



Research Article

Molecular Characterization and Identification of *Micromonospora* Genus Bacteria from the Van Lake Basin

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Abstract: In this study, *Micromonospora* species were isolated from soil and sediment samples collected from the Van Lake Basin and characterized through a polyphasic taxonomic approach combining phenotypic, biochemical, and molecular (16S rRNA gene) analyses to elucidate their phylogenetic relationships and diversity. The dilution plate method was employed for isolation, yielding 141 purified *Micromonospora* isolates. Colonies with distinct morphologies were selected, numbered, and preserved in cryogenic tubes. The moisture and pH levels of the soil and sediment samples were also measured. Based on colony pigmentation, the isolates were classified into 11 color groups. For numerical taxonomy, 83 morphological and biochemical characteristics were assessed, and a dendrogram was constructed using the Simple Matching Coefficient (S_{SM}) in MVSP 3.1 software. As a result, 22 clusters (7 major and 15 minor) were formed based on 78% similarity. Cluster members generally showed similarity in altitude and geographic origin. Scanning Electron Microscopy (SEM) was used to analyze the spore chain morphology, contributing to more accurate identification. For molecular characterization, genomic DNA was extracted from 13 selected isolates, and the 16S rRNA gene was amplified using universal primers 27F and 1492R. Phylogenetic trees were constructed using Maximum Likelihood and Bayesian inference algorithms. The resulting topologies were largely congruent, and *Micromonospora* isolates clustered with reference strains from NCBI with high sequence homology. These findings revealed the genetic positions of the isolates and provided insight into their evolutionary relationships, contributing to the microbial diversity knowledge of alkaline environments such as the Van Lake Basin.

Keywords: *Micromonospora*, Molecular characterization, Van Lake basin, 16S rRNA

Van Gölü Havzası *Micromonospora* Cinsi Bakterilerinin Moleküler Karakterizasyonu ve Teşhisi

Öz: Bu çalışmada, Van Gölü Havzası'ndan toprak ve sediment örneklerinden izole edilen *Micromonospora* türleri, filogenetik ilişkilerini ve çeşitliliklerini ortaya koymak amacıyla fenotipik, biyokimyasal ve moleküler (16S rRNA gen bölgesi) analizleri bir arada içeren polifazik taksonomik bir yaklaşımla karakterize edilmiştir. İzolasyon için dilüsyon plak yöntemi kullanılmış ve toplam 141 saf *Micromonospora* izolatu elde edilmiştir. Farklı morfolojiye sahip koloniler seçilerek numaralandırılmış ve kriyojenik tüplerde muhafaza edilmiştir. Toprak ve sediment örneklerinin nem ve pH değerleri ölçülmüş, izolatlar koloni pigmentasyonuna göre 11 renk grubuna ayrılmıştır. Nümerik taksonomi için 83 morfolojik ve biyokimyasal karakter değerlendirilmiş ve MVSP 3.1 yazılımında Simple Matching Coefficient (S_{SM}) yöntemiyle dendrogram oluşturulmuştur. %78 benzerlik oranına göre 7'si majör, 15'i minör olmak üzere toplam 22 küme tespit edilmiştir. Aynı kümede yer alan izolatların genellikle benzer rakım ve lokalitelerden geldiği gözlemlenmiştir. Spor zinciri morfolojisi Taramalı Elektron Mikroskobu (SEM) ile incelenerek teşhis sürecine katkı sağlanmıştır. Moleküler karakterizasyon için seçilen 13 izolatın genomik DNA'sı izole edilmiş ve 16S rRNA gen bölgesi, 27F ve 1492R evrensel primerleri

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kullanılarak çoğaltılmıştır. Maximum Likelihood ve Bayesian çıkarım algoritmaları ile filogenetik ağaçlar oluşturulmuş, elde edilen ağaç topolojilerinin genellikle benzerlik gösterdiği belirlenmiştir. İzolatların NCBI'dan alınan referans türlerle güçlü bir homoloji gösterdiği ve genetik pozisyonlarının ortaya konduğu tespit edilmiştir.

Anahtar Kelimeler: *Micromonospora*, Moleküler karakterizasyon, Van Gölü havzası, 16S rRNA

1. Introduction

The genus *Micromonospora*, first described by Ørskov in 1923, constitutes an ecologically and industrially significant group within the family Micromonosporaceae, subclass Actinobacteria. These Gram-positive, chemoorganotrophic, aerobic bacteria exhibit a high G+C content and are recognized as true actinomycetes. Although traditionally considered rare in terrestrial environments, *Micromonospora* species have frequently been isolated from diverse aquatic and semi-aquatic habitats, including lake and marine sediments, freshwater streams, rice paddies, coastal sands, and peat swamps (Cross, 1981; Kawamoto, 1989; Zhao et al., 2004; Maldonado et al., 2009).

Micromonospora strains typically produce single spores directly on the substrate mycelium and generally lack aerial hyphae (Nouioui et al., 2025). Their colonies often display ambiguous morphological features, rendering differentiation from other actinomycetes difficult based solely on microscopic or phenotypic characteristics. Consequently, traditional morphological and biochemical methods prove insufficient for accurate identification. Recent advances have demonstrated the efficacy of molecular approaches such as RAPD analysis and genus-specific primers for rapid and reliable identification (Laurent et al., 1999; Moron et al., 1999; Zhi et al., 2006).

Phylogenetic analyses based on 16S rRNA gene sequences reveal a high degree of similarity among *Micromonospora* strains. However, discrimination at the species and subspecies level necessitates additional genetic methods, including DNA-DNA hybridization (DDH), Average Nucleotide Identity (ANI), and alternative marker genes such as *gyrB* and *rpoB*. Notably, Kasai et al. (2000) reported significant phylogenetic discrepancies between *gyrB*-based and 16S rRNA-based trees, underscoring the importance of multilocus or genome-wide approaches for robust taxonomic resolution.

This genus is renowned for its prolific production of secondary metabolites, particularly aminoglycoside antibiotics (e.g., gentamicin, netilmicin) and anticancer compounds (e.g., lomaiviticins, lupinacidins, anthracyclines) (Kasai et al., 2000; Jensen et al., 2005). As such, *Micromonospora* ranks as the second most important antibiotic-producing actinomycete genus after *Streptomyces*. In addition, members of this genus produce biologically active molecules such as vitamin B12 and antifungal compounds, and exhibit unique capabilities including rubber degradation and survival in radioactive environments (Wagman et al., 1969; Trujillo et al., 2006). Genomic analyses reveal that *Micromonospora* strains possess rich biosynthetic gene clusters (BGCs) encoding specialized metabolites with antibiotic, anticancer, or antiviral activities, some of which are genus-specific (Carro et al., 2018). Furthermore, numerous antibiotic compounds from various families—including gentamicin, sisomicin, verdamicin, fortimicins, neomycin, and sagamicin—have been isolated from this genus (Trujillo et al., 2014).

Ecologically, *Micromonospora* species contribute significantly to organic matter mineralization by degrading cellulose, chitin, lignin, and other complex polysaccharides via hydrolytic enzymes, thereby facilitating nutrient cycling and soil fertility (Erikson, 1941; Gacto et al., 2000). Some strains have been isolated from nitrogen-fixing root nodules of actinorhizal and leguminous plants, indicating beneficial endophytic associations. Due to their production of vitamins and plant growth-promoting hormones, these strains are considered valuable as phytostimulants or microbial inoculants (“biofertilizers”) in sustainable agriculture (Valdés et al., 2005; Trujillo et al., 2006).

The genus continues to expand with the discovery of novel species from extreme and underexplored environments such as thermal lakes, deep-sea habitats, and saline-alkaline soils.

Studies on marine and freshwater sediments have demonstrated *Micromonospora*'s dominance in deeper sediment layers, where its spores exhibit greater resilience than those of *Streptomyces* and *Nocardia*-like organisms (Johnston & Cross, 1976; Willoughby, 1969). Their prevalence in anaerobic and microaerophilic conditions further highlights their metabolic versatility.

Given their ecological ubiquity, metabolic potential, and taxonomic complexity, the isolation and characterization of *Micromonospora* species from underexplored habitats—such as the Van Lake Basin in Eastern Türkiye—offer promising opportunities for discovering novel strains with significant biotechnological and agricultural applications. The alkaline and saline nature of the Van Lake ecosystem provides an ideal environment for isolating alkaliphilic actinomycetes and offers valuable insights into microbial adaptation and secondary metabolite biosynthesis. Accordingly, enhancing the genetic diversity of this genus by targeting such environments is critical, as the discovery of new bacterial strains is likely to lead to novel bioactive compounds (Goodfellow et al., 2018).

The aim of this study was to isolate and characterize *Micromonospora* species from soil and sediment samples of the Van Lake Basin through a polyphasic taxonomic approach combining phenotypic, biochemical, and molecular analyses to assess their genetic diversity and biotechnological potential.

