Journal of Environmental Biology
p-ISSN: 0254-8704 • e-ISSN: 2394-0379 • CODEN: JEBIDP

JEB®

Original Research Paper
Volume 47 Issue 5 September 2026 pp. 1015-1023
DOI: http://doi.org/10.22438/jeb/47/5/MRN-5984

J. Environ. Biol., 2026

Journal website : www.jeb.co.in ★ E-mail : editor@jeb.co.in

Diversity metrics for the evaluation of cultivable Actinobacterial variety from the limestone soils of the Cuddapah Basin

S. Meenakshi[1], J. Hiremath[2], M.P. Shilpa[1] and S. Shivaveerakumar[1]*

[1]*Department of Microbiology, Davangere University, Shivagangothri, Davanagere-577 007, India*
[2]*Department of Food Science and Nutrition, Khaja Banda Nawaz University, Kalaburagi-585 104, India*

Received: 11 February 2026 **Revised:** 01 June 2026 **Accepted:** 23 July 2026

***Corresponding Author Email:** shiv_math1984@yahoo.com ***ORCiD:** https://orcid.org/0000-0002-0907-5610

Abstract

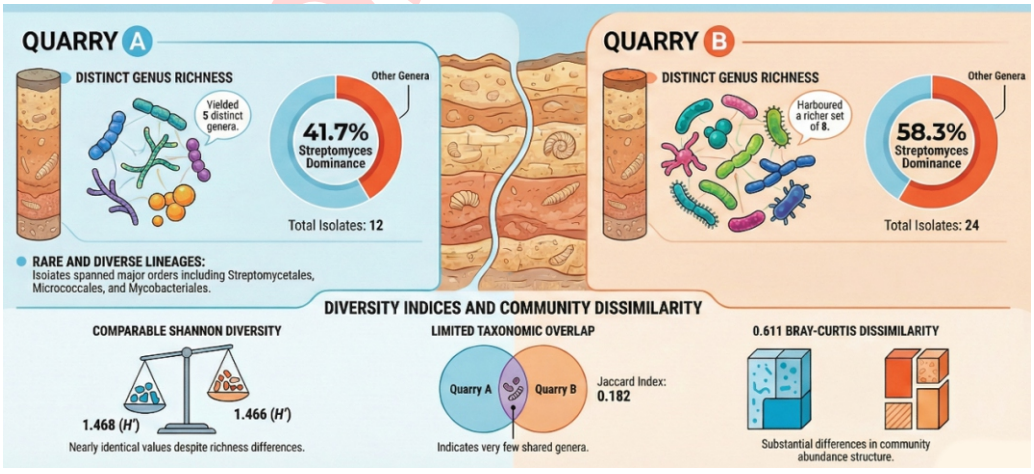
Aim: Limestone ecosystems represent geochemically unique and underexplored habitats that can harbor specialized Actinobacteria with vital applications. The present study investigates the culturable actinobacterial diversity from limestone-embedded soil of the Cuddapah Basin, India.

Methodology: A total of 36 isolates were obtained on selective media and identified by 16S rRNA gene sequencing.

Results: Quarry A yielded five genera, dominated by *Streptomyces* (41.7% of isolates), whereas Quarry B harbored eight genera, with a strong dominance of *Streptomyces* (58.3%). Despite the differences in richness, both quarries exhibited comparable Shannon diversity values of 1.468 and 1.465, respectively. Only two genera (*Streptomyces* and *Rhodococcus*) were shared between the two quarries, resulting in a low Jaccard similarity index of 0.182, indicative of limited overlap in genus composition. The Bray-Curtis dissimilarity value of 0.611 further supports noticeable differences in genus-level abundance structure between the two-quarry sites, proving dissimilarities in genus typed between two samples. Phylogenetic analysis resolved all isolates into well-supported lineage that correspond to their respective genera, reflecting broad representation across the orders *Streptomycetales, Micrococcales* and *Mycobacteriales*.

Interpretation: This study presents the first report of actinomycetes isolated from limestone quarry and provides the first baseline of combining culturedependent isolation with quantitative diversity metrics to evaluate actinobacterial diversity from the Cuddapah Basin limestone ecosystems of Southern India.

Key words: Actinobacteria, Cuddapah Basin, Culturable diversity, Limestone soil, *Streptomyces*



How to cite: Meenakshi, S., J. Hiremath, M.P. Shilpa and S. Shivaveerakumar: Diversity metrics for the evaluation of cultivable actinobacterial variety from the limestone soil of the Cuddapah basin. *J. Environ. Biol.*, **47,** 1015-1023 (2026).

Introduction

Soil serves as a complex ecological matrix, hosting a diverse community of microorganisms, with Actinobacteria being particularly significant due to their widespread distribution and vital roles in organic matter decomposition and nutrient cycling (Li *et al.,* 2024). These soil saprophytes contribute to the characteristic earthy odor of freshly plowed soil through the production of geosmin (Shivaveerakumar *et al.,* 2014). The abundance and diversity of Actinobacteria are influenced by various ecological and physico-chemical factors, including geographical location, soil temperature, pH, texture, organic matter content, aeration, nutrient availability, moisture and vegetation (Meenakshi *et al.,* 2024). Limestone quarries, especially in Southern India, represent geochemically unique and under explored ecological niches. These quarries, situated within sedimentary formations potentially older than the surrounding Deccan Plateau, offer distinctive physicochemical conditions conducive to specialized microbial populations (Sunitha, 2014). Recent investigations have identified rare, thermo-alkaliphilic Actinobacteria in these limestone ecosystems, particularly in Karnataka, emphasizes the potential for bioprospecting and discovery of novel actinomycete taxa (Quadri and Agsar, 2012; Quadri *et al*., 2015).

