Journal of Integral Sciences
[An International Open Access Journal]
Available at www.jisciences.com ISSN: 2581-5679

MOLECULAR GENOTYPING, 16S rRNA SEQUENCING, AND EVOLUTIONARY MATRIX OF *STREPTOMYCES* sp. STRAIN ANU-27 ISOLATED FROM ESTUARINE MANGROVE SEDIMENTS

ANUSHA RANI YAVVARI AND NAGALAKSHMI DEVAMMA MUNDLA*

Department of Botany, SVU College of Sciences, Sri Venkateswara University, Tirupati-517502.

Received: 11-04-2026 Revised: 03-05-2026 Accepted: 30-06-2026

ABSTRACT

Estuarine mangrove environments are unique, highly dynamic habitats harboring a rich and unexplored diversity of bioactive actinobacteria. In this study, we highlight the extensive molecular identification, genetic sequencing, and evolutionary positioning of the bacterial isolate ANU-27, which exhibited superior broad-spectrum antimicrobial efficacy during initial phenotypic screenings. Genomic DNA was successfully extracted using the CTAB-lysozyme methodology, yielding634±32 µg/gof high-integrity DNA with a distinct genomic G+C content of 55.1% calculated via thermal denaturation midpoint (T_m= 92.3C).The 16S rRNA gene locus was targeted and amplified via PCR utilizing 27F and 1492R universal primers, generating a sharp ~1500 bp amplicon. Bidirectional Sanger sequencing resolved a definitive sequence spanning 1177 nucleotides with a high ribosomal G+C ratio 59.13%. Comprehensive homology mapping via NCBI BLASTn paired with a1000-replicate Maximum Parsimony cladogram clustered the strain with 100% sequence identity(E-Value = 0.0) within the genus *Streptomyces*, revealing an immediate phylogenetic node shared with *Streptomyces maritimus* strain SBK2-IR9 and *Streptomyces rochei* strain ABU8. These findings firmly clarify the taxonomic assignment of strain ANU-27, spotlighting its high secondary metabolic and biotechnological promise.

Keywords: *Streptomyces sp. ANU-27, 16S rRNA Sequencing, Molecular Genotyping, Maximum Parsimony, Estuarine Actinomycetes.*

***Corresponding Author**
Nagalakshmi Devamma Mundla

DOI: https://doi.org/10.37022/jis.v9i2.147

Produced and Published by
South Asian Academic Publications

1. INTRODUCTION

The expansion of multi-drug resistance among human pathogens has driven accelerated exploration into extreme or geographically distinct environments for novel antimicrobial agents. Marine and estuarine mangrove sediments represent exceptional reservoirs for the phylum Actinomycetota (traditionally Actinomycetes), due to localized fluctuations in salinity, temperature, and rich organic matter processing. In a regional screening of marine environments across the south and northeast districts of Andhra Pradesh, India, a collection of 32 morphologically distinct actinobacterial isolates was established. Among these, strain ANU-27 emerged as a critical candidate of interest. While initial evaluations recorded its unique phenotypic and bioactivity properties, this paper focuses specifically on the rigorous genomic validation, 16S rRNA genotyping, base composition profile, and phylogenetic framework required to define the exact identity and evolutionary parameters of this high-performing strain.

2. MATERIALS AND METHODS

2.1 Brief Overview of Source Isolation and Phenotypic Background

The source sediment sample was acquired from the top 10–15 cm of the soil profile within the mangrove zones of Andhra Pradesh. Following multi-step processing and serial dilutions in 0.9% NaCl, isolation was carried out on selective starch casein agar plates prepared with 50% sterilized sea water and fortified with 5 µg/mL rifampicin and 25 µg/mL nystatin to suppress cross-contamination. Isolate ANU-27 was physically characterized as a Gram-positive, non-motile, non-spore-forming rod displaying a raised, circular configuration and a chalky, cream-colored surface. Preliminary modified cross-streak screening confirmed excellent antagonist activity, producing wide clea zones of inhibition (25 mm against *Bacillus subtilis and 29 mm*

against *Escherichia coli*).