2. Material and Methods

2.1. Selection of soil and sediment samples, and isolation and purification of *Micromonospora*

For this study, soil and sediment samples were collected from 15 different regions of the Van Lake basin (Table 1) and placed in sterile containers and sterile plastic bags (Figure 1). The collected soil samples were labeled according to their laboratory numbers and stored at 4°C.

Table 1. Sampling locations, coordinates, and elevations of soil and sediment samples used for *Micromonospora* isolation

No	Site	Coordinates	Elevation
N1	Karasu Stream	38°39'46N 043°19'03E	1693 m
N2	Yaylıyaka Marsh	38°49'59N 043°23'03E	1675 m
N3	Yaylıyaka Roadside	38°49'59N 043°23'09E	1678 m
N4	Çelebibağ Marsh	39°00'28N 043°18'56E	1673 m
N5	Zilan Stream	39°00'26N 043°18'58E	1670 m
N6	Erciş-Adilcevaz Highway, Van Lake Shore	38°57'35N 043°14'35E	1664 m
N7	Livit Hill	38°53'29N 043°02'30E	1707 m
N8	Adilcevaz	38°47'50N 042°42'34E	1722 m
N9	Ahlat Marshes	38°44'02N 042°26'36E	1665 m
N10	Adabağ	38°24'24N 042°26'51E	1670 m
N11	Kıy düzü Village, Tatvan	38°34'09N 042°22'31E	1710 m
N12	Tatvan	38°26'27N 042°19'04E	1748 m

Tablo 1. Sampling locations, coordinates, and elevations of soil and sediment samples used for *Micromonospora* isolation (Continued)

No	Site	Coordinates	Elevation
N13	Küçüksu Village, Tatvan	38°26'26N 042°19'37E	1737 m
N14	Balaban	38°19'58N 042°52'04E	1744 m
N15	Edremit	38°25'21N 043°15'37E	1698 m

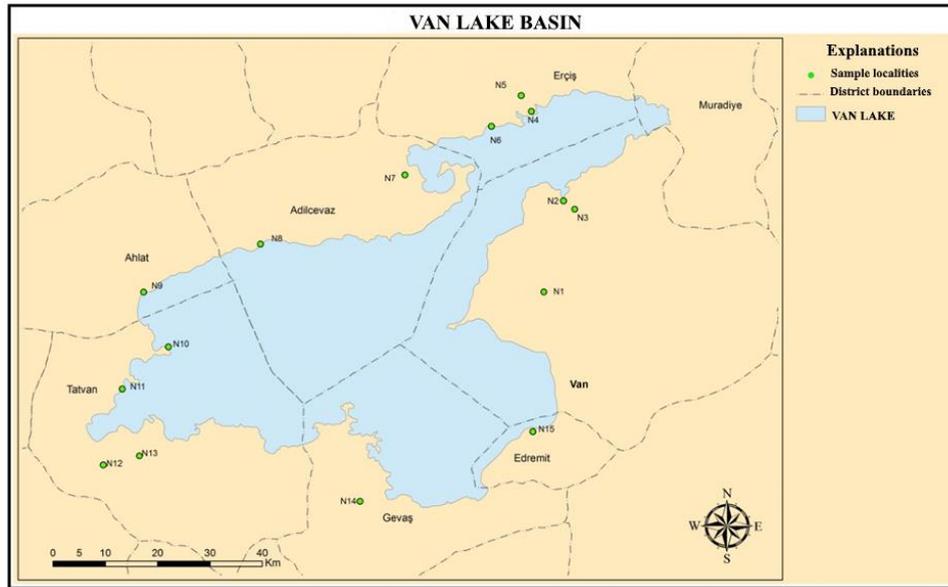


Figure 1. Map indicating the locations of soil and sediment sampling stations in the Lake Van Basin.

The pH measurement and moisture determination of the collected soil and sediment samples were initially conducted. To isolate *Micromonospora* bacteria, Medium 65 (CaCO₃ 2.0 g, Malt extract 10.0 g, Yeast extract 4.0 g, Glucose 4.0, Agar 20.0, ddH₂O 1000 ml, pH 7.2) and SM3 (Glikoz 10.0 g, Pepton 5.0 g, Tripton 3.0 g, Agar 15.0 g, ddH₂O 1000 ml, pH 7.2) culture media were prepared. Dilutions of 1/10 and 1/10,000 were made, and 100 µl of each dilution was spread thoroughly onto the surface of Medium 65 and SM3 plates. The Petri dishes were then incubated at 27°C for 7 days. After incubation, colonies that formed mycelium and substrate mycelium were evaluated as potential *Micromonospora* bacterial colonies. To obtain pure cultures, mixed cultures that developed on Medium 65 and SM3 were streaked onto sterile Bennet's agar medium (Yeast Extract 1.0 g, Lab-Lemco 0.8 g, Bacto Casitone 2.0 g, Glycerol 10 g, Agar 18 g, ddH₂O 1000 ml, pH 7.2) using the streak plate method. The pure cultures were transferred to cryogenic tubes containing 20% glycerol and stored in a deep freezer. For the identification of all isolates, color grouping was performed to determine the colors of both aerial and substrate mycelia. For further color classification of purified strains, isolates were inoculated onto oatmeal agar (ISP 3) (Shirling & Gottlieb, 1966) using the streaking method. After a 14 day incubation period at 28°C, the colors of the mycelia and substrate mycelia were determined according to a color catalog and grouped accordingly.

2.2. Color grouping

A total of 141 presumptive *Micromonospora* isolates were cultured on ISP 3 (Oatmeal agar; [Shirling & Gottlieb, 1966](#)) and incubated at 28°C for 21 days. Aerial and substrate mycelium colors were determined using a color chart and used for colony grouping.

2.3. Tests for numerical taxonomy

The isolates were analyzed for 83 phenotypic characteristics as recommended by [Williams et al. \(1983\)](#). All results were converted to binary data (1/0) and analyzed using MVSP 3.1 software (Multivariate Statistical Package). Similarity coefficients (Ssm) were calculated, and UPGMA clustering was performed.

2.4. Morphological characteristics

The isolates were transferred from glycerol stocks to SM3 agar ([Tan et al., 2006](#)) and incubated at 27°C for 14–21 days. Aerial mycelium color, substrate mycelium color, pigment production, and spore chain morphology were observed microscopically following [Shirling & Gottlieb \(1966\)](#).

2.5. Antimicrobial activity

Antimicrobial potential was evaluated against six test organisms following the method of [Williams et al. \(1983\)](#). Isolates grown on Bennet's agar were streaked on fresh media, and pathogens were cross-streaked after 7 days of incubation. Inhibition zones were recorded as an indication of sensitivity or resistance.

2.6. Degradation activities

Degradation capabilities of the isolates were tested for eight substrates (xanthine, tween 80, elastin, casein, guanine, tyrosine, esculin, and arbutin) on Bennett's agar ([Jones, 1949](#)). Clear zones after incubation at 27°C for ~5 days were scored as positive.

2.7. Physiological and chemical inhibitor tests

Isolates were evaluated for growth at varying pH values (4.3 and 7.2) and temperatures (4°C to 45°C) using Medium 65 (DSMZ). Tolerance to NaCl (4–13%), sodium azide (0.01–0.02%), phenol (0.1%), and tallus acetate (0.001–0.01%) was tested by incorporating inhibitors into the medium.

2.8. Hippurate hydrolysis

Hippuricase activity was assessed using the method of [Thirsk \(1957\)](#), modified by [Ziegler & Kutzner \(1973\)](#). Phenol red-containing hippurate agar slants were inoculated and incubated at 25°C. Pink color development indicated positive activity.

2.9. Lipolysis activity

Lipase production was determined using egg yolk agar prepared according to [Nitsch & Kutzner \(1969\)](#). Lipolysis was evidenced by the formation of clear zones (10–15 mm diameter) around colonies.

2.10. Pectin hydrolysis

Pectinolytic activity was evaluated on 0.5% pectin-containing medium (Sands et al., 1972) after 6 days of incubation at 25°C. Colonies were treated with 1% CTAB, and clear zones were recorded as positive.

2.11. Nitrate reduction and H₂S production

Nitrate reductase activity was tested using nitrate agar tubes per Williams et al. (1983). Color changes upon addition of sulfanilic acid and α -naphthylamine reagents indicated positive results. H₂S production was assessed on SIM medium by black precipitate formation along the stab line.

2.12. Nitrogen utilization tests

The ability to utilize 11 nitrogen sources was tested using the medium of Williams et al. (1983). Growth was evaluated after 7 and 14 days at 27°C and scored against positive (L-asparagine) and negative controls.

2.13. Carbon utilization tests

Utilization of 16 carbon sources was assessed using ISP 9 medium (Shirling & Gottlieb, 1966). Each source was sterilized separately and incorporated into basal media. Growth evaluation was performed on days 7 and 14 relative to glucose (positive control) and carbon-free medium (negative control).