Despite the recognized significance of Actinobacteria as sources of novel bioactive compounds, the cultivable diversity within the limestone soils of the Cuddapah Basin remains largely unexplored. Traditional culture-based methods often underestimate the natural diversity of Actinobacteria, while culture-independent assessments, particularly those utilizing 16S rRNA gene analysis, reveal complex communities that are inadequately represented in the cultivated collections (Yabe *et al.,* 2017). A systematic assessment of these communities, focusing on richness, dispersion and community structure is essential. The current investigation aims to characterize the actinobacterial community associated with the limestone deposits of the Cuddapah Basin, employing 16S rRNA gene analysis on 36 isolated strains to determine their closest type strains and compute alpha and beta diversity indices. This study represents a pioneering effort to systematically assess cultivable actinobacterial diversity in this unique limestone ecosystem, providing a valuable reference for future microbial ecological studies and bioprospecting initiatives.

Recent research has increasingly emphasized the exploration of extreme habitats, such as limestone environments, which harbor unique actinobacterial communities with significant biotechnological potential. Over the decade, microbial ecology has evolved from simple isolation and taxonomic descriptions to comprehensive diversity assessments, integrating ecological metrics and molecular characterization with environmental variables (Shirokikh *et al.,* 2026). Factors such as lithological characteristics, nutrient availability, calcium carbonate content, pH, and moisture have been shown to significantly influence the microbial community assimilate in limestone ecosystems (Tyc, *et al.,* 2017; Ndabankulu *et al.,* 2022). However, critical gaps persist in the understanding of cultivable actinobacterial communities, particularly in the limestone soils of the Cuddapah Basin. Most recent studies have focused on culture-independent metagenomic approaches or geographically distinct limestone habitats (Chakraborty *et al.,* 2022; Yang *et al.,* 2024), leaving cultivable communities inadequately characterized. Furthermore, while previous research has primarily reported species occurrence and antimicrobial potential, quantitative ecological assessments using diversity indices remain limited (Tamreihao *et al.,* 2018). The application of such metrics is crucial for a robust ecological understanding of community structure and for enabling comparisons among different habitats.

Therefore, the current investigation sought to evaluate the diversity and cultivable abundance of Actinobacteria linked to the limestone deposits of the Cuddapah Basin through culture-dependent techniques, followed by morphological, biochemical and molecular characterization of the isolates to understand the microbial diversity selected in this underexplored niche.

Materials and Methods

Sampling and isolation of actinobacteria: Limestone soil samples were collected aseptically from the selected sites of the Cuddapah Basin as illustrated in the Fig. 1 [Latitude 15°23'26.53"N to Longitude 78°13'55.00"E; and Latitude 15°23'34.62"N to Longitude 78°14'9.85"E] and brought to the laboratory in sterile containers for the analyses of physico-chemical properties recorded as reported earlier. Soil samples were air-dried and subjected for pre-treatment method before isolation (Meenakshi *et al.,* 2025).

Serial dilution plating was done to isolate Actinobacteria. Pretreated soil suspension (1ml) was suspended in 9 ml of sterile distilled water and serially diluted to 10^{-6} (Nimaichand *et al.,* 2012). Aliquots (0.1m l) from appropriate dilutions were spread on Starch Casein Agar, a selective medium originally proposed for the isolation of soil actinomycetes by Kuester and Williams (1964). Plates were incubated at 28+2°C for 2-3 weeks and morphologically discrete actinomycetal colonies were selected and purified by repeated streaking.

Morphological characterization: The purified isolates were characterized based on colony morphology, pigmentation, aerial mycelium, substrate mycelium, diffusible pigment and sporulation patterns. Morphological observations were carried out according to the methods of the International *Streptomyces* Project (ISP) as described by Shirling and Gottlieb (1966, 1972).

16S rRNA Gene Sequencing and Molecular identification: The isolates were subjected to molecular identification through amplification and sequencing of the 16SrRNA gene. Genomic, DNA was extracted using the CTAB method (Ahmadi *et al*., 2018) , quality checked by agarose gel electrophoresis, and quantified using Nano Drop 1000 (Karthik, 2024). DNA samples were stored

Fig. 1: Map locating sampling sites. Quarry A – YL; Quarry B – PL.

at -20°C until further analysis. The efficiency and specificity of 16S rRNA gene barcodes were evaluated by systematically optimizing PCR reaction parameters. The reaction mixture was prepared to a total volume of 25 µl, consisting of the following components: 1 µl of genomic DNA template (approximately 25 ng), 2 µl of 10x PCR reaction buffer, 0.5 µl of $MgCl_2$ (50 mM), 1 µl of dNTP mix (10mM for each nucleotide), 1 µl of forward primer (10 mM), 1 µl of reverse primer (10 mM), and 0.5 µl of Taq DNA polymerase (5U µ l^{-1}), with the final volume made upto 25 µl using molecular-grade water.

PCR amplification of primer sequences targeting conserved regions of the actinobacterial 16S rRNA gene was employed. Sequencing was performed using forward primer 5'-AGAGTTTGATCCTGGCTCAG-3' and the reverse primer 5'-GGTTACCTTGTTACGACTT-3' (27F and 1492R). PCR amplification was conducted with an optimized thermocycling protocol, which included an initial denaturation at 95°C for 2 min, succeeded by 30 cycles of a three-step process: denaturation at 95°C for 30 sec, annealing at 60°C for 45 sec and elongation at 72°C for 1 min. A final elongation step was performed at 72°C for 10 min to ensure complete DNA strand extension, followed by storage of reactions at 4°C for an indefinite period. This protocol reliably produced distinct single-band amplicons of the expected molecular size with minimal variation between runs. The PCR

products were checked in 1.5% agarose gel and visualized on a UV transilluminator, and the gel imaging was done using a Gel documentation system. The amplified PCR products were sequenced using same set of primers (27F' and 1492R') on ABI 3730xl analyzer (Jose and Jebakumar, 2012; McCaig *et al.,* 1999).