2.2 Extraction of Genomic DNA

To ensure high-yield extraction for downstream molecular analysis, 24-hour log-phase liquid cultures of strain ANU-27 were supplemented with 1% of a 10% (W/V) glycine solution and incubated at 37°C for an additional 24 hours to weaken the thick peptidoglycan cell walls. Cells were harvested via centrifugation (2000 rpm for 10 min), resuspended in glucose Tris-EDTA solution, and treated overnight at 37°C with 10 mg/mL concentrated lysozyme. Lysis was completed by the addition of a 10% SDS and 10 mg/mL proteinase K mixture at 55°C. Nucleic acids were purified using a CTAB-NaCl phase-separation framework coupled with repetitive 24:1 chloroform-isoamyl alcohol extractions. The genomic DNA was precipitated in ice-cold isopropanol, washed with 70% ice-cold ethanol, and reconstituted in 50 µL of Tris-EDTA (TE) buffer.

2.3 Qualitative and Quantitative Assessment of DNA

The exact purity matrix and yield of the extracted genomic DNA were evaluated using a UV-Vis spectrophotometer. Integrity was observed via horizontal agarose gel electrophoresis on a 1% agarose matrix dissolved in 1x TAE buffer (pH 8.0) and stained with ethidium bromide. Electrophoretic migration was conducted at 100 V for 1 hour, followed by imaging under a UV transilluminator.

2.4 PCR Amplification and Sanger Dideoxy Sequencing

The 16S rRNA gene locus was selectively targeted using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-CGGTTACCTTGTTACGACTT-3'). The standard 50 µL amplification reaction mixture comprised of 25 µL of 2X Taq mixture, 5 µL of 20 pmol forward primer, 5 µL of 20 pmol reverse primer, 3 µL of template DNA and 12 µL of molecular-grade water. Thermocycling was performed in a Wee32 Mini Thermocycler using the following conditions: initial denaturation at 94°C for 5 min; 40 cycles of denaturation at 94°C for 1 min, annealing at 48°C for 1 min, and extension at 72°C for 2 min; followed by a final elongation at 72°C for 10 min. Amplicons were visually checked on a 1.5% agarose gel alongside a 100 bp DNA ladder. The purified PCR output was subjected to bidirectional Sanger di-deoxy sequencing using the Big Dye Terminator v3.1 cycle sequencing kit on an ABI Prism 3700 DNA Analyzer.

2.5 Sequence Analysis and Tree Construction

The overall nucleotide distribution and G+C percentages were handled via the Biologics International server. Homology mapping was evaluated using the NCBI BLASTn engine against the GenBank non-redundant database. Selected reference sequences were collected and processed through multiple sequence alignment (MSA) using the UPGMA strong clustering method via Muscle software integrated within the MEGA-X platform. A distance matrix was resolved to evaluate evolutionary variations, from which a Maximum Parsimony phylogenetic tree was built and verified utilizing the bootstrap method with 1000 iterations.

3. RESULTS AND DISCUSSION

3.1 Genomic DNA Profile and Thermal Properties

The CTAB-lysozyme workflow successfully yielded an average total genomic DNA density quantified at 634±32 µg/g. The absorbance ratio at A260/280 reached 1.90, demonstrating outstanding chemical purity and a complete absence of co-purified proteins or cell wall debris. Agarose gel resolution confirmed a single, distinct high-molecular-weight band completely free from visible shearing or low-weight fragmentation.

The thermal denaturation midpoint (T_m) of the genomic sample was explicitly verified at 92.3°C. Using the classic empirical equation of Marmur and Doty (1962), the total genomic G+C content calculated from this midpoint stood at 55.1%:

$$\text{Mol \% G + C} = \frac{\text{Tm} - 69.3}{0.41}$$

3.2 16S rRNA Amplification and Fine Base Architecture

Targeted PCR amplification successfully resolved a high-quality, singular amplicon measuring approximately 1500 base pairs, which is the standard baseline for prokaryotic ribosomal markers.