2.14. Numerical taxonomic analysis

A total of 141 potential *Micromonospora* isolates were evaluated for numerical taxonomy based on 83 phenotypic characters recommended by Williams et al. (1983). All test strains were preserved at -20°C in 20% glycerol prior to analysis. The complete set of tests applied for identification purposes is summarized in Table 3. For each isolate, test results were recorded in a binary format, with positive results indicated by “+” and negative results by “-”.

All data were entered into the MVSP 3.1 software (Multi-Variate Statistical Package) as 1/0 and subjected to cluster analysis using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) algorithm to generate similarity dendrograms. Similarity coefficients were calculated using the Simple Matching Coefficient (S_{SM}) method.

To assess the reproducibility of tests, eight randomly selected duplicate strains (Table 2) were included in the study. Similarity analyses were performed using both S_{SM} (Sokal & Michener, 1958). Test variance (S_i²) was calculated following the method of Sneath & Johnson (1972) to identify experimental errors.

Table 2. Duplicate strains used to detect test errors in numerical taxonomy.

Duplicated Strains		
1.	N0006	N0007
2.	N0010	N0011
3.	N0023	N0024
4.	N0044	N0045
5.	N0070	N0071
6.	N0108	N0109
7.	N0120	N0121
8.	N0126	N0127

2.15. Determination of spore chain morphology by scanning electron microscopy (SEM)

Ten potential *Micromonospora* isolates, previously purified and stored in glycerol at -20°C, were selected and cultured on Medium 65 for 7 days at 27°C. The spore chain morphology of these strains was then examined using Scanning Electron Microscopy (SEM) at the Institute of Nuclear Sciences, Ankara University.

2.16. Isolation and purification of genomic DNA

Based on the color grouping and numerical analysis, 13 test organisms were selected according to their dendrogram groupings. A single colony from each strain was taken and cultured on Medium 65 agar medium at 28 °C for 7 days. For the isolation of genomic DNA from the isolated bacteria, a modified version of the method developed by Ausubel (1992) was employed (Ertas et al., 2014).

Table 3. Tests used in numerical taxonomy of *Micromonospora* isolates

<u>Aerial Spore Pigmentation</u>	<u>Growth tests</u>
1. Aerial Spore Formation	41. 4 °C
2. Dark Brown	42. 10 °C
3. Black	43. 27 °C
4. Cream	44. 37 °C
5. Pink	45. 45 °C
6. Yellow	46. pH 4.3
7. Orange	47. pH 7.2
8. Orange-Black	<u>Growth tests (%w/v)</u>
9. White	48. NaCl (4%)
10. Light Brown	49. NaCl (10%)
11. Gray	50. NaCl (13%)
12. Red	51. Sodium azide (0.01%)
<u>Substrate Mycelium Pigmentation</u>	52. Sodium azide (0.02%)
13. Brown	53. Phenol (0.1%)
14. Black	54. Tallus acetate (0.001%)
15. Orange	55. Tallus acetate (0.01%)
16. Dark Red	<u>Nitrogen sources (0.1% w/v)</u>
17. Yellow	56. L-Cysteine
18. Cream	57. L-Valine
19. White	58. L-Tyrosine
<u>Diffuse Pigment Color</u>	59. L-Phenylalanine
20. Brown	60. L-Cystine
21. Black	61. L-Arginine
22. Dark Red	62. L-Serine
<u>Antimicrobial Activity</u>	63. L-Methionine
23. <i>Bacillus subtilis</i>	64. L-Tryptophan
24. <i>Enterococcus faecalis</i>	65. L-Threonine
25. <i>Esherichia coli</i>	66. L-Asparagine
26. <i>Micrococcus luteus</i>	67. L-Hydroxyproline
27. <i>Salmonella typhinium</i>	<u>Carbon sources (1% w/v)</u>
28. <i>Staphylococcus aureus</i>	68. Sucrose
<u>Degradation tests</u>	69. Arabinose
29. Guanine	70. Inositol
30. Elastin	71. Mannitol
31. Xanthine	72. Fructose
32. Tween 80	73. Rhamnose
33. Casein	74. Mannose
34. Arbutin	75. Lactose
35. Esculin	76. Arabitol
<u>Enzyme tests</u>	77. Trehalose
36. Hippurate Hydrolysis	77. Dulcitol
37. Lipolysis	79. Galactose
38. Pectin Hydrolysis	80. Xylitol
39. Nitrate Reduction	81. Glucose
40. H ₂ S Production	82. Erythritol
	83. Ribose

2.17. Analysis of the 16S rRNA gene region

Following the sequencing of the 16S rRNA gene region, ab1 files were analyzed using Codon Code Aligner V.6.0.2. Chromatograms for each strain were carefully examined, and low-quality base sequences (ambiguous bases marked as ‘N’), typically found at the ends, were trimmed to generate contigs. The 16S rDNA nucleotide sequences of all isolates were used to construct phylogenetic dendrograms. Species closely related to each strain were identified via BLAST searches in NCBI, and sequences were compared both among themselves and with database sequences. Sequences were imported in FASTA format into MEGA7 software and aligned using Clustal W to identify conserved regions. The resulting alignment file was converted to Phylip and Nexus formats using the ALTER platform. Maximum Likelihood (ML) trees were constructed using RAxML-HPC BlackBox on the CIPRES Science Gateway V.3.3. jModelTest determined GTR+G+I as the best-fit model for Bayesian analysis in MrBayes, where 1.000.000 trees were generated with a burn-in of 250.000. The remaining 750,000 trees were used to produce the most suitable Bayesian tree. Comparative analyses were performed between the resulting ML and Bayesian phylogenetic trees.

3. Results

3.1. Physicochemical properties of soil and sediment samples

The pH and moisture contents of soil and sediment samples were measured prior to bacterial isolation. Soil pH values ranged from 6.80 (sample N4) to 9.06 (sample N6), indicating variation in soil types. Moisture content varied between 18.35% (N6) and 64.12% (N1), with most samples exhibiting high moisture levels. Sediment samples showed similar pH values (7.15–7.57) regardless of elevation. Moisture levels in sediments were generally consistent, except for sample N6, which showed lower moisture due to its high sand content. All values are summarized in Table 4.

Table 4. Determination of moisture content and pH in soil and sediment samples

Lab. No	Moisture (%)	pH
N1	64.12	7.07
N2	48.73	8.55
N3	19.38	7.51
N4	53.66	6.80
N5	60.60	6.86
N6	18.35	9.06
N7	33.28	7.50
N8	28.65	7.64
N9	41.78	7.46
N10	39.80	7.40
N11	40.42	7.51
N12	42.94	7.41
N13	60.16	7.15
N14	35.14	7.57
N15	45.28	7.43

3.2. Isolation and purification of *Micromonospora*

A total of 141 presumptive *Micromonospora* strains were isolated from 15 soil and sediment samples using SM3 Agar and Medium 65 supplemented with cycloheximide and nystatin (50 µg/mL each). Colonies exhibiting characteristic mycelial and pigment production were selected and purified on Medium 65. Enumeration of total bacteria and *Micromonospora* colonies was performed separately for each medium and presented in Figures 2. All isolates were stored at –20°C in 20% glycerol. Colony morphology on both media is shown in Figure 3.

3.3. Color grouping

A total of 141 purified presumptive *Micromonospora* isolates were inoculated onto oatmeal agar, resulting in the classification of test isolates into 11 distinct color groups (Table 5). The grouping was based on both pigment production and substrate mycelium coloration. While some color groups included fewer than five isolates, the majority comprised more than five test organisms.

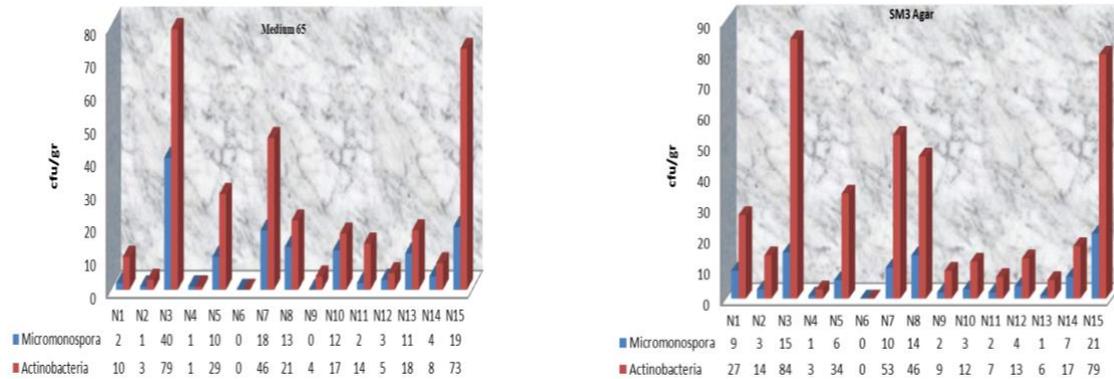


Figure 2. Graph showing the total counts of *Micromonospora* and Actinobacteria (cfu/g) isolated from Medium 65 and SM3 media during the isolation study.