Phylogenetic analysis and taxonomic classification: The resulting 16S rRNA gene sequences were read (~300–1400 bp) from actinobacterial isolates to determine the phylogenetic connections and taxonomy classifications. Sequences with ≥97% nucleotide similarity were categorized into phylogenetic groupings to determine species boundaries and evolutionary relationships. Multiple sequence alignment was done using CLUSTALx version 2.1 (Larkin *et al.,* 2007), and phylogenetic reconstruction followed. MEGA 12 version maximum-likelihood method was used to create a phylogenetic tree (Kumar *et al.,* 2024), and the Kimura two-parameter model was used to calculate evolutionary distances to account for multiple substitutions at nucleotide Bootstrap analysis with 1,000 replications was used to evaluate the statistical support and reliability of internal nodes in the phylogenetic tree, with branch point values indicating clade topology confidence. The phylogenetic tree was visualized and edited to show clade relationships and evolutionary distances among isolates, labeling terminal taxa by genus classification from sequence homology searches against curated reference databases (GenBank NCBI). The phylogenetic location of isolates was compared with type strains and reference sequences in the actinobacterial lineage to determine their genus and species. Actinobacterial community composition was defined by phylogenetic clustering patterns, with isolate distributions among clades indicating genetic and phenotypic variability within and across samples (Baig *et al.,* 2021).

Biodiversity indices for assessing cultivable actinobacterial diversity: Diversity assessment of cultivable actinobacteria was done by ecological diversity indices. Abundance data of purified isolates were used to calculate the species richness, Shannon-Wiener diversity index (H), Simpson's diversity index (D) and Pielou's evenness index (J). These indices were used to assess the community structure and distribution patterns at the sampling sites (Cazzolla Gatti *et al.,* 2020). Combined, these metrics provide a comprehensive understanding of actinomycete composition and ecological health (Kim *et al.,* 2017). The biodiversity indices were calculated using the following equations:

Alpha Biodiversity Indices

Species Richness Estimator: A point estimator derived from observed richness, with an additional term that estimates the number of unobserved taxa due to sequencing technology limitations, based on the ratio of singletons to doubletons (Cassol *et al.,* 2025). The Chao1 richness estimator was computed as (Chao and Lee, 1992).

$$\hat{S}_{Chao1} = S_{obs} + \frac{f_1^2}{2f_2}$$

where,

S_{obs} – number of observed taxa; f_1 and f_2 are the counts of singletons and doubletons, respectively.

Relative Abundance: The relative abundance of each genus was computed as the proportion of isolates belonging to that genus in relation to the total number of isolates. It was expressed as a percentage using the following formula:

$$\text{Relative Abundance (\%)} = \frac{\text{No. of Isolates per Genus}}{\text{Tot No. of Isolates}} \times 100$$

This measure reflects the degree to which particular genera dominate or contribute to the overall culturable actinobacterial community.

Shannon-Wiener Diversity Index (H`): Shannon diversity index (H') (also called Shannon–Wiener index) represents the entropy measure of species diversity within a community (Shannon, 1948). This index accounts both for the number of species and their relative abundances, giving more weight to rare species than to abundant ones. Higher Shannon values indicate greater diversity and community stability; besides, it is also used to quantify both the richness and evenness of the actinobacterial community. It was calculated by the formula:

$$H' = -\sum_{i=1}^{s} \cdot p_i \ln (p_i)$$

where, p_i represents the proportion of individuals (isolates) belonging to the i^{th} genus. This index increases with both the number of genera and the equitability of their distribution. Shannon's index is sensitive to the presence of rare genera and is particularly useful in comparing communities with varying levels of richness and dominance.

Simpson's Diversity Index (1-D): Simpson's index (Simpson, 1949) provides a measure of diversity that assigns more weight to dominant taxa. Emphasizes on dominant species rather than rare ones, making it sensitive to the most abundant taxa in the community. This index produces values between 0 and 1, where values closer to 1 indicate higher diversity. A lower value reflects greater dominance by a few genera. It was calculated by the formula:

$$D = \sum_{i=1}^{s} \cdot p_i^2 \quad \text{Simpson's Index of Diversity} = 1 - D$$

where, p_i is the proportion of isolates belonging to genus ith OUT and Simpson's diversity as 1 - *D*.

Pielou's evenness index (E): Pielou's evenness index (Pielou, 1966) was calculated to evaluate the homogeneity of isolate distribution among genera. It quantifies the evenness of species distribution within a community, with values spanning from 0 to 1. This score is unaffected by species richness and indicates whether species abundances are evenly distributed or predominantly controlled by a limited number of taxa. Values approaching 1 signify a more equitable distribution of species

Table 1: Isolate molecular characterization, closest type strain match, sequence similarity percentage based on 16S rRNA sequence analysis, and GenBank accession number are mentioned

