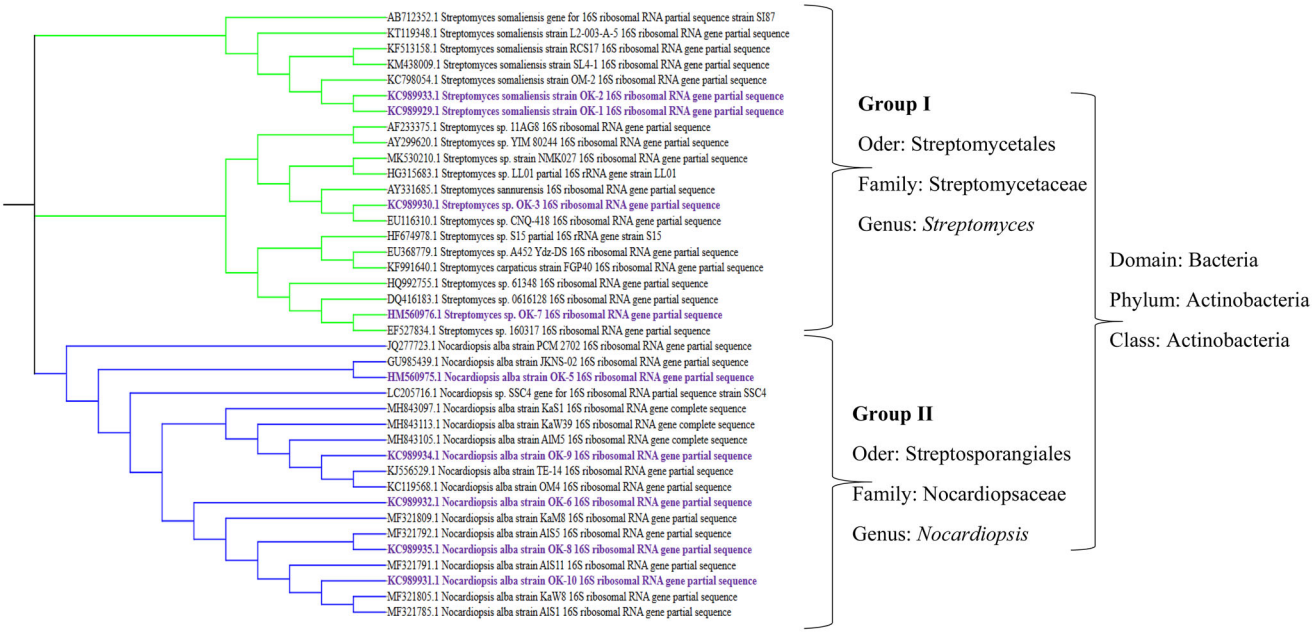


**Figure 2.** Phylogenetic tree constructed in MEGA 11 software using the Maximum Parsimony method.

**Table 3.** Details of the 16S rDNA sequences of the haloalkaliphilic actinobacteria.

| Actinobacterial Strain and Gen Bank Accession No. | Length | %G + C content | % Similarity and Gen Bank Accession No. |
|---|---|---|---|
| *Streptomyces somaliensis* strain OK-1 KC989929.1 | 1448 | 59.46 | 99.44 *Streptomyces somaliensis* strain OM-2 KC798054.1 |
| *Streptomyces somaliensis* strain OK-2 KC989933.1 | 1434 | 59.41 | 99.52 *Streptomyces somaliensis* strain OM-2 KC798054.1 |
| *Streptomyces sp.* strain OK-3 KC989930.1 | 1483 | 59.00 | 97.55 *Streptomyces sp.* CNQ-418 EU116310.1 |
| *Nocardiopsis alba strain* OK-5 HM560975.1 | 1506 | 58.69 | 100 *Nocardiopsis alba* strain JKNS-02 GU985439.1 |
| *Nocardiopsis alba* strain OK-6 KC989932.1 | 1469 | 58.61 | 99.93 *Nocardiopsis alba* strain KaM8 MF321809.1 |
| *Streptomyces sp.* strain OK-7 HM560976.1 | 1405 | 59.00 | 100 *Streptomyces sp.* 160317 EF527834.1 |
| *Nocardiopsis alba* strain OK-8 KC989935.1 | 1464 | 58.53 | 99.79 *Nocardiopsis alba* strain KaM8 MF321809.1 |
| *Nocardiopsis alba* strain OK-9 KC989934.1 | 1462 | 58.82 | 100 *Nocardiopsis alba* strain AIM5 MH843105.1 |
| *Nocardiopsis alba* strain OK-10 KC989931.1 | 1461 | 58.59 | 99.79 *Nocardiopsis alba* strain AIS11 MF321791.1 |

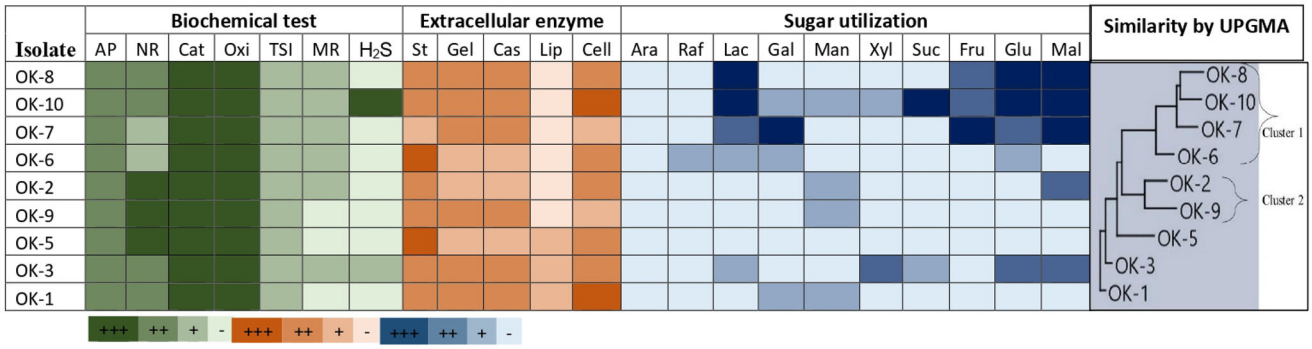


**Figure 3.** Heatmap of the biochemical characteristics, production of the extracellular enzymes, and sugar fermentation along with the phylogram constructed by PAST 4 software.

based on their sugar utilization was variable, suggesting its significance in the survival strategies of the actinobacteria of different marine origins.

### Ecological diversity indices and analysis of the phenogram

The phenogram is a diagrammatic representation of the taxonomic relationships of the actinobacteria based on the overall similarity of many non-genetical characteristics. Based on the physiochemical characteristics, the phylogram was constructed using PAST4 software. Among the generated cluster, the actinobacterial strains; OK-6, OK-7, OK-8, and OK-10 belonged to cluster one and thus shared the closest similarity. While actinobacterial strains; OK-2 and OK-9 belonged to cluster two, the strains; OK-5, OK-3, and OK-1 displayed no similarity with each other (Figure 3). The physicochemical characteristics would help to explore diversity among marine actinobacterial species.

The diversity index is a mathematical measure of the species diversity in a particular community. It provides information about the community structure and composition rather than just the species richness (Sharma et al. 2021). The alpha diversity in terms of Pielous evenness index (J), Species richness (S), Simpson (1-D), and Shannon (H) were calculated. The evenness of the microbial community is numerically measured by the Pielous evenness index. Evenness is the count of individual species with different metabolic abilities, such as biochemical traits, sugar utilization, and enzyme production. The calculated Pielous evenness values in the present study range from 0 to 1. A value near 0 means no evenness, and close to 1 means increasingly greater evenness (Travlos et al. 2018). Based on the Pielous evenness values, the maximum evenness was observed for the biochemical characteristics followed by the enzyme secretion. While the differences among the Pielous evenness index were mainly observed with respect to the sugar utilization by the actinobacteria. The higher species richness was mainly observed with TSI, oxidase, catalase, and cellulose hydrolyzing ability, as shown in Figure 4(B).

The Shannon Diversity Index measures actinobacterial diversity based on their metabolic potentials. The values of the Shannon diversity Index near zero indicate a similar metabolic potential of the species, whereas a higher value suggests species diversity (Travlos et al. 2018). The higher Shannon index of the above two was observed in the present study with respect to the biochemical traits; TSI and oxidase, as well as hydrolytic enzyme secretion, mainly cellulase, and caseinase. The patterns indicate the diverse metabolic potential of the isolated actinobacteria (Figure 4(C)). The Simpson's diversity index (1-D), ranging from 0 to 1, describes the similarity probability between two randomly selected species. The values near 1 suggest higher diversity. In this study, a higher Simpson diversity index (1-D) was observed with biochemical tests and extracellular enzyme production. Whereas a diverse Simpson diversity index (1-D) was evident for sugar utilization by the actinobacterial species (Figure 4(D)). Among the calculated diversity indices, significant differences were observed in the sugar utilization ability of actinobacterial species.