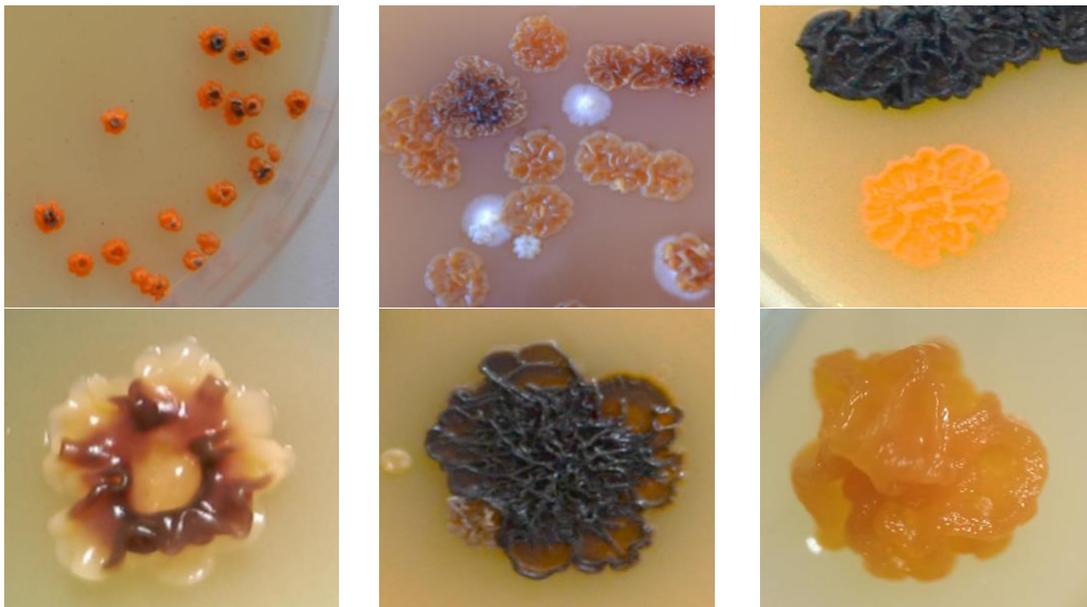


Figure 3. *Micromonospora* colonies grown on SM3 Agar and Medium 65 after 14 days of incubation at 27°C.

3.4. Antimicrobial activity assay

A total of 141 purified *Micromonospora* strains were screened for antimicrobial activity against six pathogenic and non-pathogenic test microorganisms using Bennet's agar. After 7 days of incubation at 27 °C, followed by 24 hours at 37 °C with test strains applied in a cross-streak manner, inhibition zones were evaluated. Antagonistic activity was observed in 36.87% to 58.86% of isolates. The highest inhibition rate was against *Bacillus subtilis* (58.86%), followed by *Escherichia coli* (55.31%), *Micrococcus luteus* (48.93%), *Salmonella typhimurium* (46.09%), *Staphylococcus aureus* (39.71%), and *Enterococcus faecalis* (36.87%) (See Table 5).

3.5. Degradation activities

A total of 141 presumptive *Micromonospora* isolates were tested for degradation of Xanthine (0.4%), Tween 80 (1%), Elastin (0.3%), Casein (1%), Guanine (0.05%), Tyrosine (0.5%), Esculin (0.5%), and Arbutin (1%) on Bennet's agar. Each compound was added individually, and 7 µL of standardized inoculum was applied. Plates were incubated at 27°C for 5 days. Clear zones around colonies indicated substrate utilization. Degradation was observed for Guanine (42.55%), Elastin (41.84%), Xanthine (38.29%), Tween 80 and Casein (17.02% each), Arbutin (32.62%), and Esculin (14.89%). Eleven isolates (N0015, N0018, N0019, N0048, N0054, N0082, N0095, N0096, N0100, N0106, N0112) did not degrade any of the tested substrates (See Table 5).

3.6. Growth tests

Micromonospora isolates were evaluated for growth under different temperatures, pH levels, and chemical inhibitors. All strains grew at 27°C, while no growth was observed at 4°C, 10°C, or 45°C. Nine isolates (N0010, N0011, N0021, N0047, N0062, N0063, N0070, N0071, N0109) showed growth at 37°C. All isolates grew at pH 7.3, but none at pH 4.3 (Table 5).

In media supplemented with NaCl (4%, 10%, 13%), no growth was detected. Growth was observed in seven isolates (N0023, N0024, N0090, N0123, N0126, N0127, N0136) in 0.01% sodium azide, while no isolates grew in 0.02% sodium azide. In the presence of 0.1% phenol, 35.46% of the isolates showed growth. Thallous acetate (0.01%) supported the growth of seven isolates, whereas no growth occurred at 0.001% concentration (Table 5).

3.7. Hippurate hydrolysis, lipolysis, and pectin hydrolysis

Among the isolates, 43.97% tested positive for hippurate hydrolysis, indicated by a pink color on phenol red-containing hippurate agar. Lipolytic activity, identified by shiny zones (10–15 mm) on egg yolk agar, was observed in 26.95% of isolates. Pectin hydrolysis, detected by clear zones after CTAB treatment on 0.5% pectin agar, was positive in 41.13% of the isolates (Table 5).

3.8. Utilization of nitrogen sources

Among 12 tested nitrogen sources, *Micromonospora* isolates showed variable growth. Positive utilization rates were: L-cysteine (75.17%), L-tyrosine (35.46%), L-valine (71.63%), L-phenylalanine (79.43%), L-cystine (77.30%), L-arginine (90.78%), L-serine (76.59%), L-methionine (79.43%), L-tryptophan (84.39%), L-threonine (64.53%), and L-hydroxyproline (70.21%). Isolates N0042, N0122, and N0138 utilized all tested nitrogen sources (Table 5).

3.9. Utilization of carbon sources

Micromonospora isolates exhibited varying abilities to utilize sixteen different carbon sources. Positive utilization rates were observed as follows: sucrose (78.01%), arabinose (74.46%), inositol (73.75%), mannitol (71.63%), fructose (78.72%), rhamnose (71.63%), mannose (74.46%), lactose (81.56%), arabitol (71.63%), trehalose (76.59%), dulcitol (71.63%), galactose (80.14%), xylitol (75.17%), erythritol (65.24%), and ribose (52.48%). Nine isolates (N0013, N0026, N0032, N0033, N0046, N0117, N0122, N0131, N0140) utilized all tested carbon sources (Table 5).

3.10. Numerical analysis results

The results of 83 phenotypic tests performed on 141 presumptive *Micromonospora* isolates were subjected to UPGMA (Unweighted Pair Group Method with Arithmetic Average) clustering analysis using Simple Matching Coefficient (SSM) in MVSP 3.22 (Multi-Variate Statistical Package; Appendix IV). The resulting dendrogram is presented in Figure 4. Isolates showed more than 70% similarity and were grouped into two main clusters. Group I comprised 7 clusters and 1 singleton, while Group II

consisted of 15 clusters and 5 singletons. The clusters and test isolates of the analyzed *Micromonospora* isolates are shown in Table 6.

Table 5. Test results applied to the isolated *Micromonospora* isolates

TESTS	N0001	N0002	N0003	N0004	N0005	N0006	N0007	N0008	N0009	N0010	N0011	N0012	N0013	N0014	N0015	N0016	N0017	N0018	N0019	N0020	N0021	N0022	N0023	N0024	N0025	N0026	N0027	N0028	N0029	N0030	N0031	N0032	N0033	N0034	N0035	N0036	N0037	N0038	N0039	N0040	N0041										
Morphology and Pigmentation																																																			
1. Aerial mycelium production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
2. Dark brown	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
3. Black	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
4. Cream	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	-	-	+	-	+				
5. Pink	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-			
6. Yellow	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-			
7. Orange	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	+	+	+	+	-	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-				
8. Orange-Black	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
9. White	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
10. Light brown	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
11. Grey	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
12. Red	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Substrate Mycelium																																																			
13. Brown	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
14. Black	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
15. Orange	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	-	+	+	-	+	-	+	-	+	-	+	-	-	-	-	-	-	+	-	+	-	+	-	-			
16. Dark Red	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
17. Yellow	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
18. Cream	-	-	-	-	-	-	-	+	-	+	+	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-		
19. White	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Diffused Pigment																																																			
20. Brown	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
21. Black	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
22. Dark Red	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Antimicrobial Activity																																																			
23. <i>Bacillus subtilis</i>	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	+	+	-	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
24. <i>E. Coli</i>	+	+	+	+	-	-	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25. <i>S. Aureus</i>	-	-	+	+	-	+	+	+	-	+	+	-	-	-	+	-	-	-	+	-	+	+	-	-	-	-	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 5. Test results applied to the isolated *Micromonospora* isolates (Continued)