Genus	Strain	Close type strain	Similarity (%)	Accession Number
Streptomyces	SMS 1	*Streptomyces longisporoflavus*	99.63%	PV097146
Streptomyces	SMS 2	*Streptomyces griseoincarnatus*	98.75%	PV097147
Streptomyces	SMS 3	*Streptomyces flavogriseus*	98.91%	PV097148
Rhodococcus	SMS 4	*Rhodococcus baikonurensis*	98.95%	PV097149
Bifidobacterium	SMS 5	*Bifidobacterium pseudocatenulatum*	99.13%	PV097150
Nocardia	SMS 6	*Nocardia wallacei*	98.74%	PV097151
Gordonia	SMS 7	*Gordonia sihwensis*	99.06%	PV097152
Rhodococcus	SMS 8	*Rhodococcus erythropolis*	97.96%	PV097153
Gordonia	SMS 9	*Gordonia sihwensis*	99.89%	PV097154
Streptomyces	SMS 10	*Streptomyces luridiscabiei*	98.22%	PV097155
Bifidobacterium	SMS 11	*Bifidobacterium pseudocatenulatum*	99.09%	PV097156
Streptomyces	SMS 12	*Streptomyces rochei*	98.09%	PX699887
Micromonospora	SMS 13	*Micromonospora rosaria*	97.78%	PV097158
Streptomyces	SMS 14	*Streptomyces pratensis*	99.33%	PV097159
Rhodococcus	SMS 15	*Rhodococcus qingshengii*	98.89%	PV097160
Streptomyces	SMS 16	*Streptomyces flavovirens*	99.93%	PV097161
Streptomyces	SMS 17	*Streptomyces albus*	98.70%	PV097162
Nocardiopsis	SMS 18	*Nocardiopsis dassonvillei*	99.56%	PV097163
Streptomyces	SMS 19	*Streptomyces griseus*	99.99%	PV097164
Rhodococcus	SMS 20	*Rhodococcus erythropolis*	99.65%	PV097165
Streptomyces	SMS 21	*Streptomyces pratensis*	99.18%	PV097166
Blastococcus	SMS 22	*Blastococcus saxobsidens*	99.12%	PV097167
Micrococcus	SMS 23	*Micrococcus endophyticus*	97.98%	PV097168
Streptomyces	SMS 24	*Streptomyces albus*	98.45%	PV097169
Streptomyces	SMS 25	*Streptomyces venezuelae*	98.81%	PV097170
Streptomyces	SMS 26	*Streptomyces tendae*	99.26%	PV097171
Streptomyces	SMS 27	*Streptomyces parvulus*	99.20%	PV097172
Streptomyces	SMS 28	*Streptomyces cirratus*	99.72%	PV097173
Streptomyces	SMS 29	*Streptomyces misionensis*	98.92%	PV097174
Nocardiopsis	SMS 30	*Nocardiopsis dassonvillei*	99.65%	PV097175
Kocuria	SMS 31	*Kocuria rhizophila*	99.70%	PV097176
Glutamicibacter	SMS 32	*Glutamicibacter uratoxydans*	96.90%	PV097177
Glutamicibacter	SMS 33	*Glutamicibacter endophyticus*	98.07%	PV097178
Streptomyces	SMS 34	*Streptomyces pratensis*	99.77%	PV097179
Streptomyces	SMS 35	*Streptomyces daghestanicus*	99.21%	PV097180
Streptomyces	SMS 50	*Streptomyces olivaceus*	98.71%	PV097191

abundances. it was calculated by the following equation:

$$E = \frac{H'}{\ln(S)}$$

where, *H'* is the Shannon diversity index and S is the species richness.

Beta Diversity Indices

Jaccard Diversity index: Jaccard Index (Jaccard, 1912) measures the portion of shared species between two samples.

$$J(X,Y) = \frac{\|X \cap Y\|}{\|X \cup Y\|}$$

Where, X∩Y, is the intersection of sets X and Y, i.e. elements common to both sets; X∪Y, is the union of sets X and Y, i.e. all unique elements from both sets combined.

Bray-Curtis Index: Measures the dissimilarity of species abundances between two samples (Bray and Curtis, 1957).

$$Bcij = 1 - \frac{2Cij}{Si + Sj}$$

Where, C_{ij} the sum of the absolute differences in abundances between corresponding species in samples *i* and *j*; S_i in the total abundance or sum of species abundances in sample *i*; S_j in the total abundance or sum of species abundances in sample *j*. Higher the dissimilarity value, the greater is the difference in species composition or abundance.

Results and Discussion

Total 36 cultivable actinobacterial isolates were obtained from limestone soil samples collected from two quarries of the Cuddapah Basin. Twelve isolates were obtained from Quarry A

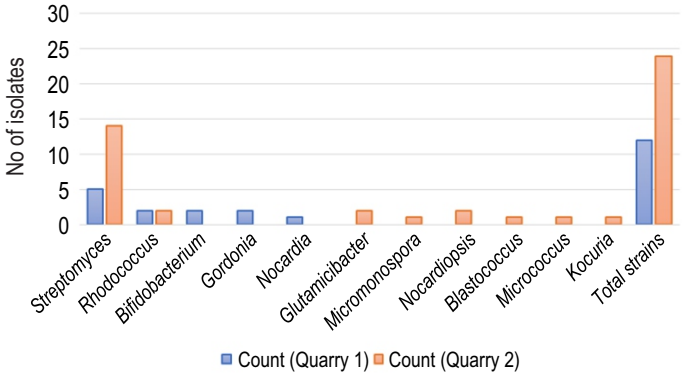


Fig. 2: Comparative Genus-level counts of isolates in each quarry (n=12 for Quarry A; n=24 for Quarry B as represented).Quarry A has5 genera (total n=12 isolates) and Quarry B had 8 genera (n = 24 in this subset).