The distance similarity among the actinobacterial species, NMDS (Non-metric Dimensional Scaling) using Bary-Curties dissimilarity was calculated based on the biochemical tests and hydrolytic properties of the isolates. Based on the positive results of the biochemical characteristics, sugar utilization, and enzyme hydrolysis, the actinobacterial strains; OK-6, OK-7, OK-8, and OK-10 were found to be closely related, whereas distinct clusters were observed for the strains OK-2 and OK-9 and strains OK-1 and OK-3 (NMDS stress value = 0.1236) (Figure 5(A,B)). The raffinose lies on −1 and xylose and lipid hydrolysis on +1 on the X-axis, indicating the diverse metabolic potential of actinobacteria based on characteristic data (Figure 5(A)). Similarly, the NMDS plot was created using cumulative characteristic data (test). The results show the highest variation for using sugars, followed by extracellular enzymes and biochemical tests (Figure 5(B)).

### Effect of pH and NaCl concentrations on the growth of the actinobacteria

Physico-chemical parameters, temperature, pH, and salt are considered the most significant limiting factors in species distribution and ecological function in marine environments. Therefore, NaCl tolerance of the actinobacterial strains was examined at 0–20% NaCl (w/v). Among the studied isolates, the actinobacterial strains OK-3, OK-5, and OK-6 could grow on starch agar supplemented with 15% NaCl (w/v) while none of the actinobacterial strains could grow in 20% NaCl (w/v). Moreover, the isolated actinobacteria grew optimally in 5% and 10% NaCl (w/v) (Figure 6(A)). Similarly, a pH range of 7–12 was used to assess the effect of pH. All the strains except OK-1 and OK-2 grew well in starch broth with 5% NaCl (w/v) at pH 11, while OK-1 and OK-2 grew up to pH 10 (Figure 6(A)). Further, based on the optimum pH and NaCl (w/v) for the growth of the marine actinobacteria, a scatter plot of the Principal Component Analysis (PCA) was constructed using PAST4 Software (Figure 6(A)). The actinobacterial strains OK-2, OK-9, OK-8, and OK-10 were closely located, indicating their similarity in terms of their optimum pH and NaCl concentration. Whereas OK-7 and OK-6 are distantly located from each other, suggesting their distant relationship.

### DNA extraction and 16S rDNA amplification with the universal and specific primers (U1, U2, Step B/F, StepB/E, NF/R)

The spectrophotometric analysis demonstrated the purity of the extracted DNA based on the ratio of the $A_{260}/A_{280}$ between $1.4-1.8$. Among the nine actinobacterial strains, 16S rDNA of 5 isolates (55.55%) of the Okha study site were amplified with the universal

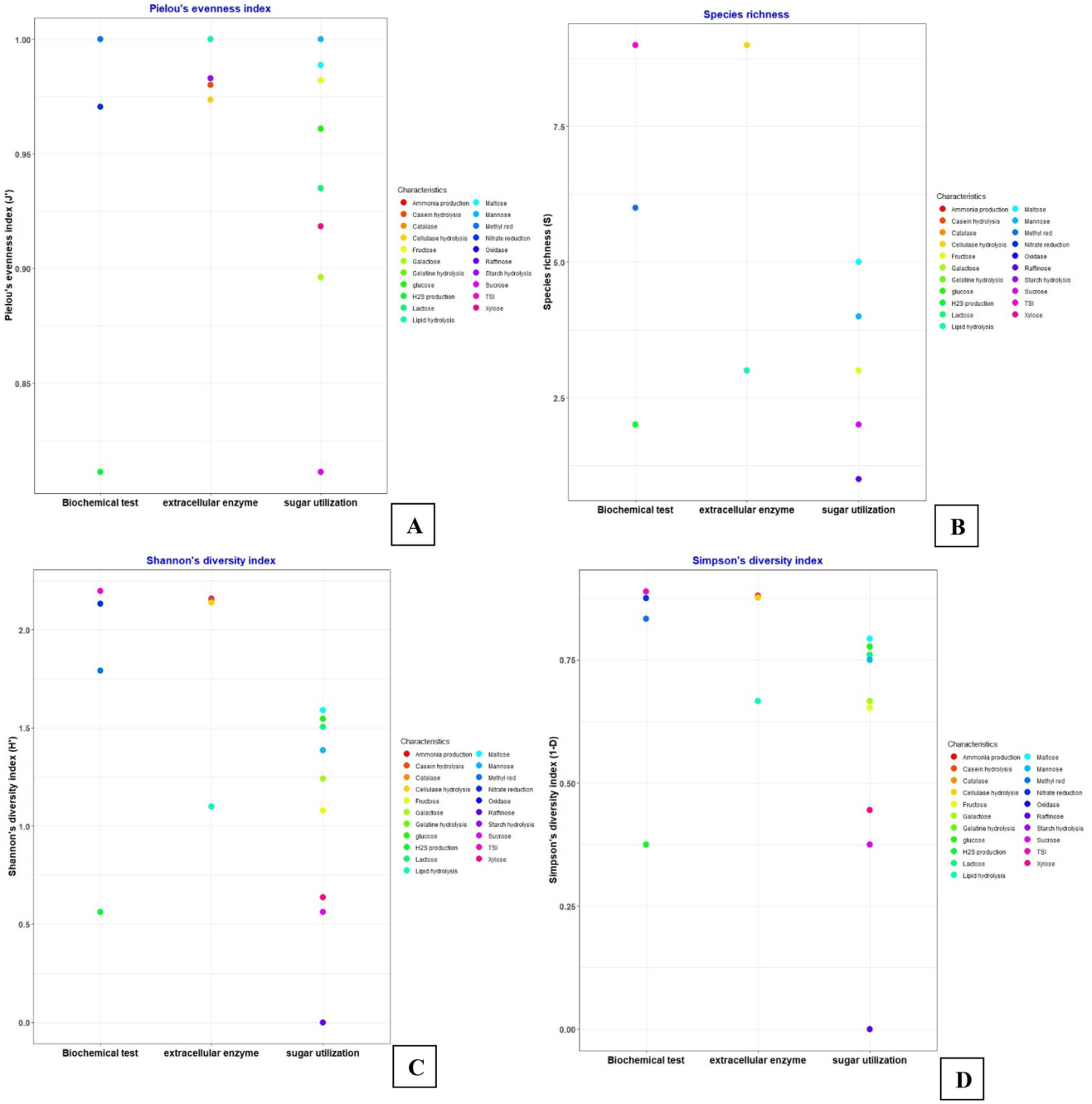


**Figure 4.** Alpha diversity indices of the actinobacteria isolated from the Okha Coast, Gujarat, India. (A) Pielous evenness index J. (B) Species richness S. (C) Simpson 1-D. (D) Shannon H.

primer, U1. The amplified 16S rDNA were of 1500 bp length. However, the actinobacterial strains OK-1, OK-5, OK-6, and OK-7 were not amplified with U1 primer. Similarly, OK-1 and OK-5 were not amplified with U2 primer. Annealing temperatures (Ta) play a significant role in gene amplification. Therefore, three gradients of the annealing temperature: 53.3 °C, 56.3 °C, and 59.3 °C, were used given the melting temperature of U1 primer (Figure 7(A,F)), while 52.7 °C, 55.9 °C, and 59.2 °C were used for U2 primer (Figure 7(B,F)). Genus-specific primer sets were also used to amplify the 16S rDNA. The *Streptomyces*-specific primer, StrepB/E amplified the 16S rDNA of actinobacteria at 50.7 °C, 53.9 °C, 56.7 °C Ta (Figure 7(C)), whereas StrepB/F primer amplified 16S rDNA of actinobacteria at 54.1 °C, 58.1 °C, 60.0 °C Ta (Figure 7(D)). Based on the agarose gel electrophoresis, a 520 bp band of StrepB/E amplified product was obtained, whereas an 1170 bp-sized band was obtained with StrepB/F primer. Among the strains, 04 isolates; OK-1, OK-2, OK-3, and OK-8 were amplified with StrepB/E while OK-1, OK-2, OK-3, OK-8, and OK-9 were amplified with StrepB/F primer (Figure 7(C,D,F)). With the *Nesterenkonia*-Specific primer N-F/R, an 1120 bp gene product was amplified at 53.3 °C, 56.4 °C, and 60.0 °C Ta. The 16S rDNA of actinobacterial strains OK-1 and OK-8 were amplified using N-F/R primer.