TESTS	N0001	N0002	N0003	N0004	N0005	N0006	N0007	N0008	N0009	N0010	N0011	N0012	N0013	N0014	N0015	N0016	N0017	N0018	N0019	N0020	N0021	N0022	N0023	N0024	N0025	N0026	N0027	N0028	N0029	N0030	N0031	N0032	N0033	N0034	N0035	N0036	N0037	N0038	N0039	N0040	N0041			
75. Lactose	+	+	+	+	-	-	-	-	-	+	+	-	+	+	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+			
76. Arabitol	+	+	+	+	-	+	+	-	-	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	+	-	-	+	+	-	-	-		
77. Trahalose	+	+	+	-	-	+	+	-	-	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+		
78. Dulcitol	+	-	+	-	-	+	+	-	-	-	-	+	+	+	+	+	+	-	-	+	-	-	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-	+	+	-	
79. Galactose	+	+	+	-	-	-	-	-	-	+	+	+	+	-	+	-	-	-	+	+	+	-	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	-	+	+	
80. Xylitol	+	+	-	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+
81. Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
82. Erythritol	-	-	-	+	-	-	-	-	-	+	+	-	+	+	-	+	+	-	-	+	+	-	+	+	+	+	+	+	-	+	-	-	+	+	+	+	-	-	+	-	+	+	+	
83. Ribose	+	+	+	-	-	-	-	-	-	+	+	-	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	-	+	-	-	+	

Table 5. Test results applied to the isolated *Micromonospora* isolates (Continued)

TESTS	N0083	N0084	N0085	N0086	N0087	N0088	N0089	N0090	N0091	N0092	N0093	N0094	N0095	N0096	N0097	N0098	N0099	N0100	N0101	N0102	N0103	N0104	N0105	N0106	N0107	N0108	N0109	N0110	N0111	N0112	N0113	N0114	N0115	N0116	N0117	N0118	N0119	N0120	N0121	N0122	N0123
75. Lactose	+	-	+	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	-	+	+	-	+	+	-	+	+	
76. Arabitol	+	+	+	+	+	-	+	+	+	+	+	+	-	+	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-
77. Trahalose	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-
78. Dulcitol	+	+	+	-	-	+	+	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	-
79. Galactose	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
80. Xylitol	+	-	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
81. Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
82. Erythritol	+	+	+	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	-	+	+	+	+	+	-	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+
83. Ribose	-	+	-	-	+	+	+	-	+	-	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	-	+	-	+	+	+	+	-

Table 5. Test results applied to the isolated Micromonospora isolates (Continued)

TESTS	N0124	N0125	N0126	N0127	N0128	N0129	N0130	N0131	N0132	N0133	N0134	N0135	N0136	N0137	N0138	N0139	N0140	N0141
Morphology and Pigmentation																		
1. Aerial mycelium production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2. Dark brown	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3. Black	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4. Cream	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-	+
5. Pink	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
6. Yellow	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7. Orange	-	-	-	-	-	+	-	+	-	-	-	-	-	+	+	-	-	-
8. Orange-Black	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9. White	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-
10. Light brown	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
11. Grey	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12. Red	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Substrate Mycelium																		
13. Brown	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-
14. Black	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
15. Orange	-	-	-	-	-	+	-	+	-	-	-	-	-	-	+	+	-	-
16. Dark Red	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
17. Yellow	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18. Cream	-	+	-	-	+	-	+	-	-	-	+	+	-	-	-	-	-	+
19. White	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Diffused Pigment																		
20. Brown	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21. Black	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
22. Dark Red	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Antimicrobial Activity																		
23. <i>Bacillus subtilis</i>	+	-	+	+	-	-	+	-	+	+	-	+	-	+	-	-	-	+
24. <i>E. coli</i>	+	-	+	+	-	-	+	-	+	-	-	+	-	+	+	+	-	+
25. <i>S. aureus</i>	-	-	-	-	-	-	+	-	-	+	-	+	-	+	+	+	-	+

Table 5. Test results applied to the isolated *Micromonospora* isolates (Continued)

TESTS	N0124	N0125	N0126	N0127	N0128	N0129	N0130	N0131	N0132	N0133	N0134	N0135	N0136	N0137	N0138	N0139	N0140	N0141
Antimicrobial Activity																		
26. <i>Micrococcus luteus</i>	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+
27. <i>Salmonella typhinum</i>	-	-	-	-	-	-	-	-	+	+	-	+	-	+	+	+	-	+
28. <i>Enterococcus faecalis</i>	-	-	+	+	-	-	+	-	+	+	-	+	-	+	+	+	-	+
Degradation Tests																		
29. Guanine	+	+	-	-	-	+	+	+	+	+	+	-	+	-	+	+	+	+
30. Elastin	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+
31. Xanthine	+	-	+	+	-	-	+	+	-	-	+	+	+	-	+	+	+	+
32. Tween 80	+	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	+
33. Casein	-	-	-	-	-	-	+	-	-	+	-	+	-	+	+	+	+	-
34. Arbutin	+	+	-	-	-	+	-	-	-	+	-	+	+	-	+	+	+	+
35. Esculin	+	+	-	-	-	+	+	+	+	+	+	-	+	-	+	-	-	+
Enzyme Tests																		
36. Hippurate Hydrolysis	-	-	-	-	-	+	-	-	+	+	-	+	-	-	-	-	-	-
37. Lipolysis	-	+	-	-	-	+	-	-	-	-	-	-	+	+	-	+	-	+
38. Pectin Hydrolysis	-	+	+	+	-	+	-	+	-	+	-	-	+	-	-	+	-	+
39. Nitrate Reduction	-	+	-	-	-	+	-	-	-	-	-	-	+	+	-	-	-	+
40. H ₂ S Production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth tests																		
41. 4 °C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42. 10 °C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43. 27 °C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
44. 37 °C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45. 45 °C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46. Ph 4.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47. Ph 7.2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth (% w/v)																		
48. NaCl (4)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 5. Test results applied to the isolated *Micromonospora* isolates (Continued)

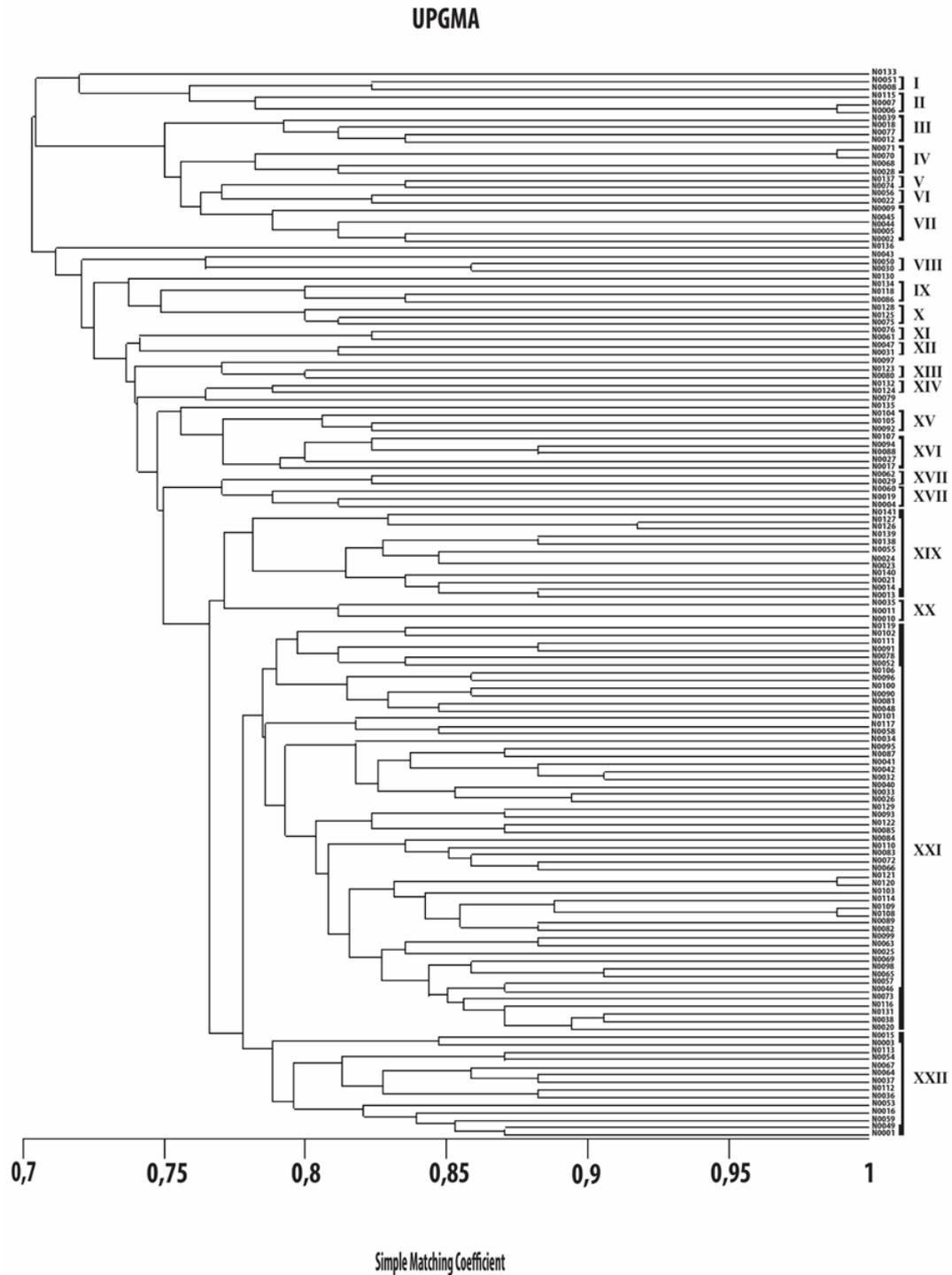
TESTS	N0124	N0125	N0126	N0127	N0128	N0129	N0130	N0131	N0132	N0133	N0134	N0135	N0136	N0137	N0138	N0139	N0140	N0141
Growth (% w/v)																		
49. NaCl (10)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
50. NaCl (13)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
51. Sodium azide (0.01)	-	-	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-
52. Sodium azide (0.02)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
53. Phenol (0.1)	+	+	-	-	-	+	-	-	+	+	-	-	-	-	+	-	-	-
54. Thallus acetate (0.001)	+	-	+	+	-	-	-	-	-	-	-	+	-	-	-	-	+	-
55. Thallus acetate (0.01)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitrogen Sources (0.1 % w/v)																		
56. L-Asparagine	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
57. L - Cysteine	+	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+
58. L-Tyrosine	+	+	-	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-
59. L - Valine	+	-	+	+	-	+	+	+	+	+	+	-	+	-	+	+	+	+
60. L - Phenylalanine	+	+	+	+	-	+	-	+	-	-	+	+	+	-	+	+	+	+
61. L - Cystine	+	-	-	+	-	+	-	+	+	+	+	-	-	+	+	+	+	+
62. L - Arginine	+	+	+	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+
63. L - Serine	+	+	+	+	-	+	+	+	+	-	+	+	+	-	+	+	+	+
64. L - Methionine	+	-	+	+	-	+	-	+	+	+	+	+	+	-	+	+	-	+
65. L - Tryptophan	+	+	+	+	-	+	+	+	+	-	+	+	+	-	+	+	+	-
66. L - Threonine	+	-	+	+	+	-	+	+	-	+	-	+	+	+	+	-	-	+
67. L - Hydroxyproline	-	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	+	+
Carbon Sources (1 % w/v)																		
68. Sucrose	+	+	+	+	-	+	-	+	+	-	-	+	+	-	+	+	+	+
69. Arabinose	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+
70. Inositol	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
71. Mannitol	-	-	+	+	-	+	-	+	+	+	-	+	-	+	-	-	+	+
72. Fructose	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	+	+	+
73. Rhamnose	+	+	+	+	-	+	-	+	+	+	+	-	-	+	+	-	+	+
74. Mannose	+	-	-	-	-	+	-	+	-	+	-	+	+	-	+	+	+	-