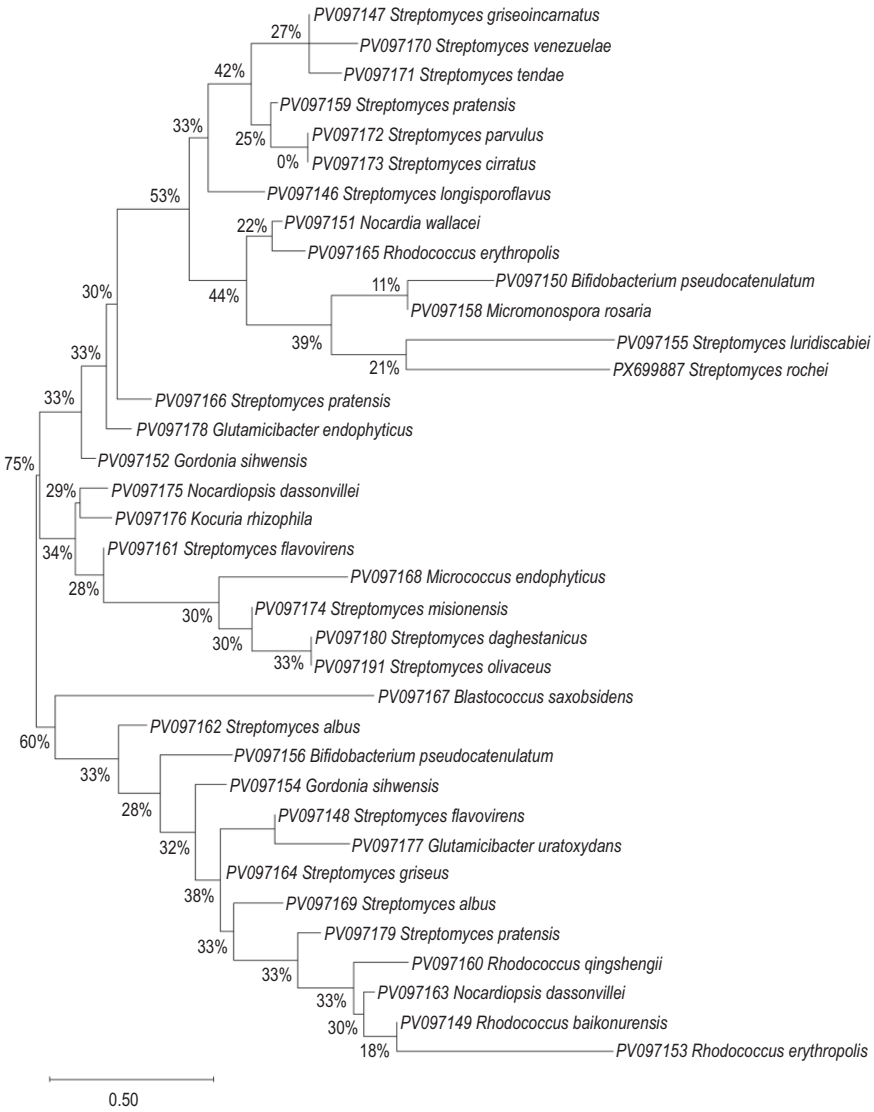


Fig. 3: Phylogenetic tree shows the phylogenetic relationship of isolates with related genera and inferred using the Maximum Likelihood method.

from five genera and 24 isolates from Quarry B from eight genera. Molecular identification by 16S rRNA gene sequencing showed >97% sequence similarity to known actinobacterial taxa confirming the affiliation of all isolates to known genera. Phylogenetic analysis improved the confidence in these identifications with isolates forming defined genus-specific clades, corresponding to members of the orders *Streptomycetales, Micrococcales* and *Mycobacteriales.* The strong phylogenetic clusters obtained were consistent with the taxonomic assignments, which suggest that the molecular identification is reliable and emphasizes wide taxonomic diversity of cultivable actinobacteria living in limestone ecosystems (Spratt, 2004).

The isolates were members of dominant and relatively rare actinobacterial genera such as *Streptomyces, Rhodococcus, Gordonia, Glutamicibacter, Micromonospora, Nocardiopsis, Micrococcus, Kocuria, Blastococcus, Nocardia* and *Bifidobacterium* (Table 1). The diversity of taxonomic lineages demonstrated that limestone soils offer a range of ecological niches that can accommodate phylogenetically distinct actinobacteria (Fig 3). This diversity is often linked to environmental heterogeneity, mineral-rich substrates, alkaline pH and nutrient-limited conditions favoring microorganisms with specific metabolic adaptations. Similarly, recent studies have shown that extreme and underexplored habitats are reservoirs of taxonomically diverse actinobacteria with huge ecological and biotechnological potential (Guevara-Luna *et al.,* 2024).

Diversity analysis revealed significant differences in community structure between the two quarries. Quarry A was moderately rich but had a high evenness (Shannon diversity index 1.468; Simpson diversity index 1−D 0.736). High Pielou's evenness value (0.912) suggested that the genera retrieved were fairly and evenly distributed, without any single taxon being strongly dominant. Chao1 richness estimate (5.167) was slightly higher than observed richness, indicating that the sampling effort likely captured most of the cultivable genera present in this habitat. The occurrence of only one singleton genus further substantiates a relatively complete representation of the dominant cultivable community.

By contrast, Quarry B was more taxonomically diverse, with eight observed genera and a Chao1 estimate of 10.667, suggesting that additional rare taxa were likely present, but not detected. This higher richness resulted in a comparable Shannon diversity index (1.466) to Quarry A. These contradictory findings highlight the strong effect of community evenness on diversity estimates. Due to strong dominance of *Streptomyces* (14 of the 24 isolates), the community structure in Quarry B was much more uneven, as indicated by lower Pielou's evenness value (0.705) and Simpson index (0.632). The increase in richness did not result in an increase in total diversity, because the abundance distribution was strongly dominated by one genus. Similar patterns have been reported in limestone and other oligotrophic environments where, *Streptomyces* often dominates cultivable communities due to their efficient sporulation, broad metabolic versatility and exceptional tolerance to environmental stress (Vijayakumar *et al.,* 2007). Extremely similar Shannon values observed in both quarries suggest that it was the evenness, and not the richness, which was the main contributor to the diversity of these communities. Evenness of taxa was similar across quarry A, whereas diversity was similar across quarry B due to extreme dominance of *Streptomyces*, despite higher richness. The computed diversity indices indicate that the cultivable actinobacterial communities are unevenly distributed throughout the limestone soils, implying habitat-specific selection of taxa. These results confirm the notion that differences in microbial diversity and uniformity reflect ecological specialization and could affect the functional capabilities of bacterial communities linked to limestone, comparable to the observations made by Stone *et al*. (2021) and Zhang *et al.* (2026) regarding soil bacterial diversity and ecosystem functioning.