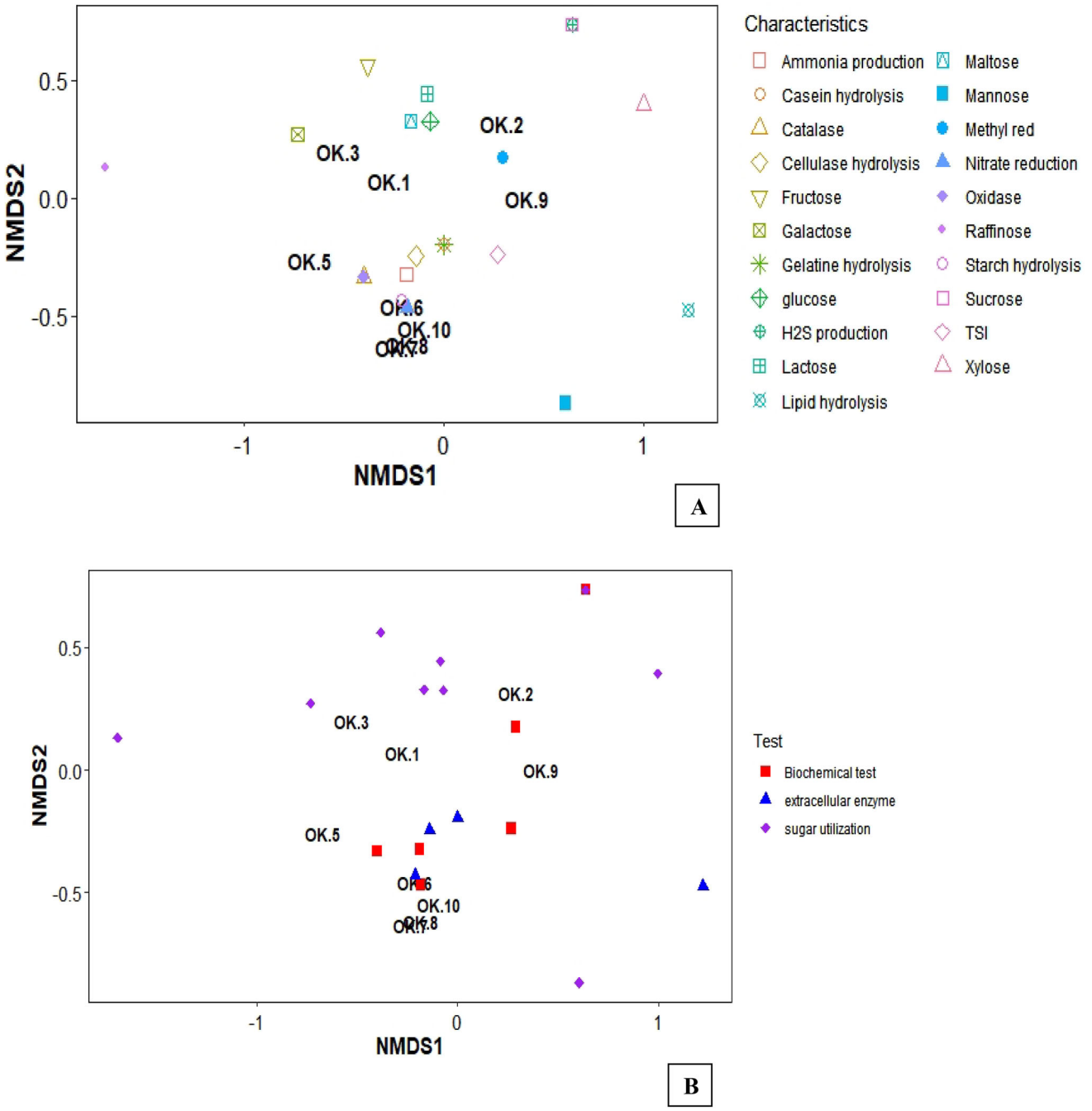


**Figure 5.** NMDS plot of Bray–Curtis distance calculated on the basis of the metabolic potential of the actinobacteria with NMDS stress = 0.1236, (A) Individual test results derived on the basis of biochemical characteristics, enzyme hydrolysis profile, and sugar utilization traits of the actinobacteria. (B) Overall results of the three clusters involving biochemical test, enzyme hydrolysis profile, and sugar utilization traits of the actinobacteria.

However, the concentrations of the amplified product were poor in OK-8 (Figure 7(E,F)). The amplification profile of the 16S rDNA using all five primer sets showed maximum amplification with U2 primer (77.77%) followed by StrepB/F (55.55%), U1 (55.55%), StrepB/E (33.33%), and NF/R (22.22%). Based on 16S rDNA amplification using five different primer sets, a dendrogram was constructed using the UPGMA method. Two different clusters were formed in which the actinobacterial strains OK-1, OK-5, OK-6, OK-7, and OK-10 shared the same cluster, whereas OK-2, OK-3, OK-8, and OK-9 formed another cluster (Figure 7(F)).

### *Analysis of the DGGE patterns with universal (U1, U2) and species specific (Strep B/E, Strep B/F, NF/R) primers*

The sensitivity of the DGGE to resolve 16S rDNA fragments with different sequences was compared. The distinct DGGE patterns of U1 and U2 primers were observed, as revealed in Figure 8(A). However, two dominating bands were observed in the majority of the isolates, suggesting that each isolate belongs to the phylum Actinobacteria and variation among the groups at the genus and species level. Interestingly, a similar DGGE band pattern was evident for the isolates amplified with StrepB/E primer suggesting

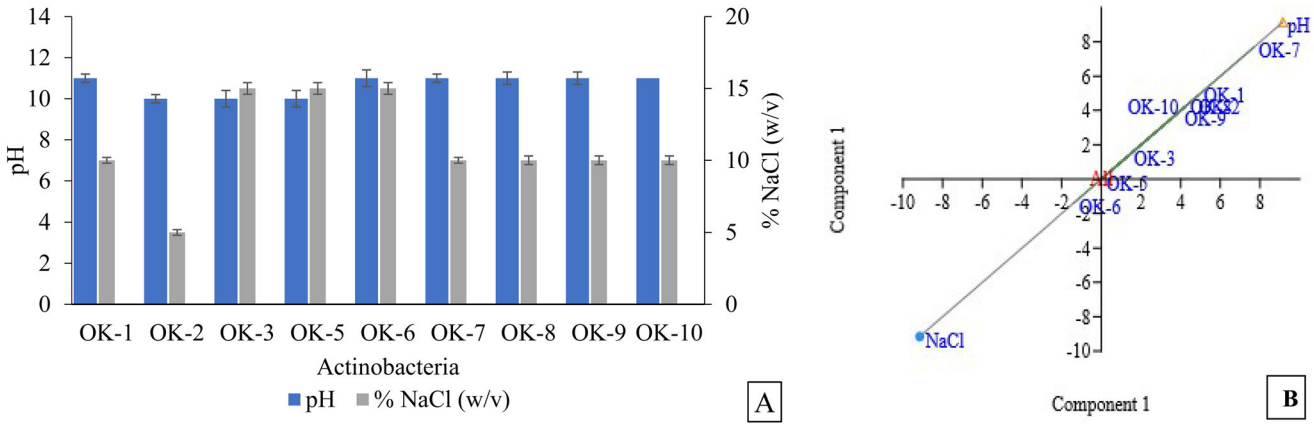


**Figure 6.** (A) Salt profiling of the isolates on a starch agar plate supplemented with 0–20% NaCl (w/v) and pH profile of the isolates on a starch agar plate at 7–11 pH. (B) Assessment of the NaCl and pH optima of the actinobacteria through the Principal Component Analysis using PAST 4 software.

species of common genera *Streptomyces*. However, a distinct band pattern for the strains; OK-1, OK-2, OK-3, and OK-8 amplified with StrepB/F primer indicated different actinobacterial species (Figure 8(B)). Similarly, the DGGE profile of the isolates amplified with *Nesternkonia*- specific primer NF/R, generated a similar band pattern for OK-1 and OK-8, indicating their affiliation with the common genus (Figure 8(C)). Further, three clusters were obtained based on the UPGMA clustering analysis of the Jaccard distance coefficient (Figure 8(D)). The diversity based on DGGE band patterns using In-Silico cluster analysis suggested the highest similarity among the strains OK-1, OK-5, OK-6, and OK-7. Similarly, OK-8, OK-9, and OK-10 shared a similar group, while OK-2 and OK-3 belonged to another distinct group.

## Discussion

Marine habitats are the untapped sources for harnessing new and diverse microorganisms of pharmaceutical and biotechnological significance. Among the microbial communities, actinobacteria are a potential source for producing secondary metabolites, various enzymes, and novel bioactive compounds (Majithiya and Gohel 2022b). Adaptations of actinobacteria and other microorganisms to extreme and nutrient scare environments provoke the production of diverse metabolites and enzymes for survival. To interpret their adaptation strategies in discrete habitats of extreme nature, the phenotypic, genotypic, and molecular characteristics of the microorganisms play a significant role (Kikani et al. 2023; Kikani and Singh 2022; Majithiya and Gohel 2022c; Rathore et al. 2021). In the present study, physio-chemical attributes of the saline habitats and molecular approaches were investigated to explore the diversity of the actinobacteria in the Okha Coast, Gujarat, India.