Table 5. Test results applied to the isolated *Micromonospora* isolates (Continued)

TESTS	N0124	N0125	N0126	N0127	N0128	N0129	N0130	N0131	N0132	N0133	N0134	N0135	N0136	N0137	N0138	N0139	N0140	N0141
Carbon Sources (1 % w/v)																		
75. Lactose	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	+	+	+
76. Arabitol	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+
77. Trahalose	+	+	+	+	+	-	+	+	+	+	+	-	-	-	+	+	+	+
78. Dulcitol	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
79. Galactose	+	+	+	+	-	+	+	+	+	-	+	-	-	+	+	+	+	+
80. Xylitol	-	-	+	-	-	+	-	+	+	-	-	+	-	-	-	+	+	-
81. Glucose	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
82. Erythritol	+	+	+	+	-	+	-	+	+	-	-	-	-	+	+	+	+	+
83. Ribose	-	-	-	-	-	+	-	+	-	+	-	+	-	-	-	-	+	-

Table 6. Clusters and test isolates generated based on 83 phenotypic characteristics of 141 *Micromonospora* isolates, as analyzed using the MVSP 3.22 (Multi-Variate Statistical Package) software. The dendrogram was constructed using the Simple Matching Coefficient (S_{SM}) and UPGMA clustering algorithm.

Cluster No	Lab No
Singleton	N0133
I	N0008, N0051
II	N0115, N0007, N0006
III	N0039, N0018, N0077, N0012
IV	N0071, N0070, N0068, N0028
V	N0137, N0074
VI	N0056, N0022
VII	N0009, N0045, N0005, N0044, N0002
Singleton	N0136
Singleton	N0043
VIII	N0050, N0030
Singleton	N0130
IX	N0134, N0118, N0086
X	N0128, N0125, N0075
XI	N0076, N0061
XII	N0047, N0031
Singleton	N0097
XIII	N0123, N0080
XIV	N0132, N0124
Singleton	N0079
Singleton	N0135
XV	N0104, N0105, N0092
XVI	N0107, N0094, N0088, N0027, N0017
XVII	N0062, N0029
XVII	N0060, N0019, N0004
XIX	N0141, N0127, N0126, N0139, N0138, N0055, N0024, N0023, N0140, N0021, N0014, N0013
XX	N0035, N0011, N0010
XXI	N0119, N0102, N0111, N0091, N0078, N0052, N0106, N0096, N0100, N0090, N0081, N0048, N0101, N0117, N0058, N0034, N0095, N0087, N0041, N0042, N0032, N0040, N0033, N0026, N0129, N0093, N0122, N0085, N0084, N0110, N0066, N0083, N0072, N0121, N0120, N0103, N0114, N0109, N0108, N0089, N0082, N0099, N0063, N0025, N0069, N0098, N0065, N0057, N0046, N0073, N0116, N0131, N0038, N0020
XXII	N0015, N0003, N0113, N0054, N0067, N0064, N0037, N0112, N0036, N0053, N0016, N0059, N0049, N0001



Şekil 4. Dendrogram based on 83 phenotypic characteristics of 141 *Micromonospora* test isolates, constructed using MVSP 3.22 (Multi-Variate Statistical Package). Clustering was performed using the UPGMA method and the Simple Matching Coefficient (S_{SM}).

At a 78% similarity threshold, a total of 22 clusters were identified: 9 clusters with 2 members, 6 clusters with 3 members, 2 clusters with 4 members, 2 clusters with 5 members, and single clusters containing 12, 14, and 54 members, respectively, along with 6 single-member clusters. Seven major clusters and 15 minor clusters formed at this threshold. The seven major clusters encompassed 70% of the isolates, with cluster XXI, containing 54 isolates, being the largest. Clusters XX and XXI exhibited 80% similarity. Additionally, pairs of isolates N0131-N0038, N0098-N0065, and N0043-N0042 showed 80% similarity. Most isolates in cluster XXII originated from the same locality. Singletons N0133 and

N0136 were isolated from the marshland area in the center of Ahlat. Overall, clustering patterns correlated with geographic origin (Table 6). The cophenetic correlation coefficient (r) was calculated as 0.705, indicating a good level of agreement between the original similarity matrix and the dendrogram structure. This result suggests that the UPGMA clustering reliably represents the phenotypic relationships among the studied isolates.

Eight duplicate strains selected for error estimation exhibited an average similarity above 98%. Duplicate pairs N0010-N0011, N0023-N0024, and N0044-N0045 showed 100% similarity. Members within clusters generally demonstrated concordance in terms of altitude and collection locality.

3.11. Determination of spore chain morphologies by scanning electron microscopy (SEM)

The spore chain morphologies of selected strains were examined using Scanning Electron Microscopy (SEM) (Figure 5).