This finding highlights the importance of considering multiple measures of diversity in the interpretation of microbial communities, since richness alone may not be sufficient to reflect the ecological complexity. Recent evaluations of alpha-diversity metrics have also pointed out that Shannon diversity is highly affected by both richness and abundance distribution and thus, offers a more holistic view on community organization than richness estimates alone (Cassol *et al.,* 2025).

Beta-diversity analysis also showed significant differentiation between the two limestone quarries. Jaccard similarity index was low at 0.182, implying that the two sites shared only two genera (*Streptomyces* and *Rhodococcus*) out of a total of eleven genera identified in both locations. Likewise, the Bray-Curtis dissimilarity value of 0.611 revealed significant differences in the patterns of genus abundance. These results indicate that local-scale environmental heterogeneity has a strong effect on the assembly of actinobacterial communities even within geographically connected limestone formations. Differences in the soil physico-chemical properties, moisture availability, organic matter, mineral composition and microhabitat characteristics may selectively promote different actinobacterial taxa, leading to different community structure (Riquelme *et al.,* 2015). Subtle environmental gradients result in marked shifts in microbial composition in calcareous and arid ecosystems, and similar observations have been reported in these environments (Stach *et al.,* 2003; Weeraphan *et al.,* 2025). A diversity values obtained in the present investigation were relatively low as compared to culture independent studies. High-throughput sequencing studies report soil actinobacteria to have Shannon indices higher than 4.0 and Simpson values close to 1.0, indicative of many rare and uncultivable taxa. Lower diversity observed here is likely due to the intrinsic limitations of culture-based methodologies, which favor the recovery of fast growing and easy to cultivate organisms, but ignore a large proportion of microbial diversity (Zhang *et al.,* 2019). The Chao1 estimates of species richness, especially in Quarry B, that exceed observed richness also suggest the presence of unknown taxa in these

limestone habitats. This observation aligns with the current knowledge that most environmental microorganisms are still uncultured and taxonomically undescribed even with advances in molecular ecology and microbial systematics (Weiss *et al.,* 2017).

The dominance of *Streptomycetes* and the recovery of other genera such as *Micromonospora, Nocardiopsis, Rhodococcus* and *Gordonia* are of particular importance as these groups are known to produce a variety of bioactive secondary metabolites. Limestone ecosystems are increasingly recognized as promising reservoirs of novel actinobacteria with potential applications in antimicrobial, anticancer and industrial biotechnology research (Helmi, 2025). The isolates recovered in this study are widely phylogenetically distributed, indicating not only significant ecological diversity, but also significant potential for future natural-product discovery. It is expected that combining culture-dependent isolation with modern culture-independent methods, such as amplicon sequencing and metagenomic analyses, will further uncover hidden diversity and enable the identification of new actinobacterial taxa from the limestone soils of the Cuddapah Basin.

The results suggest that limestone quarries of the Cuddapah Basin are inhabited by taxonomically diverse and compositionally different cultivable actinobacterial communities. Quarry A had better taxonomic balance, but Quarry B showed higher richness with a strong dominance of *Streptomyces*. This research recognizes limestone soils as important repositories of several actinobacteria with considerable biotechnological applications. The findings establish a foundation for the selective isolation of new bioactive metabolite-producing strains, promote the conservation of microbial biodiversity, and facilitate prospective uses in pharmaceutical development, agriculture, environmental restoration, and industrial biotechnology. In addition, the produced phylogenetic and diversity data serve as reference for upcoming ecological and taxonomic studies of microbial communities associated with limestone.

Acknowledgment

The authors sincerely acknowledge the financial support to Ms. S. Meenakshi by the Department of Science and Technology, Karnataka Science and Technology Promotion Society (KSTePS), Karnataka (DST/KSTePS/Ph.D.Fellowship/LIF-14:2023-24, dated: 23.01. 2024).

Authors' contribution: S. Meenakshi: Conceptualization, methodology design, investigation, data analysis, and preparation of the original manuscript draft; **J. Hiremath:** Preliminary data processing and biostatistics analysis; **M.P. Shilpa:** Contributed in draft preparation; **S. Shivaveerakumar:** Supervision, validation, critical review and final approval of the manuscript.

Funding: Not applicable.

Research content: The research work presented here is original and has not been published elsewhere.

Ethical approval: Not applicable.

Conflict of interest: The authors declare that there is no conflict of interest associated with this publication.

Data availability: The 16S rRNA sequence of all the isolates are available on GenBank databases with the GenBank accession numbers(https://www.ncbi.nlm.nih.gov/nuccore?term=PV097146+%3A+PV097192%5Baccn%5D&cmd=DetailsSearch& https://www.ncbi.nlm.nih.gov/nuccore/PX699887.1/). Other data and analyses generated during the current study are included in this published article.

Consent to publish: All authors have read, approved, and provided their consent for the publication of this manuscript.

References

Ahmadi, E., M. Kowsari, D. Azadfar and G.S. Jouzani: Rapid and economical protocols for genomic and metagenomic DNA extraction from oak (*Quercus brantii* Lindl.). *Ann. For. Sci.*, **75**, 1-14 (2018).