The biochemical attributes, catalase, oxidase, and nitrate reduction, were strictly positive in all actinobacterial strains. A positive nitrate reduction has been earlier reported for different actinobacteria, including *Streptomyces, Actinomarinicola,* and *Nocardiopsis* (He et al. 2020; Malviya et al. 2014; Qiao et al. 2012). However, in certain rare and novel actinobacterial species, for instance, *Nocardiopsis ansamitocini* sp. nov., nitrate reduction was found negative (Zhang YG et al. 2016). Further, the generation of the hydrogen sulfide was also negative in most of the species of the genera *Nocardiopsis* and *Streptomyces* (Li WJ et al. 2006; Yoon et al. 2006). While in the present study, the actinobacterial strain OK-10 belonging to the genera *Nocardiopsis*was positive for hydrogen sulfide and hence can be considered different from other *Nocardiopsis* sp. The ammonia production by the actinobacterial species of Okha corroborated an earlier finding (Yadav et al. 2018). Previously, it has been reported that microorganisms form a unique association with marine flora and fauna. The associated microorganisms secret signaling molecules, growth-promoting substances, quorum-sensing chemicals, and other effective molecules which play a crucial role in various developmental stages of seaweed, including the promotion of spore settlement, spore germination, and correct morphogenesis in adults (Singh and Reddy 2014). Similarly, actinobacteria associated with lower sessile animals protects marine host by secretion of bioactive compounds (Majithiya and Gohel 2022a, 2022b). Nitrate reduction and ammonia production are significant parameters for enhancing plant growth (Chauhan and Gohel 2022; Vaghela and Gohel 2023). In the present study, actinobacterial strains OK-1, OK-2, OK-3, OK-5, OK-8, OK-9, and OK-10 were positive for ammonia production and nitrate reduction, indicating the potential and significance of these actinobacterial strains in plant growth promotion.

The trends of the production of the hydrolytic enzymes in marine actinobacteria in this study were in parallel with some earlier reports on *Streptomyces* and *Nocardiopsis* (Dastager et al. 2006; Gohel and Singh 2018; Yadav et al. 2018). The proteases of *N. alba* from marine habitats are thermostable, salt-tolerant, and alkaliphilic in nature (Chauhan et al. 2021; Gohel and Singh 2012; Sharma et al. 2020; Thakrar et al., 2023; Thakrar and Singh, 2019). More recently, the Co-production of amylase and protease in marine *S. lopnurensis* KaM5 has been reported under haloalkaliphilic conditions (Rathore and Singh 2021). *Streptomyces* sp. Al-Dhabi-49 isolated from Saudi Arabian soil produced lipase and protease with enhanced activities in the presence of magnesium and calcium ions, respectively (Al-Dhabi, Esmail, Ghilan, Arasu 2019). With respect to the present study, the marine actinobacterial strains produced amylase,

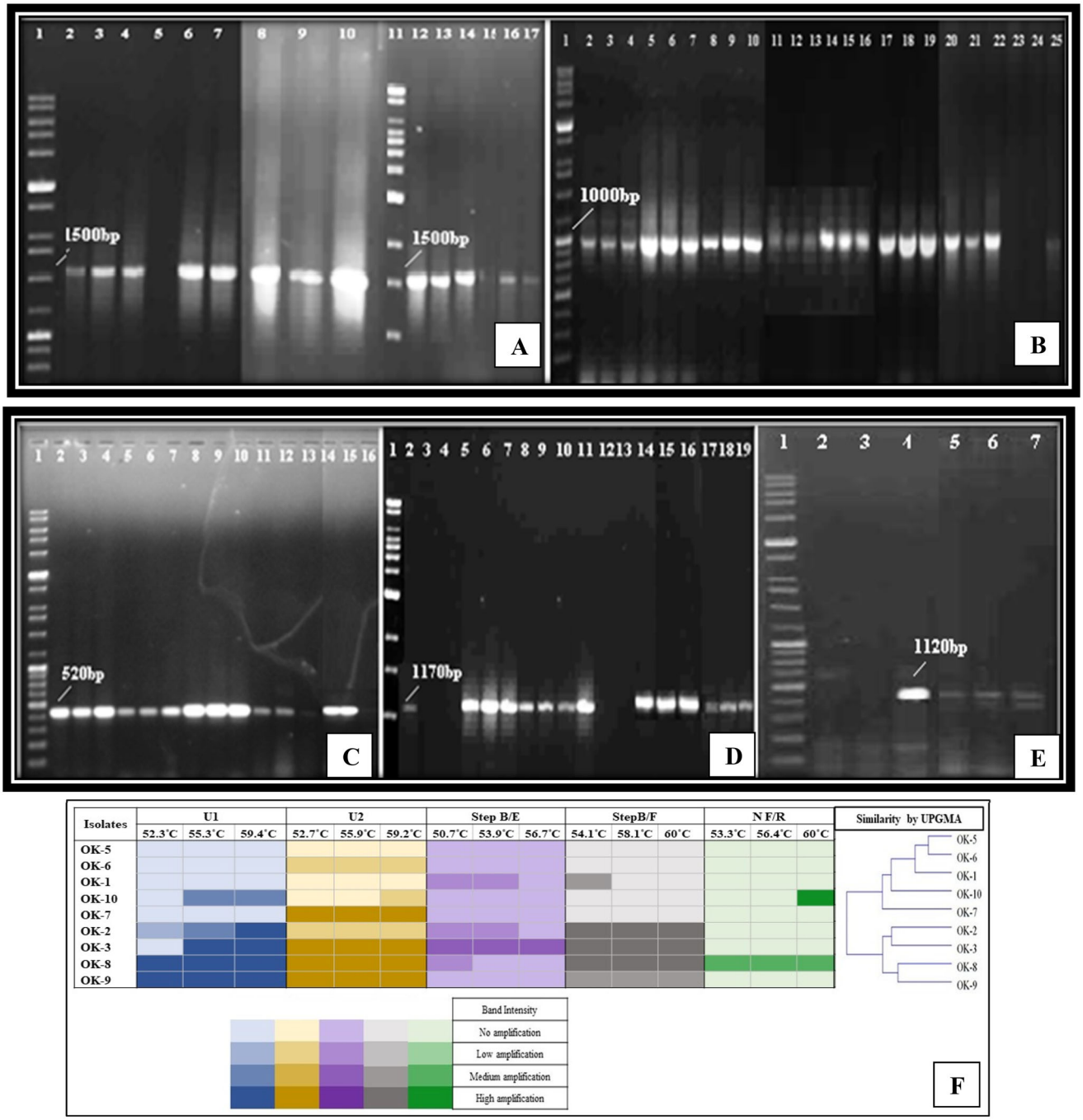


**Figure 7.** (A) U1 primer: lane-1 Medium range DNA ruler, lane-2 OK-2 (52.3 °C), lane-3 OK-2 (55.3 °C), lane-4 OK-2 (59.4 °C), lane-5 OK-3 (52.3 °C), lane-6 OK-3 (55.3 °C), lane-7 OK-3 (59.4 °C) lane- 8 OK-8 (52.3 °C), lane-9 OK-8 (55.3 °C), lane-10 OK-8 (59.4 °C), lane-11 Super Mix DNA ladder, lane-12 OK-9 (52.3 °C), lane-13 OK-9 (55.3 °C), lane-14 OK-9 (59.4 °C), lane-15 OK-10 (52.3 °C), lane-16 OK-10 (55.3 °C), lane-17 OK-10 (59.4 °C). (B) U2 primer: lane-1 High range DNA ruler, lane-2 OK-2 (52.7 °C), lane-3 OK-2 (55.9 °C), lane-4 OK-2 (59.2 °C), lane-5 OK-3 (52.3 °C), lane-6 OK-3 (55.9 °C), lane-7 OK-3 (59.2 °C), lane-8 OK-5(52.3 °C), lane-9 OK-5 (55.9 °C), lane-10 OK-5 (59.2 °C), lane-11 OK-6(52.3 °C), lane-12 OK-6(55.9 °C), lane-13 OK-6 (59.2 °C), lane-14 OK-7(52.3 °C), lane-15 OK-7 (55.9 °C), lane-16 OK-7 (59.2 °C), lane-17 OK-8 (52.3 °C), lane-18 OK-8(55.9 °C), lane-19 OK-8 (59.2 °C), lane-20 OK-9 (52.3 °C), lane-21 OK-9 (55.9 °C), lane-22 OK-9 (59.2 °C), lane-23 OK-10 (52.3 °C), lane-24 OK-10 (55.9 °C), lane-25 OK-10 (59.2 °C). (C) StrepB/E: lane-1 High range marker (10 kb), lane-2 OK-1 (50.7 °C), lane-3 OK-1(53.9 °C), lane-4 OK-1 (56.7 °C), lane-5 OK-2 (50.7 °C), lane-6 OK-2 (53.9 °C), lane-7 OK-2 (56.7 °C), lane-8 OK-3 (50.7 °C), lane-9 OK-3 (53.9 °C), lane-10 OK-3 (56.7 °C), lane-14 OK-8 (50.7 °C), lane-15 OK-8 (53.9 °C), lane-16 OK-8 (56.7 °C). (D) StrepB/F: lane-1 Super mix DNA ladder, lane-2 OK-1 (54.1 °C), lane-3 OK-1 (58.1 °C), lane-4 OK-1 (60.0 °C), lane-5 OK-2 (54.1 °C), lane-6 OK-2 (58.1 °C), lane-7 OK-2 (60.0 °C), lane-8 OK-3 (54.1 °C), lane-9 OK-3 (58.1 °C), lane-10 OK-3 (60.0 °C), lane-14 OK-8 (54.1 °C), lane-15 OK-8 (58.1 °C), lane-16 OK-8 (60.0 °C), lane-17 OK-9 (54.1 °C), lane-18 OK-9 (58.1 °C), lane-19 OK-9 (60.0 °C). (E) N-F/Rprimer: lane-1 high range marker (10 kb), lane-2 OK-1 (53.3 °C), lane-3 OK-1 (56.4 °C), lane-4 OK-1 (60.0 °C), lane-5 OK-8 (54.1 °C), lane-6 OK-8 (58.1 °C), lane-7 OK-8 (60.0 °C). (F) Heat map of PCR amplification conditions and expected base pair amplification of five different primer sets along with phylogram constructed using PAST4 software.

protease, and cellulase, while only a few produced lipase along with protease, amylase, and cellulase. The results, therefore, reflect the potential of these actinobacteria for multi-enzyme production.