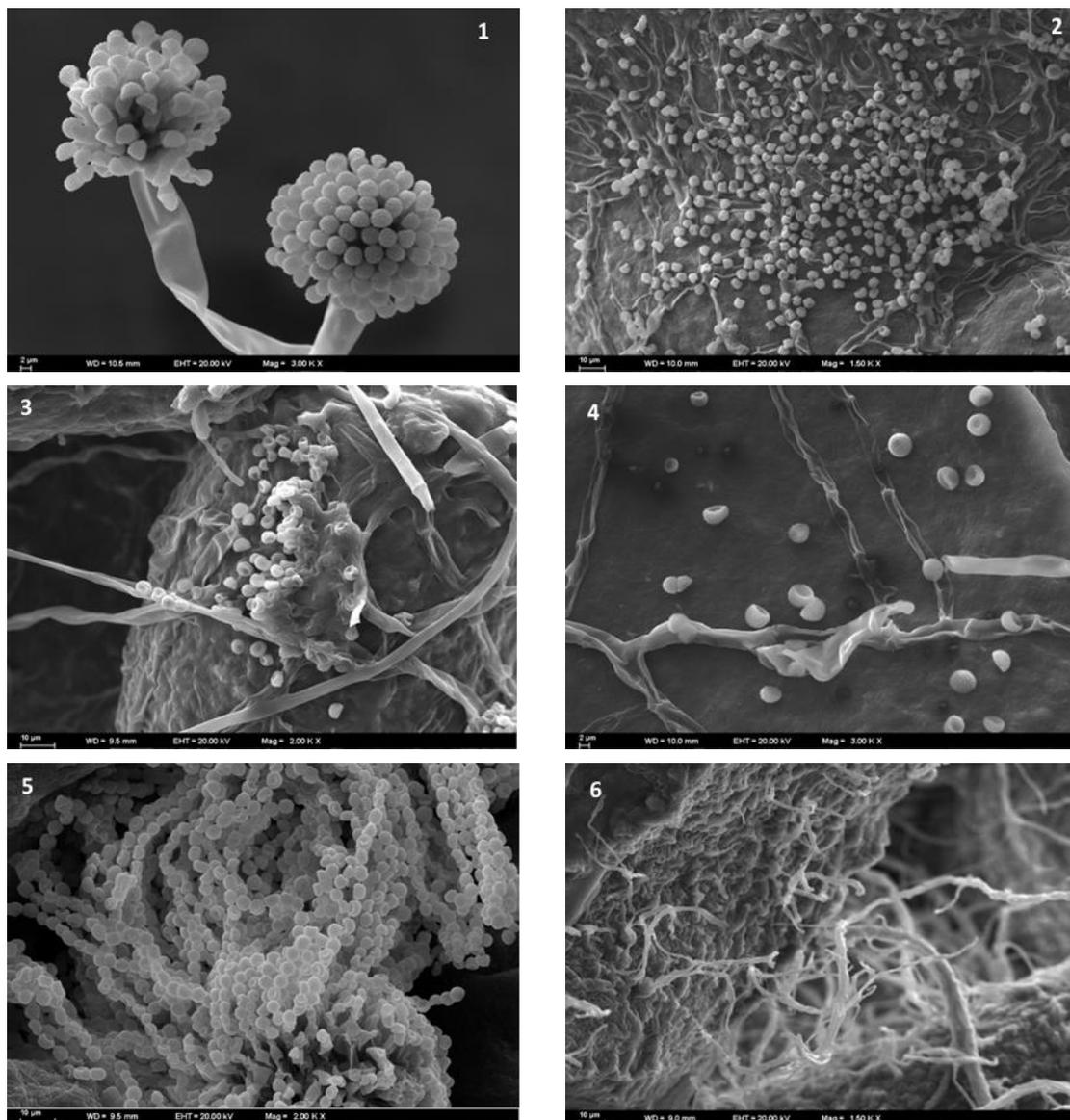


Figure 5. SEM images showing the spore chain morphologies of *Micromonospora* isolates N0019, N0074, N0022, N0068, N0119, and N0047 (1, 2, 3, 4, 5, and 6, respectively).

3.12. 16S rRNA gene analysis – phylogenetic inference

Phylogenetic analyses based on 16S rRNA gene sequences were performed using Maximum Likelihood (ML) and Bayesian methods. The resulting phylogenetic trees were highly consistent with one another, both supporting two major clades. The first major clade included species from the genus *Micromonospora*, while the second comprised species of *Amycolatopsis*, *Saccharothrix*, and *Nocardia* (Figure 6).

Within the *Micromonospora* clade, isolates grouped into four distinct subclusters in the Bayesian tree. The largest subgroup included isolates N0017, N0022, N0074, N0052, N0019, N0068, and multiple reference species such as *M. lupini*, *M. purpureochromogenes*, and *M. saelicesensis*. The other three subgroups were composed of closely related species and isolates, with isolate N0001, N0016, and *M. echinofusca* forming one cluster, while *M. rhizosphaerae* formed a distinct group. Notably, *M. rhizosphaerae* was placed in a separate clade in the Bayesian tree, whereas in the ML tree it clustered within the second subclade, highlighting a minor topological difference between the two analyses (Figure 7).

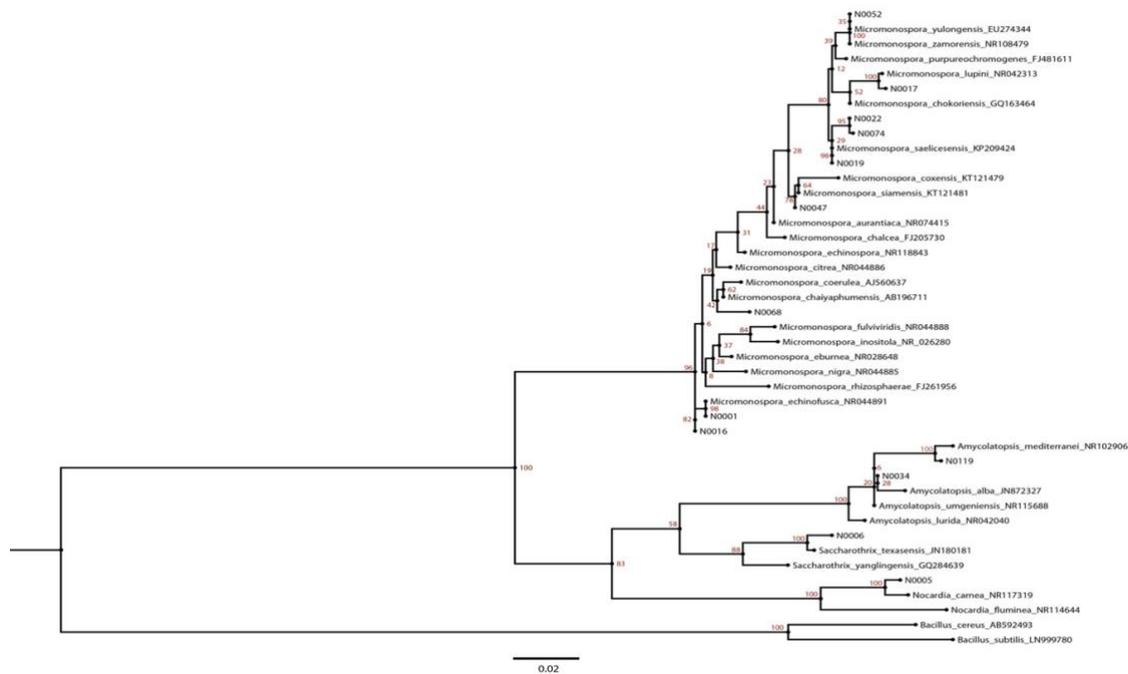


Figure 6. Maximum Likelihood (ML) phylogenetic tree based on 16S rDNA sequences of *Micromonospora* isolates and reference strains. The tree was constructed using 1000 bootstrap replications to assess branch support.

(Abdel-Mageed et al., 2021; Deng et al., 2025). Furthermore, the genetic makeup of these extreme-tolerant strains often includes genes for Plant Growth Promoting (PGP) features (Nouioui et al., 2025). This suggests our isolates have a high potential to be used as biostimulants to mitigate salt stress in agriculture, a role previously confirmed for species like *M. echinaurantiaca* in wheat (Aghaei Dargiri et al., 2025).

Molecular identification was conducted by sequencing the 16S rRNA gene of 13 selected isolates. Phylogenetic trees were constructed using both Maximum Likelihood (ML) and Bayesian inference methods, with highly consistent topologies observed between them. This concordance strengthens the reliability of the results and underscores the effectiveness of 16S rRNA gene sequencing in bacterial taxonomy (Woese, 1987; Garrity & Holt, 2001). However, the presence of isolates in two distinct major clades suggests that the genus *Micromonospora* may be paraphyletic, raising questions about its current taxonomic delineation (Kasai et al., 2000).

One of the notable findings was the strong phylogenetic support (MrBayes = 1; ML = 100) for *Micromonospora* isolate N0017 as a sister group to *M. lupini*, yet genetically distinct, indicating the potential for novel species designation. Similarly, isolates N0022, N0074, and N0047 formed distinct branches, implying divergence from known species. Isolate N0019, though phylogenetically close to *M. saelicesensis*, was placed on a different node, again suggesting the possibility of taxonomic novelty. These results align with earlier findings where closely related strains showed high 16S rRNA similarity but were later reclassified based on additional genetic markers (Kasai et al., 2000). Interestingly, several morphologically identified *Micromonospora* isolates (N0119, N0034, N0006, N0005) clustered closely with other genera such as *Amycolatopsis*, *Saccharothrix*, and *Nocardia*, indicating misclassification at the genus level or a paraphyletic grouping. These discrepancies underscore the need for multi-gene phylogenies (e.g., *gyrB*, *rpoB*) or genomic methods like average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) for more precise species delineation (Coenye et al., 2005; Tamura et al., 2001). Collectively, the findings suggest that the genus *Micromonospora* harbors significant cryptic diversity. The presence of potentially novel species among only 13 sequenced isolates strongly indicates that the remaining 128 isolates may also include taxonomically undescribed strains. Given this high level of diversity, the Van Lake basin emerges as a promising reservoir of biotechnologically valuable actinobacteria.