Following manual editing and assembly, the definitive 16S rRNA sequence of strain ANU-27 was determined to span exactly **1177 nucleotides**. The comprehensive composition metrics of the base architecture are structured in Table 01.

Table 01: 16S rRNA Ribosomal Nucleotide Profile of Strain ANU-27

S. No	Structural Parameter / Base Type	Base Count (bp)	Percentage (%)
1.	Adenine (A)	269	22
2.	Thymine (T)	212	20
3.	Guanine (G)	395	33
4.	Cytosine (C)	301	25
5.	Total subunit length	1177	
6.	Total combined G+C Bases	696	59.13

This elevated ribosomal G+Cdistribution (59.13%) serves as an definitive taxonomic marker characteristic of the phylum Actinomycetota. In environmental actinobacteria, high internal (G+C) concentrations structurally stabilize the secondary orientation of functional rRNA, ensuring structural survival and cellular fitness across thermal or highly saline estuarine environments.

3.3 Multiple Sequence Alignment (MSA) and Evolutionary Divergence

NCBI BLASTn parsing of the 1177 bp sequence matched strain ANU-27 firmly within the genus

Streptomyces, showing a 100% identity metric (E-value = 0.0) with established reference species.

Thirteen highly homologous *Streptomyces* entries were retrieved from GenBank to formulate a definitive multiple alignment matrix. The precise alignment parameters generated are outlined in the below table 02:

Table 02: precise alignment parameters for homologous *Streptomyces*

S. No	Parameter	No. of Sites	% of Sites
1.	Total Aligned Sites	1477	-
2.	Conserved Sites	1452	98.31
3.	Variable Sites	16	1.08
4.	Parsimony-Informative Sites	1(0.07)	0.07
5.	Singleton Sites	14	0.95
6.	Overall Mean Distance Matrix Index	0.00	-

The distance calculations revealed an evolutionary divergence score of **0.0000** between strain ANU-27 and its absolute closest matching types, indicating high conservation across the tracked ribosomal space.

3.4 Phylogenetic Clustering and Taxonomic Identification

The Maximum Parsimony cladogram clearly positioned strain ANU-27 into a well-supported, robust evolutionary group comprising valid marine *Streptomyces* representatives.

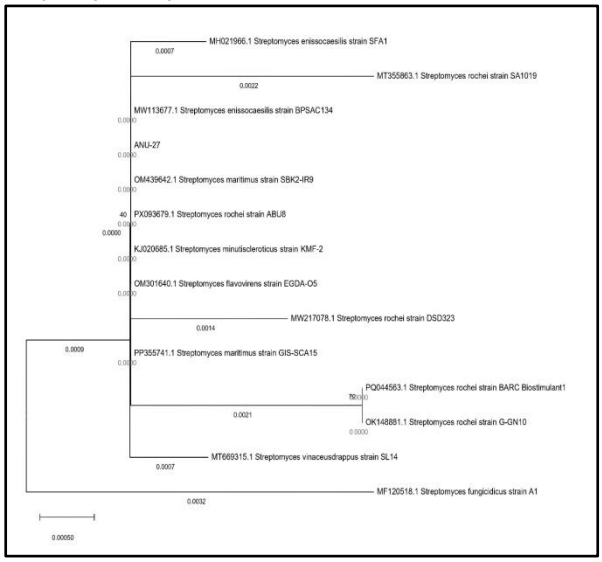

Figure 01: Outline Topology of the Maximum Parsimony Cladogram for Isolate ANU-27 based on 16S rRNA Genotyping.