The pattern of the sugar fermentation reflected vast differences among the marine actinobacterial. As per an earlier study, most *Streptomyces* sp. utilized maltose and fructose (Silva et al. 2016). Similarly, most *Nocardiopsis* utilized

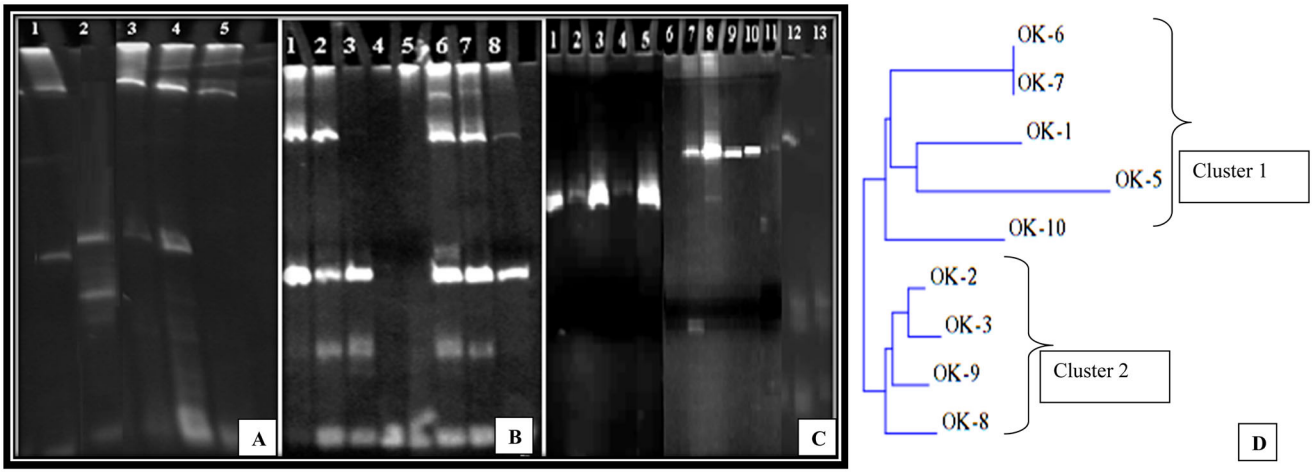


**Figure 8.** DGGE patterns of the 16S rRNA-amplified products with (A) Universal primer U1: lane-1 OK-2, lane-2 OK-3, lane-3 OK-8, lane-4 OK-9, lane-5 OK-10. (B) Universal primer (U2): lane-1 OK-2, lane-2 OK-3, lane-4 OK-6, lane-5 OK-7, lane-6 OK-8 lane-7 OK-9, lane-8 OK-10. (C) *Streptomyces* specific primer (StrepB/StrepE): lane-1 OK-1, lane-2 OK-2, lane-3 OK-3, lane-5 OK-8, *Streptomyces* specific primer (StrepB/StrepF): lane 6 OK-1 lane 7 OK-2, lane 8 OK-3, lane 10 OK-8, lane 11 OK-9, *Nesterenkonia* specific primer: lane-12 OK-8, lane-13 OK-10. (D) Dendrogram constructed on the basis of the multi-band DGGE using 16S rDNA gene-specific primers through Jaccard similarity.

sucrose, glucose, maltose, and lactose. In contrast, raffinose and xylose were utilized by only a few *Nocardiopsis*and *Streptomyces* species (Li X et al. 2021; Risdian et al. 2021). Similarly, among the isolated *Nocardiopsis alba* species, strain OK-6 utilized raffinose, and strain OK-10 utilized xylose. For instance, *N. alba* DSM 4337$^{T}$ utilized galactose, fructose, mannose, mannitol, and xylose (Yassin et al. 1997). Consequently, utilizing a wide variety of disaccharides compared to monosaccharides indicates the adaptation of actinobacteria in different environmental conditions for energy generation using different metabolic pathways. Therefore, according to the morphological and physiological characteristics, most actinobacterial strains appeared as *Streptomyces* and *Nocardiopsis*. However, the genotypic characteristics, as illustrated further in the discussion, would provide more profound insight.

For most salt-loving bacterial communities, salt is an essential requirement for their growth. For instance, the marine actinobacteria, *Salinospora* is an obligate halophile (Kim et al. 2020; Tuttle et al. 2022;; Vaghela and Gohel 2023). Similarly, *Nesterenkoniana tronophila* sp. nov isolated from a marine saltern grew at 10–38 °C, pH 7.5–12.0, and in the presence of 1–12 % NaCl (w/v), with its optimum growth at pH 10, 5 % NaCl (w/v) and 30 °C (Machin et al. 2019). A corresponding growth pattern was reported for *Brachystreptospora xinjiangensis* OM-6 (Gohel and Singh 2013). In the present study, all actinobacterial strains displayed haloalkaliphilic nature with their ability to grow at 5–10% NaCl (w/v) and pH 9–11. Recently, *S. tirandamycinius* sp. isolated from a marine sponge in China was able to survive at a pH range of 6–12 (optimum pH 8) and 0–7% NaCl, optimally at 3% NaCl (w/v) (Huang et al. 2019). The production of thermally stable proteolytic enzymes with the ability to catalyze under a wide range of pH, salt concentrations, and organic solvents is an essential feature of the haloalkaliphilic bacteria (Bhatt and Singh 2020; Chauhan et al. 2021; Purohit et al. 2022; Sharma et al. 2020; Thakrar and Singh 2019). Recently, a thermostable protease from a marine *Nocardiopsis alba* OM 5 was reported to possess stability at 70 °C with 4 M NaCl (Chauhan et al. 2021). In the present study, the actinobacterial strains OK-3, OK-5, and OK-6 could grow in the presence of 15% NaCl (w/v) in an alkaline starch medium. They thus can be considered as a potential source for haloalkaliphilic hydrolytic enzymes.

Molecular approaches have emerged as highly effective tools to characterize bacterial populations in a given habitat. It primarily includes 16S rDNA sequences and genomic fingerprinting techniques. The genomic fingerprinting techniques provide valuable tools for characterizing complex microbial communities in the environment. In this study, molecular methods, such as the 16S rDNA amplification using universal and species-specific primers in combination with DGGE were used as a fingerprinting technique along with the cultural and physiological characters of the salt-tolerant alkaliphilic actinobacteria to evaluate diversity.

The PCR amplification using universal and species-specific primers provides information regarding the 16S rDNA sequences. Therefore, the 16S rDNA amplification profile of the actinobacterial isolates was generated using five different primers that included U1 and U2, the universal primers, and genus-specific primers. The patterns of the 16S rDNA amplification profile generated by the universal primers suggest that the PCR-based assay described in this study is a reliable tool for the detection and diversification of marine actinobacteria. This is particularly important since recent work indicates considerable diversity and phylogenetic relatedness within the salt-tolerant alkaliphilic actinobacteria. The identification of *Streptomyces* sp. from Mount Semeru using a U1 primer to amplify the 1529 bp 16S rDNA has been reported (Juniastuti et al. 2016). Whereas, the 16S rDNA in *Nocardiopsis valliformis* sp. nov., an inhabitant of alkaline habitat, was amplified by the U1 primer at 52 °C Ta (Yang et al. 2008). Analysis of the bacterial diversity and cloning of the 16S rDNA using U1 primer from colon mucosa and stool sample from immunodeficiency virus-infected macaques demonstrated the dominance of

Helicobacteraceae and Ruminococcaceae, respectively (Zevin et al. 2017). Similarly, in the current study, the 16S rDNA in most actinobacterial strains were amplified by U1 primer at 59.4 °C annealing temperature while 88.88% of the actinobacterial 16S rDNA were amplified with universal primer U2. The finding was in accordance with the diversity trends reflected in *Bacillus* sp. based on 16S rDNA amplification using BOX, ERIC, U2, and GTG5 primers to study the genomic differences among the species (Tolieng et al. 2018).