Baig, U., N. Dahanukar, N. Shintre, K. Holkar, A. Pund, U. Lele, T. Gujarathi, K. Patel, A. Jakati, R. Singh, H. Vidwans, V. Tamhane, N. Deshpande and M. Watve: Phylogenetic diversity and activity screening of cultivable actinobacteria isolated from marine sponges and associated environments from the western coast of India. *Access Microbiol.,* **3**, 1-13 (2021).

Bray, J.R. and J.T. Curtis: An ordination of the upland forest communities of southern Wisconsin. *Ecol. Monogr.,* **27**, 325–349 (1957).

Cassol, I., M. Ibañez and J. Bustamante: Key features and guidelines for the application of microbial alpha diversity metrics. *Sci. Rep.,* **15**, 1-13 (2025).

Cazzolla Gatti, R., N. Amoroso and A. Monaco: Estimating and comparing biodiversity with a single universal metric. *Ecol. Model.,* **424**, 109020 (2020).

CD Genomics: Diversity analysis of actinomycetes. Available at: https://www.cd-genomics.com/microbioseq/diversity-analysis-of-actinomycetes.html (accessed 21 November 2025).

Chakraborty, J., K. Palit and S. Das: Metagenomic approaches to study the culture-independent bacterial diversity of a polluted environment—A case study on north-eastern coast of Bay of Bengal, India. In: Microbial Biodegradation and Bioremediation. 2nd Edn., Elsevier, pp. 81–107 (2022).

Chao, A. and S.-M. Lee: Estimating the number of classes via sample coverage. *J. Am. Stat. Assoc.,* **87**, 210–217 (1992).

Guevara-Luna, J., I. Arroyo-Herrera, E.Y. García, P. Estrada-de los Santos, A. Ortega-Nava and M.S. Vásquez-Murrieta: Diversity and biotechnological potential of cultivable halophilic and halotolerant bacteria from the "Los Negritos" geothermal area. *Microorganisms,* **12**, 482 (2024).

Helmi, N. R.: Exploring the diversity and antimicrobial potential of actinomycetes isolated from different environments in Saudi Arabia: A systematic review. *Front. Microbiol.*, **16**, 1568899 (2025).

Jaccard, P.: The distribution of the flora in the alpine zone. *New Phytol.,* **11**, 37–50 (1912).

Jose, P. and S.R. Jebakumar: Phylogenetic diversity of actinomycetes cultured from coastal multipond solar saltern in Tuticorin, India.