Strain ANU-27 directly consolidates on an immediate shared evolutionary branch characterized by a negligible branch distance (0.0000) with *Streptomyces maritimus* strain SBK2-IR9 (OM439642.1) and *Streptomyces rochei* strain ABU8 (PX093679.1). This structural topology mathematically confirms that strain ANU-27 possesses an identical or nearly identical genetic profile in this diagnostic locus and shares a very recent common ancestor with these validated marine isolates. Taxonomic placement within this distinct clade matches its broad-spectrum antimicrobial profile. Members of the *Streptomyces maritimus* complex are known for the biosynthesis of marinomycins, which are potent polyene compounds displaying rich anti-cancer and antimicrobial activity. Similarly, the *Streptomyces rochei* group is a documented source of elite therapeutic molecules, including borrelidin and lankacidin. The exact molecular classification of strain ANU-27 validates its ecological role within estuarine sediment communities and underscores its immense potential for metabolic scale-up and pharmaceutical discovery.

4. CONCLUSION

In the present study, the bioactive estuarine bacterial isolate ANU-27 was successfully characterized via multi-level genotyping. Spectrophotometric and electrophoretic diagnostics proved the extraction of high-quality, high-integrity genomic DNA. 16S rRNA amplification and Sanger sequencing resolved a clean 1177 bp sequence showing a characteristic high G+C ratio of 59.13%. Evolutionary distance mapping and Maximum Parsimony tree construction firmly placed the isolate within a validated *Streptomyces* cluster, demonstrating direct alignment with *S. maritimus* and *S. rochei*. This genomic baseline securely classifies the strain and supports further studies into its specific secondary metabolome for biomedical applications.

5. ACKNOWLEDGEMENT

The authors express sincere gratitude to the laboratory and institutional infrastructure for supporting the molecular characterization and sequencing phases of this research project.

6. CONFLICT OF INTEREST

The authors declare that there are no commercial or financial conflicts of interest related to this work.

7. REFERENCES

1. Bredholdt, H., Galatenko, O. A., Engelhardt, K., Fjaervik, E., Terekhova, L. P., & Zotchev, S. B. (2007). Rare actinomycete bacteria from the shallow water sediments of the Trondheim fjord, Norway: isolation, diversity, and biological activity. *Environmental Microbiology*, *9*(11), 2756-2764.
2. Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, *39*(4), 783-791.
3. Jensen, P. R., Dwight, R., &Fenical, W. (2005). Distribution of actinomycetes in near-shore tropical marine sediments. *Applied and Environmental Microbiology*, *61*(4), 1102-1108.
4. Kämpfer, P. (2012). Genus *Streptomyces*: The Actinobacteria. In M. Goodfellow, P. Kämpfer, H. J. Busse, M. E. Trujillo, K. Suzuki, W. Ludwig, & W.

B. Whitman (Eds.), *Bergey's Manual of Systematic Bacteriology* (pp. 1455-1767).

5. Kwon, H. C., Kauffman, C. A., Jensen, P. R., &Fenical, W. (2006). Marinomycins A–D, antitumor-antibiotics of a new structure class from a marine actinomycete of the recently discovered genus "Marinispora". *Journal of the American Chemical Society*, *128*(5), 1622-1633.
6. Marmur, J., & Doty, P. (2012). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *Journal of Molecular Biology*, *5*(1), 109-118.
7. Mizrahi-Man, O., Davenport, E. R., & Gilad, Y. (2013). Taxonomic classification of bacterial 16S rRNA genes using short sequencing reads: evaluation of effective study designs. *PLoS ONE*, *8*(1), e53608.
8. Olano, C., Méndez, C., & Salas, J. A. (2008). Antitumor compounds from marine actinomycetes. *Marine Drugs*, *6*(1), 25-44.
9. amura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, *30*(12), 2725-2729.
10. Ventura, M., Canchaya, C., Tauch, A., Chandra, G., Fitzgerald, G. F., Chater, K. F., & van Sinderen, D. (2007). Genomics of Actinobacteria: tracing the evolutionary history of an ancient phylum. *Microbiology and Molecular Biology Reviews*, *71*(3), 495-548.