Further, xylanase and amylase-producing alkaliphilic and thermophilic *Bacillus* sp. KH-13 isolated from the Caspian Sea, Iran Coast sediment demonstrated amplification of 1468 bp gene by U2 primer at 55 °C Ta (Khodayari et al. 2014). The identification and amplification of the 16S rDNA of 400 soil isolates from the campus perimeter of Kyonggi University, Suwon, South Korea were carried out using 27 F/1492R and U2F/800R universal primers. The identification revealed that most species belonged to Actinobacteria, Chlorobi, Nitrospirae, Fusobacteria, Acidobacteria, and Proteobacteria (Chaudhary et al. 2019). Further, *Nesternkonia jeotgali* nov. had 16S rDNA amplification using the universal primers U1 and U2 (Yoon et al. 2006). Whereas 1494 bp of 16S rDNA sequence was obtained from *Nocardia* and *Streptomyces* sp. using a universal primer set (Rashidian and Goodfellow 2003; Roth et al. 2003). The diversity analysis of the actinobacteria using species-specific primers included two *Streptomycetes*-specific and one *Nesterenkonia*-specific primer sets in the present study. About 55% of the isolates demonstrated 16S rDNA amplification with *Streptomycetes* specific primers, StepB/E and StepB/F, at the annealing temperatures of 53.9 and 54.1 °C, respectively. In an earlier study with *Streptomyces* sp. strain MAR01, the 16S rDNA was amplified using Step B/F primer at 53 °C Ta (El-Naggar et al. 2006). Similarly, 16S rDNA of *Strepomyces vilaceusniger*s train HAL64 was amplified using StrepB/F primer (El-Naggar 2007). In *Streptomyces longisporuber,* a 426 bp 16S rDNA sequence was amplified using StepF/R at 53 °C Ta for 60 sec (Yassien et al. 2014). In yet another instance, the *Streptomyces rimosus* 16S rDNA was amplified with 1.5 kbps product by StrepF/R primer at 53 °C Ta (Atta et al. 2011). A draft genome sequence of *Streptomyces avermitilis* strain SA51 possessing the plant growth-promoting traits was reported earlier. In this organism, the 16S rDNA was amplified by the StepB/E primer at 55 °C Ta for 60 sec (Vurukonda et al. 2020). In the microbiota of *Sarcoptes scabiei* namely *Streptomyces species,* the 16S rDNA was amplified by the genus-specific primers; StepB/F and StepE/R leading to a 519 bp product, while the primer set StepF/R yielded a 1079 bp product (Swe et al. 2019). Earlier, 16S rDNA from 54 actinobacterial strains were amplified using *Streptomyces*-specific primer Step B/F followed by the restriction digestion by *Bst*YI. All actinobacterial strains were identified to belong to *Streptomyces* except three strains that showed false positive amplification with the StepB/F primer (Rintala et al. 2001). Therefore, optimizing the PCR conditions can prevent interference and, thus the generation of pseudo-positive results. The *Nesterenkonia*- specific primer with the ability to amplify 1120 bp specific DNA fragment was helpful in the identification of novel *Nesterenkonia jeotgali* sp. isolated from Korean traditional fermented food (Yoon et al. 2006). In a similar context, *Nesterenkonia* sp. was identified based on the amplification of the 16S rDNA using NF/R primer with a 1120 bp PCR product (Zhi et al. 2008). In the present study of the manuscript, 22% of the isolates could amplify the 16S rDNA using a *Nesterenkonia*-specific primer.

The 16S rDNA based DGGE analysis is important to study the microbial diversity at the species level of poorly studied matrices. The present study incorporates DGGE fingerprinting analysis to project diversity among the actinobacteria strains based on band patterns. The cluster analysis helped elucidate the alpha diversity (Majed et al. 2018). The sequence heterogeneity of the 16S rDNA using universal and species-specific primers was evaluated by the DGGE patterns. The presence of two common bands in the strains indicated their common belongingness to the actinobacteria. Recently, culture-independent and culture-dependent bacterial diversity was reported based on the PCR-DGGE approaches that included a detailed analysis of the microbial biota that emerged from the Next Generation Sequencing and DGGE techniques (Bruni et al. 2022). The sequencing of the DGGE bands compared with the NGS sequencing revealed the identification of *Oceanobacillus* sp., *Bacillus borbori,* and *Streptococcus* sp. (Bruni et al. 2022).

An insect microbiota was reported using metagenomics and PCR-DGGE approaches. Mainly, *Alloiococcus, Bacillus, Geobacillus, Corynebacterium, Lysobacter, Vibrio,* and *Acinetobacter* genera were reported from the silkworm pupae, rhino beetles, black ants, mole crickets, and giant water bugs. Among the bacterial genera, *Clostridium* and *Paenibacillus apiaris* were detected only by PCR-DGGE in the giant water bug and silkworm, respectively. Whereas the genus *Vibrio* was reported based on metagenomic and PCR-DGGE analysis in combination (Jin et al. 2019).

The 16S rDNA amplification profile generated from the species-specific and universal primer suggests that all the isolated actinobacteria species belonged to *Streptomyces* and *Nocardiopsis* genera. However, PCR-DGGE analysis showed variation in the band patterns for similarly identified species, likely due to the heterogeneity. The heterogeneity in the bacterial species is mainly mediated by the horizontal gene transfer through conjugative plasmids. The gene flux mediated by the conjugative plasmids also plays a significant role in the genetic diversity of the same bacterial species (Gohel and Singh 2018). The banding pattern generated by the *Nocardiopsis alba* strains OK-6 and OK-8 using the PCR-DGGE suggests differences between the two. Based on the 16S rDNA amplification profile, both strains displayed similar homology. Therefore, genotypically similar bacteria may have heterogeneity in their phenotypic characteristics. Thus, the variation in DGGE analysis of similar microbial groups could be due to mutations and adaptation for their survival in that particular habitat (Nimnoi et al. 2011). Therefore, DGGE analysis provides enough clues for the genetic diversity of the actinobacterial species belonging to the same genus. In this study, PCR-based DGGE analysis using

universal and genus-specific primers resulted in the generation of specific clusters indicating the sequence heterogeneity among the isolated actinobacteria.

## Conclusion

This study revealed actinobacterial diversity in the marine habitats of Coastal Gujarat. The actinobacteria varied in their biochemical properties and sugar utilization ability. The actinobacteria produced extracellular amylase, gelatinase, cellulase, and lipase that would play a significant role in the food, detergent, paper, pulp, and leather industries. Strains OK-1, OK-3, and OK-5 could also produce lipases. Further, many strains were positive for ammonia production and nitrate reduction, reflecting their potential as plant growth promoters. The molecular diversity of the marine cultivable actinobacteria was evaluated by the optimization and analysis of the PCR reactions using universal and genus-specific PCR primers in combination with the DGGE. These approaches distinguished different species belonging to the same genus and thus established their significance in microbial ecology from a methodological perspective. Moreover, due to the ease of the performance, the DGGE appears quite significant in identifying the extended numbers of the isolates besides being a powerful fingerprinting method to reveal the sequence heterogeneity in 16S rDNA. The foremost objective of this study was to develop and assess methods for analyzing the molecular diversity of the salt-tolerant alkaliphilic actinobacteria and their tentative assignment with the help of the metabolic properties.

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## Disclosure statement

No potential conflict of interest was reported by the author(s).

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## References

Al-Dhabi NA, Esmail GA, Ghilan AKM, Arasu MV. 2019. Isolation and screening of *Streptomyces* sp. Al-Dhabi-49 from the environment of Saudi Arabia with concomitant production of lipase and protease in submerged fermentation. Saudi J Biol Sci 27(1):474–479.

Al-Dhabi NA, Esmail GA, Ghilan AKM, Arasu MV, Duraipandiyan V, Ponmurugan K. 2019. Characterization and fermentation optimization of novel thermo stable alkaline protease from *Streptomyces* sp. Al-Dhabi-82 from the Saudi Arabian environment for eco-friendly and industrial applications. J King Saud Uni Sci 32(1):1258–1264.

Atta HM, El-Sayed AS, El-Desoukey MA, Mona-Hassan M, Manal-El-Gazar M. 2011. Screening, identification, phylogenetic characterization and optimization of antimicrobial agents biosynthesis production by *S. rimosus*. World Rural Obs 3:40–52.

Bhatt HB, Singh SP. 2020. Cloning, Expression and structural elucidation of a biotechnologically potential alkaline serine protease from a newly isolated Haloalkaliphilic *Bacillus lehensis* JO-26. Front Microbiol 11:941.

Bruni L, Milanovic V, Tulli F, Aquilanti L, Parisi G. 2022. Effect of diets containing full-fat *Hermetiaillucens* on rainbow trout microbiota: a dual cultivation-independent approach with DGGE and NGS. Aquaculture 553:738109.

Chaudhary DK, Khulan A, Kim J. 2019. Development of a novel cultivation technique for uncultured soil bacteria. Sci Rep 9(1):6666.

Chauhan JV, Gohel SD. 2022. Exploring plant growth-promoting, biocatalytic, and antimicrobial potential of salt tolerant rhizosphericGeorgenia soli strain TSm39 for sustainable agriculture. Braz J Microbiol 53(4):1817–1828.

Chauhan JV, Mathukiya RP, Singh SP, Gohel SD. 2021. Two steps purification, biochemical characterization, thermodynamics and structure elucidation of thermostable alkaline serine protease from *Nocardiopsis alba* strain OM-5. Int J Biol Macromol 169:39–50.

Dastager SG, Li WJ, Dayanand A, Tang SK, Tian XP, Zhi XY, Xu LH, Jiang CL. 2006. Seperation, identification and analysis of pigment (melanin) production in Streptomyces. Afr J Biotechnol 5(11):1131–1134.