Aquat. Biosyst., **8**, 23 (2012).
Karthik, L.: Protocols of Actinomycetes: Microbiology to Gene Editing. 1st Edn., CRC Press, Boca Raton, 376 pages (2024).
Kim, B.R., J. Shin, R.B. Guevarra, J.H. Lee, D.W. Kim, K.H. Seol, J.H. Lee, H.B. Kim and R.E. Isaacson: Deciphering diversity indices for a better understanding of microbial communities. *J. Microbiol. Biotechnol.,* **27**, 2089–2093 (2017).
Kuester, E. and S. T. Williams: Selection of media for isolation of *Streptomycetes. Nature,* **202**, 928–929 (1964).
Kumar, S., G. Stecher, M. Suleski, M. Sanderford, S. Sharma and K. Tamura: MEGA12: Molecular Evolutionary Genetics Analysis version 12 for adaptive and green computing. *Mol. Biol. Evol.,* **41**, 1–9 (2024).
Larkin, M.A., G. Blackshields, N.P. Brown, R. Chenna, P.A. McGettigan, H. McWilliam, F. Valentin, I.M. Wallace, A. Wilm, R. Lopez, J.D. Thompson, T.J. Gibson and D.G. Higgins: Clustal W and Clustal X version 2.0. *Bioinformatics,* **23**, 2947–2948 (2007).
Li, C., X. Chen, Z. Jia, L. Zhai, B. Zhang, U. Grüters, S. Ma, J. Qian, X. Liu, J. Zhang and C. Müller: Meta-analysis reveals the effects of microbial inoculants on the biomass and diversity of soil microbial communities. *Nat. Ecol. Evol.,* **8**, 1270–1284 (2024).
McCaig, A.E., L.A. Glover and J.I. Prosser: Molecular analysis of bacterial community structure and diversity in unimproved and improved upland grass pastures. *Appl. Environ. Microbiol.,* **65**, 1721–1730 (1999).
Meenakshi, S., J. Hiremath, M.H. Meenakshi and S. Shivaveerakumar: Actinomycetes: Isolation, cultivation and its active biomolecules. *J. Pure Appl. Microbiol.,* **18**, 118–143 (2024).
Ndabankulu, K., S. O. Egbewale, Z. Tsvuura and A. Magadlela: Soil microbes and associated extracellular enzymes largely impact nutrient bioavailability in acidic and nutrient poor grassland ecosystem soils. *Scien. Rep.,* **12**, 12601 (2022).
Nimaichand, S., W.-Y. Zhu, L.-L. Yang, H. Ming, G.-X. Nie, S.-K. Tang, D. S. Ningthoujam and W.J. Li: Streptomyces manipurensis sp. nov., a novel actinomycete isolated from a limestone deposit site in Manipur, India. *Antonie van Leeuwenhoek,* **102**, 133–139 (2012).
Pielou, E.C.: The measurement of diversity in different types of biological collections. *J. Theor. Biol.,* **13**, 131–144 (1966).
Quadri, S.R. and D. Agsar: Detection of melanin producing thermosalkaliphilic Streptomyces from limestone quarries of the Deccan traps. *World J. Sci. Technol.*, **2**, 08-12 (2012).
Quadri, S.R., X.P. Tian, J. Zhang, J. Li, G.X. Nie, S.K. Tang, J. Al-Ruwaili, D. Agasar, W.J. Li and S.G. Dastager: *Nonomuraea indica* sp. nov., novel actinomycete isolated from a limestone open pit mine, India. *J. Antibiot.,* **68**, 491–495 (2015).
Riquelme, C., J.J.J. Marshall Hathaway, M.D.L.N. Enes Dapkevicius, A.Z. Miller, A. Kooser, D.E. Northup, V. Jurado, O. Fernandez, C. Saiz-Jimenez and N. Cheeptham: Actinobacterial diversity in volcanic caves and associated geomicrobiological interactions. *Front. Microbiol.,* **6**, 1342 (2015).
Shannon, C.E.: A mathematical theory of communication. *Bell Syst. Tech. J.,* **27**, 379–423 (1948).
Shirling, E.B. and D. Gottlieb: Cooperative description of type strains of Streptomyces. V. Additional descriptions. *Int. J. Syste. Bacteriol.,* **22**, 265–394 (1972).
Shirling, E.B. and D. Gottlieb: Methods for characterization of *Streptomyces* species. *Int. J. Syst. Bacteriol.,* **16**, 313–340 (1966).
Shirokikh, I.G., N.A. Bokov, S.E. Mokrushina, A.S. Makhlacheva and A. A. Shirokikh: Characteristics of actinomycete complexes on outcrops of reef limestone. *Eura. Soil Sci.,* **59**, 61 (2026).
Shivaveerakumar, S., D.N. Madhusudhan, H. Raghavendra and A. Dayanand: Screening of Streptomyces and process optimization for the production of tyrosinase. *J. Pure Appl. Microbiol.*, **8,** 32453252 (2014).
Silavery, M., H. Jyothi, P. Shilpa and S. Shivaveerakumar: Impact of soil physicochemical properties on the diversity of limestone-acquired actinomycetes. *Res. J. Biotechnol.,* **20**, 10–16 (2025).
Simpson, E.H.: Measurement of diversity. *Nature,* **163**, 688 (1949).
Spratt, D. A.: Significance of bacterial identification by molecular biology methods. *Endod. Topics*., **9**, 5-14 (2004).
Stach, J.E.M., L.A. Maldonado, D.G. Masson, A.C. Ward, M. Goodfellow and A.T. Bull: Statistical approaches for estimating actinobacterial diversity in marine sediments. *Appl. Environ. Microbiol.,* **69**, 6189–6200 (2003).
Stone, B. W., J. Li, B.J. Koch, S.J. Blazewicz, P. Dijkstra, M. Hayer, K.S. Hofmockel, X.J.A. Liu, R.L. Mau, E.M. Morrissey, J. Pett-Ridge, E. Schwartz and B.A. Hungate: Nutrients cause consolidation of soil carbon flux to small proportion of bacterial community. *Nat. Commun*., **12**, 3381 (2021).
Sunitha, V., B.M. Reddy and M.R. Reddy: Mineral resources of the Cuddapah Basin. *J. Biol. Chem. Res*., **31**, 226-235 (2014).
Tamreihao, K., S. Nimaichand, S.B. Chanu, K.A. Devi, R. Lynda, N. Jeeniita and D.S. Ningthoujam: Acidotolerant *Streptomyces* sp. MBRL 10 from limestone quarry site showing antagonism against fungal pathogens and growth promotion in rice plants. *J. King Saud Univ. Sci.,* **30**, 143–152 (2018).
Tyc, O., C. Song, J. S. Dickschat, M. Vos and P. Garbeva: The ecological role of volatile and soluble secondary metabolites produced by soil bacteria. *Tren. Microbiol.,* **25**, 280–292 (2017).
Vijayakumar, R., C. Muthukumar, N. Thajuddin, A. Panneerselvam and R. Saravanamuthu: Studies on the diversity of actinomycetes in the Palk Strait region of Bay of Bengal, India. *Actinomycetologica,* **21**, 59–65 (2007).
Weeraphan, T., C. Chou, N. Chanthathamrongsiri, T. Sirirak, S. Putchakarn, S. Chamni and W. Phongsopitanun: Microbiome profiling and Actinomycetes isolation from tropical marine sponges. *AIMS Microbiol.,* **11**, 182–205 (2025).
Weiss, S., Z.Z. Xu, S. Peddada, A. Amir, K. Bittinger, A. Gonzalez, C. Lozupone, J.R. Zaneveld, Y. Vázquez-Baeza, A. Birmingham, E.R. Hyde and R. Knight: Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome,* **5**, 27 (2017).
Yabe, S., Y. Sakai, K. Abe and A. Yokota: Diversity of Ktedonobacteria with actinomycetes-like morphology in terrestrial environments. *Micro. Environ.,* **32**, 61–70 (2017).
Yang, S., W. Zhang, B. Yang, X. Feng, Y. Li, X. Li and Q. Liu: Metagenomic evidence for antibiotic-associated actinomycetes in the Karamay Gobi region. *Front. Microbiol.,* **15**, 1330880 (2024).
Zhang, B., X. Wu, X. Tai, L. Sun, M. Wu, W. Zhang, X. Chen, G. Zhang, T. Chen, G. Liu and P. Dyson: Variation in actinobacterial community composition and potential function in different soil ecosystems of the arid Heihe River Basin, Northwest China. *Front. Microbiol.,* **10**, 2209 (2019).
Zhang, J., J. Huang, Y. Li, X. Wu and F. Guo: The Actinobacteria-to-Proteobacteria (A/P) ratio: A novel oral microbial marker for metabolic syndrome. *Int. Dent. J*., **76**, 109499 (2026).