Edwards U, Rogall T, Blöcker H, Emde M, Böttger EC. 1989. Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal DNA. Nucleic Acids Res 17(19):7843–7853. http://www.academicjournals.org/AJB.

El-Naggar MY. 2007. Kosinostatin, a major secondary metabolite isolated from the culture filtrate of *Streptomyces violaceusniger* strain HAL64. J Microbiol 45(3):262–267.

El-Naggar MY, El-Assar SA, Abdul-Gawad SM. 2006. Meroparamycin production by newly isolated *Streptomyces* sp. strain MAR01: taxonomy, fermentation, purification and structural elucidation. J Microbiol 44(4):432–438.

Gohel SD, Singh SP. 2012. Purification strategies, characteristics and thermodynamic analysis of a highly thermostable alkaline protease from a salt-tolerant alkaliphilicactinomycete, *Nocardiopsis alba* OK-5. J Chromatogr B 889–890(890):61–68.

Gohel SD, Singh SP. 2013. Characteristics and thermodynamics of a thermostable protease from a salt-tolerant alkaliphilic actinomycete. Int J Biol Macromol 56:20–27.

Gohel SD, Singh SP. 2015. Thermodynamics of a $Ca^{2+}$-dependent highly thermostable alkaline protease from a haloalkliphilicactinomycete. Int J Biol Macromol 72:421–429.

Gohel SD, Singh SP. 2018. Molecular Phylogeny and diversity of the salt-tolerant alkaliphilic actinobacteria inhabiting coastal Gujarat, India. Geomicrobiol J 35(9):775–789.

He YQ, Chen RW, Li C, Shi SB, Cui LQ, Long LJ, Tian XP. 2020. *Actinomarinicolatropica* gen. nov. sp. nov., a new marine actinobacterium of the family Iamiaceae, isolated from South China Sea sediment environments. Int J Syst Evol Microbiol 70(6):3852–3858.

Hinsu AT, Panchal KJ, Pandit RJ, Koringa PG, Kothari RK. 2021. Characterizing rhizosphere microbiota of peanut (*Arachishypogaea* L.) from pre-sowing to post-harvest of crop under field conditions. Sci Rep 11(1):17457.

Huang X, Kong F, Zhou S, Huang D, Zheng J, Zhu W. 2019. *Streptomyces tirandamycinicus* sp. nov., a novel marine sponge-derived actinobacterium with antibacterial potential against *Streptococcus agalactiae*. Front Microbiol 10:482.

Jin N, Lu X, Wang X, Liu Q, Peng D, Jian H. 2019. The effect of combined application of *Streptomyces rubrogriseus* HDZ-9-47 with soil biofumigation on soil microbial and nematode communities. Sci Rep 9(1):16886.

Juniastuti T, Kurnijasanti R, Sudjarwo SA. 2016. Isolasi dan identifikasi gen 16s rrna Streptomyces sp. isolat tanah gunungsemeru. International Seminar on Molecular in Veterinary Medicine. Faculty of Veterinary Medicine Airlangga University, Surabaya, p80–85. ISBN 978-602-7043-81-7

Khodayari F, Cebeci Z, Ozcan B. 2014. Optimization of xylanase and α-amylase production by alkaline and thermophilic *Bacillus* isolate KH-13. J EntomolZool Stud 2(5):295–303.

Kikani BA, Patel RK, Thumar JT, Bhatt HB, Rathore DR, Koladiya G, Singh SP. 2023. Solvent tolerant enzymes in extremophiles: adaptations and applications. Int J Biol Macromol 238:124051.

Kikani BA, Singh SP. 2022. Amylases from thermophilic bacteria: structure and function relationship. Crit Rev Biotechnol 42(3):325–341.

Kim H, Kim S, Kim M, Lee C, Yang I, Nam SJ. 2020. Bioactive natural products from the genus Salinospora: a review. Arch Pharm Res 43(12):1230–1258.

Leutou AS, Yang I, Le TC, Hahn D, Lim KM, Nam SJ, Fenical W. 2018. Fluvirucin B6, a new macrolactam isolated from a marine-derived actinomycete of the genus *Nocardiopsis*. J Antibiot 71(6): 609–612.

Li WJ, Kroppenstedt RM, Wang D, Tang SK, Lee JC, Park DJ, Kim CJ, Xu LH, Jiang CL. 2006. Five novel species of the genus *Nocardiopsis* isolated from hypersaline soils and emended description of *Nocardiopsissalina* Li et al. 2004. Int J Syst Evol Microbiol 56(Pt 5): 1089–1096.

Li F, Xie Q, Zhou S, Kong F, Xu Y, Ma Q, Wu W, Huang D, Zhao Y, Huang X. 2021. *Nocardiopsiscoralli sp*. nov. a novel actinobacterium isolated from the coral *Galaxeaastreata*. Int J Syst Evol Microbiol 71(6):004817.

Lu JJ, Perng CL, Lee SY, Wan CC. 2000. Use of PCR with universal primers and restriction endonuclease digestions for detection and identification of common bacterial pathogens in cerebrospinal fluid. J Clin Microbiol 38(6):2076–2080.

Machin EV, Asem MD, Salam N, Iriarte A, Langleib M, Li WJ, Menes RJ. 2019. *Nesterenkonianatronophila* sp. nov., an alkaliphilic actinobacterium isolated from a soda lake, and emended description of the genus *Nesterenkonia*. Int J Syst Evol Microbiol 69(7):1960–1966.

Majed D, Wahiba Z, Fadhel BM, Sghaier AM. 2018. Characterization of the dominant bacterial communities associated with terrestrial isopod species based on 16S rDNA analysis by PCR-DGGE. OJE 08(09):495–509.

Majithiya VR, Gohel SD. 2020. Isolation and screening of extracellular enzymes producing actinobacteria associated with seaweed. Proceedings of the National Conference on Innovations in Biological Sciences (NCIBS), p127–135.

Majithiya VR, Gohel SD. 2022a. Isolation and characterization of marine actinobacteria associated with the seaweeds, *Codiumdwarkense* and *Sargassumcinereum*, collected from the Veraval coastline, Gujarat, India. J Mar Biol Ass India 64(1):33–37.

Majithiya VR, Gohel SD. 2022b. Actinobacteria associated with marine invertebrates: diversity and biological significance. In: Hozzein, WN, editor. Actinobacteria-Diversity, Applications and Medical Aspects. London: Intechopen.

Majithiya VR, Gohel SD. 2022c. Diversity, distribution and biotechnological potential of marine actinobacteria. In: Vala, AK, Kumar, KS, editors. Current Trends in Marine Biology. Cambridge: Cambridge Scholars Publishing, p183–209.

Malviya N, Yandigeri MS, Yadav AK, Solanki MK, Arora DK. 2014. Isolation and characterization of novel alkali-halophilic actinomycetes from the Chilika brackish water lake, India. Ann Microbiol 64(4):1829–1838.

Myers RM, Larin Z, Maniatis T. 1985. Detection of single base substitutions by ribonuclease cleavage at mismatches in RNA: DNA duplexes. Science 230(4731):1242–1246.

Nimnoi P, Pongsilp N, Lumyong S. 2011. Actinobacterial community and diversity in rhizosphere soils of Aquilariacrassna Pierre ex Lec assessed by RT-PCR and PCR-DGGE. BiochemSystEcol 39(4–6): 509–519.

Pankaj A. 2022. Taxonomic study of Faviidae family-quaternary corals of Okha-*Mandal* Taluka, Saurashtra Coast, Gujarat. J Geol Soc India 98(5):669–677.

Pernodet JL, Boccard F, Alegre MT, Gagnat J, Guerineau M. 1989. Organization and nucleotide sequence analysis of a ribosomal RNA gene cluster from *Streptomyces ambofaciens*. Gene 79(1):33–46.

Purohit MK, Rathore DS, Koladiya G, Pandey S, Singh SP. 2022. Comparative analysis of the catalysis and stability of the native, recombinant and metagenomic alkaline proteases in organic solvents. Environ Sci Pollut Res Int 29(53):80968–80982.

Qiao J, Chen L, Li Y, Wang J, Zhang W, Chen S. 2012. Whole-genome sequence of *Nocardiopsis alba* strain ATCC BAA-2165, associated with honeybees. J Bacteriol 194(22):6358–6359.

Raiyani N, Singh SP. 2023. Microbial community and predictive functionalities associated with the marine sediment of Coastal Gujarat. Environ Sci Pollut Res. 30:43245–43266.

Rashidian E, Goodfellow M. 2003. Sequence-based differentiation of strains in the *Streptomyces cyaneus* species-group. Iran J Biotechnol 1(4):224–233.

Rathore DS, Sheikh MA, Gohel SD, Singh SP. 2021. Genetic and phenotypic heterogeneity of the *Nocardiopsis alba* strains of seawater. Curr Microbiol 78(4):1377–1387.

Rathore DS, Singh SP. 2021. Kinetics of growth and co-production of amylase and protease in novel marine actinomycete, *Streptomyces lopnurensis* KaM5. Folia Microbiol 66(3):303–316.

Rintala H, Nevalainen A, ROnka E, Suutari M. 2001. PCR primers targeting the 16S rRNA gene for the specific detection of streptomycetes. Mol Cell Probes 15(6):337–347.

Risdian C, Landwehr W, Rohde M, Schumann P, Hahnke RL, Sproer C, Bunk B, Kampfer P, Schupp PJ, Wink J. 2021. *Streptomyces bathyalis* sp. nov., an actinobacterium isolated from the sponge in a deep sea. Antonie Van Leeuwenhoek 114(4):425–435.

Roth A, Andrees S, Kroppenstedt RM, Harmsen D, Mauch H. 2003. Phylogeny of the genus *Nocardia* based on reassessed 16S rRNA gene sequences reveals underspeciation and division of strains classified as *Nocardiaasteroides* into three established species and two unnamed taxons. J Clin Microbiol 41(2):851–856.

Ryu MJ, Hillman PF, Lee J, Hwang S, Lee EY, Cha SS, Yang I, Oh DC, Nam SJ, Fenical W. 2021. Antibacterial meroterpenoids, merochlorins G–J from the marine bacterium *Streptomyces* sp. Mar Drugs 19(11):618.

Salazar O, Gonzalez I, Genilloud O. 2002. New genus-specific primers for the PCR identification of novel isolates of the genera *Nocardiopsis* and *Saccharothrix*. Int J Syst Evol Microbiol 52(Pt 4): 1411–1421.

Sambrook J. 1989. Molecular Cloning: A Laboratory Manual. Synthetic Oligonucleotides. Cold Spring Harbor, NY: Cold spring Harbour Labouratry Press.

Sharma AK, Kikani BA, Singh SP. 2021. Diversity and phylogeny of actinomycetes of Arabian Sea along the Gujarat Coast. Geomicrobiol J 38(4):347–364.

Sharma AK, Kikani BA, Singh SP. 2020. Biochemical, thermodynamic and structural characteristics of a biotechnologically compatible alkaline protease from a haloalkaliphilic, *Nocardiopsisdassonvillei* OK-18. Int J Biol Macromol 153:680–696.

Shirling EB, Gottlieb D. 1966. Methods for characterization of Streptomyces species. Int J Syst Bacteriol. 16(3):313–340.

Silva FSP, Souza DT, Zucchi TD, Pansa CC, de Figueiredo Vasconcellos RL, Crevelin EJ, de Moraes LAB, Melo IS. 2016. *Streptomyces atlanticus* sp. nov., a novel actinomycete isolated from marine sponge Aplysinafulva (Pallas, 1766). Antonie Van Leeuwenhoek 109(11):1467–1474.

Singh RP, Reddy CRK. 2014. Seaweed–microbial interactions: key functions of seaweed-associated bacteria. FEMS Microbiol Ecol 88(2): 213–230.

Sunish KS, Biji M, Rosamma P, Sudheer NS, Sreedharan K, Mohandas A, Singh IB. 2020. Marine actinomycetes*Nocardiopsis alba* MCCB 110 has immunomodulatory property in the tiger shrimp Penaeus monodon. Fish Shellfish Immunol 102:125–132.

Suutari MH, Rintala H, Ronak E, Nevalainen A. 2002. Oligonucleotide primers for detection of Streptomyces species. Patent WO-02092848-A1.

Swe PM, Zakrzewski M, Waddell R, Sriprakash KS, Fischer K. 2019. High-throughput metagenome analysis of the *Sarcoptesscabiei* internal microbiota and in-situ identification of intestinal *Streptomyces* sp. Sci Rep 9(1):11744.

Thakrar FJ, Koladiya G, Singh SP. 2023. Heterologous expression and structural elucidation of a highly thermostable alkaline serine protease from haloalkaliphilic actinobacterium, *Nocardiopsis* sp. Mit-7. Appl Biochem Biotechnol. 2023:01–20.

Thakrar FJ, Singh SP. 2019. Catalytic, thermodynamic and structural properties of an immobilized and highly thermostable alkaline protease from a haloalkaliphilicactinobacteria, *Nocardiopsis alba*Tata-5. Bioresour Technol 278:150–158.

Thakur MC, Reddy CRK, Jha B. 2008. Seasonal variation in biomass and species composition of seaweeds stranded along Port Okha, northwest coast of India. J Earth Syst Sci 117(3):211–218.

Tolieng V, Booncharoen A, Nuhwa R, Thongchul N, Tanasupawat S. 2018. Molecular identification, L-lactic acid production, and antibacterial activity of *Bacillus strains* isolated from soils. J Appl Pharm Sci 8(10):098–105.

Travlos IS, Cheimona N, Roussis I, Bilalis DJ. 2018. Weed-species abundance and diversity indices in relation to tillage systems and fertilization. Front Environ Sci 6:11.

Tuttle RN, Rouse GW, Castro-Falcon G, Hughes CC, Jensen PR. 2022. Specialized metabolite-mediated predation defense in the marine ActinobacteriumSalinispora. Appl Environ Microbiol 88(1): e01176-21.

Vaghela N, Gohel S. 2023. Medicinal plant-associated rhizobacteria enhance the production of pharmaceutically important bioactive compounds under abiotic stress conditions. J Basic Microbiol 63(3–4):308–325.

Vurukonda SSKP, Mandrioli M, D'Apice G, Stefani E. 2020. Draft genome sequence of plant growth-promoting *Streptomyces* sp. strain SA51, isolated from olive trees. Microbiol Resour Announc 9(1): e00768-19.

Wolińska A, Górniak D, Zielenkiewicz U, Kuźniar A, Izak D, Banach A, Błaszczyk M. 2019. Actinobacteria structure in autogenic, hydrogenic and lithogenic cultivated and non-cultivated soils: a culture-independent approach. Agronomy 9(10):598.

Yadav AN, Verma P, Kumar S, Kumar V, Kumar M, Sugitha TCK, Singh BP, Saxena AK, Dhaliwal HS. 2018. Actinobacteria from rhizosphere: molecular diversity, distributions, and potential biotechnological applications. In: Singh, BP, Gupta, VK, Passari, AK, editors. New and future developments in microbial biotechnology and bioengineering. Amsterdam: Elsevier, p13–41.

Yang R, Zhang LP, Guo LG, Shi N, Lu Z, Zhang X. 2008. *Nocardiopsisvalliformis* sp. nov., an alkaliphilic actinomycete isolated from alkali lake soil in China. Int J Syst Evol Microbiol 58(Pt 7): 1542–1546.

Yassien MAM, Jiman-Fatani AAM, Asfour HZ. 2014. Production, purification and characterization of cellulase from *Streptomyces* sp. Afr J Microbiol Res 8(4):348–354. http://www.academicjournals.org/AJMR.

Yassin AF, Rainey FA, Burghardt J, Gierth D, Ungerechts J, Lux I, Seifert P, Bal C, Schaal KP. 1997. Description of *Nocardiopsissynnemataformans sp*. nov., elevation of *Nocardiopsis alba subsp. prasina* to *Nocardiopsisprasina* comb. nov., and designation of *Nocardiopsisantarctica* and *Nocardiopsisalborubida* as later subjective synonyms of *Nocardiopsisdassonvillei*. Int J Syst Bacteriol 47(4):983–988.

Yoon JH, Jung SY, Kim W, Nam SW, Oh TK. 2006. *Nesterenkoniajeotgali* sp. nov., isolated from jeotgal, a traditional Korean fermented seafood. Int J Syst Evol Microbiol 56(Pt 11): 2587–2592.

Zevin AS, Hensley-McBain T, Miller C, Smith E, Langevin S, Klatt NR. 2017. Antibiotic treatment disrupts bacterial communities in the colon and rectum of simian immunodeficiency virus-infected macaques. FEMS Microbiol Lett 364(23):fnx228.

Zhang X, Chen S, Zhang L, Zhang Q, Zhang W, Chen Y, Zhang W, Zhang H, Zhang C. 2021. Dassonmycins A and B, polycyclic thioalkaloids from a marine sponge-derived *Nocardiopsisdassonvillei* SCSIO 40065. Org Lett 23(8):2858–2862.

Zhang YG, Liu Q, Wang HF, Park DJ, Guo JW, Kim CJ, Zhang YM, Li WJ. 2016. *Nocardiopsisansamitocini* sp. nov., a new producer of ansamitocin P-3 of the genus *Nocardiopsis*. Int J Syst Evol Microbiol 66(1):230–235.

Zhao K, Li J, Shen M, Chen Q, Liu M, Ao X, Liao D, Gu Y, Xu K, Ma M, et al. 2018. Actinobacteria associated with the Chinaberry tree are diverse and show antimicrobial activity. Sci Rep 8(1):1–10.

Zhi XY, Tang SK, Li WJ, Xu LH, Jiang CL. 2006. New genus-specific primers for the PCR identification of novel isolates of the genus *Streptomonospora*. FEMS Microbiol Lett 263(1):48–53.

Zhi X, Yang L, Wang Y, Tang S, Lou K, Li W. 2008. Genus-specific PCR for molecular identification of novel isolates of the genus *Nesterenkonia*. Acta Microbiol Sinica 48:644–650.