In conclusion, the integration of classical (phenotypic and biochemical) and molecular (16S rRNA-based phylogeny) approaches in this study contributed to the reliable identification and classification of *Micromonospora* isolates. The presence of phylogenetically distinct strains with potential novelty highlights the importance of polyphasic taxonomy in microbial systematics and underscores the role of alkaline ecosystems as critical reservoirs of cryptic microbial diversity. Moreover, these isolates, highly adapted to the unique environmental pressures of the Van Lake Basin, represents valuable biological resources/strains for future studies on primary and secondary metabolite production, with potential applications in pharmaceutical, agricultural (PGP agents), and industrial biotechnology.

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Author Contributions

Metin Ertaş: Conceptualization, Methodology, Data Analysis, Original Draft Preparation/Writing, and Theorization/Theory Development. **Kerem Özdemir:** Supervision, Data Analysis, and Theorization/Theory Development.

Conflict of Interest

The authors declare no conflict of interest.

Ethics Statement

The authors declare that the materials and methods used in this study did not require ethics committee approval and/or specific legal permission.

AI Usage Statement

The authors declare that they have not used any type of generative artificial intelligence in the writing of this article, or in the creation of the visuals, figures, tables, or corresponding captions.

References

- Abdel-Mageed, W. M., Al-Wahaibi, L. H., Lehri, B., Al-Saleem, M. S., Goodfellow, M., Kusuma, A. B., & ... Karlyshev, A. V. (2021). Biotechnological and ecological potential of *Micromonospora provocatoris* sp. nov., a gifted strain isolated from the challenger deep of the mariana trench. *Marine Drugs*, 19(5), 243. <https://doi.org/10.3390/md19050243>
- Aghaei Dargiri, S., Naeimi, S. & Khayam Nekouei, S. M. (2025). Evaluation of the application of endophytic bacteria *Micromonospora echinaurantiaca* and *Sphingomonas aquatilis* in enhancing wheat tolerance to salinity stress. *Environmental Stresses in Crop Sciences*, <https://doi:10.22077/escs.2025.7874.2298>
- Ausubel, F. M. (1994). Preparation of genomic DNA from bacteria. *Current protocols in molecular biology*, 2-4.
- Carro, L., Nouioui, I., Sangal, V., Meier-Kolthoff, J. P., Trujillo, M. E., Montero-Calasanz, M. D. C., ... Aghaei & Goodfellow, M. (2018). Genome-based classification of micromonosporae with a focus on their biotechnological and ecological potential. *Scientific reports*, 8(1), 525. <https://doi.org/10.1038/s41598-017-17392-0>
- Coenye, T., Gevers, D., Van de Peer, Y., Vandamme, P., & Swings, J. (2005). Towards a prokaryotic genomic taxonomy. *FEMS Microbiology Reviews*, 29, 147–167. <https://doi.org/10.1016/j.fmrre.2004.11.004>
- Cross, T. (1981). Aquatic actinomycetes: a critical survey of the occurrence, growth and role of actinomycetes in aquatic habitats. *Journal of Applied Bacteriology*, 50(3), 397-423. <https://doi.org/10.1111/j.1365-2672.1981.tb04245.x>
- Deng, Y., Li, C. J., Chen, H. H., Yu, L. Y., Cai, M., & Zhang, Y. Q. (2025). Description of two novel *Micromonospora* species: *Micromonospora psammae* sp. nov. from desert soil and *Micromonospora lacuserhaii* sp. nov. from lake sediment. *International Journal of Systematic and Evolutionary Microbiology*, 75(9), 006922. <https://doi.org/10.1099/ijsem.0.006922>.
- Erikson, D. (1941). Studies on some lake-mud strains of *Micromonospora*. *Journal of Bacteriology*, 41, 277-300.
- Ertas, M., Ozdemir, K., & Atalan, E. (2014). Use of random amplified polymorphic DNA PCR to distinguish *Micromonospora* species isolated from soil. *Journal of Animal and Veterinary Advances* 13(16), 974-977. <https://doi.org/10.36478/javaa.2014.974.977>
- Gacto, M., Vicente-Soler, J., Cansado, J., & Villa, T. G. (2000). Characterization of an extracellular enzyme system produced by *Micromonospora chalcea* with lytic activity on yeast cells. *Journal of Applied Microbiology*, 88, 961-967. <https://doi.org/10.1046/j.1365-2672.2000.01065.x>
- Garrity, G. M., & Holt, J. G., 2001. The road map to the manual, p. 119–166. In G. M. Garrity (ed), *Bergey's manual of systematic bacteriology*. Springer-Verlag, New York, N.Y.
- Goodfellow, M., Nouioui, I., Sanderson, R., Xie, F., & Bull, A. T. (2018). Rare taxa and dark microbial matter: novel bioactive actinobacteria abundant in Atacama Desert soils. *Antonie Van Leeuwenhoek*, 111, 1315-1332. <https://doi.org/10.1007/s10482-018-1088-7>
- Jensen, H. L., 1930. The genus *Micromonospora* Ørskow -a little known group of soil microorganism. *Proc. Linnean Soc. N. S. Wales.*, 55, 231-248.
- Jensen, P.R., Mincer, T.J., Williams, P.G., & Fenical, W. (2005). Marine actinomycete diversity and natural product discovery. *Antonie Van Leeuwenhoek*, 87, 43-48. <https://doi.org/10.1007/s10482-004-6540-1>

- Jiang, Y., Li, W. J., Xu, P., Tang, S. K., & Xu, L. H. (2006). Study on diversity of actinomycetes under salt and alkaline environments. *Wei Sheng Wu Xue Bao= Acta Microbiologica Sinica*, 46(2), 191-195.
- Johnston, D. W., & Cross, T. (1976). The occurrence and distribution of actinomycetes in lakes of the English Lake District. *Freshwater Biology*, 6(5), 457-463. <https://doi.org/10.1111/j.1365-427.1976.tb01635.x>
- Jones, K. L. (1949). Fresh isolates of actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristic. *Journal of bacteriology*, 57(2), 141-145.
- Kasai, H., Tamura, T., & Harayama, S. (2000). Intrageneric relationships among *Micromonospora* species deduced from *gyrB*-based phylogeny and DNA relatedness. *Int. J. Syst. Evol. Microbiol.*, 50, 127-134. <https://doi.org/10.1099/00207713-50-1-127>
- Kawamoto, I. (1989). Genus *Micromonospora* Orskov. *Bergey's Manual of Systematic Bacteriology*, 4, 2442-2450.
- Lam, K. S. (2006). Discovery of novel metabolites from marine actinomycetes. *Curr Opin Microbiol*, 9, 245-51. <https://doi.org/10.1016/j.mib.2006.03.004>
- Laurent, F. J., Provost F., & Boiron, P. (1999). Rapid identification of clinically relevant *Nocardia* species to genus level by 16S rRNA gene PCR. *J. Clin. Microbiol.*, 37, 99–102. <https://doi.org/10.1128/jcm.37.1.99-102.1999>
- Lu, D., Shen, H. L., Wang, L., & Wan, C. X. (2023). *Micromonospora profundus* TRM 95458 converts glycerol to a new osmotic compound. *Frontiers in Microbiology*, 14, 1236906. <https://doi.org/10.3389/fmicb.2023.1236906>
- Maldonado, L. A., Fragoso-Yáñez, D., Pérez-García, A., Rosellón-Druker, J., & Quintana, E. T. (2009). Actinobacterial diversity from marine sediments collected in Mexico. *Antonie Van Leeuwenhoek*, 95, 111-120. <https://doi.org/10.1007/s10482-008-9294-3>
- Moron, R., Gonzalez, I., & Genilloud, O. (1999). New genus-specific primers for the PCR identification of members of the genera *Pseudonocardia* and *Saccharopolyspora*. *Int. J. Syst. Bacteriol.*, 49, 149–162. <https://doi.org/10.1099/00207713-49-1-149>
- Nitsch, B., & Kutzner, H. J. (1969). Decomposition of oxalic acid and other organic acids by streptomycetes as a taxonomic aid. *Zeitschrift für allgemeine Mikrobiologie*, 9(8), 613-632. <https://doi.org/10.1002/jobm.19690090804>
- Nouioui, I., Zimmermann, A., Gomez Escribano, J. P., Jando, M., Pötter, G., Neumann-Schaal, M., & Mast, Y. (2025). Taxonomic description of *Micromonospora reichwaldensis* sp. nov. and its biosynthetic and plant growth-promoting potential. *Microbiology Spectrum*, 13(4), e02129-24. <https://doi.org/10.1128/spectrum.02129-24>
- Öztürk, E. (2000). *Termofilik Streptomyces'lerin İzolasyonu ve Nümerik Taksonomisi*. (Master). Ondokuz Mayıs Üniversitesi, Fen-Edebiyat Fakültesi, Biyoloji Bölümü, Samsun, Türkiye.
- Postec, A., Yumoto, I., Morales-Barrera, L., Gessesse, A., & McMillan, D. G. (2025). Microbial Ecology and Biotechnological Potential of Alkaline Environments. *Frontiers in Microbiology*, 16, 1726999. <https://doi.org/10.3389/fmicb.2025.1726999>
- Sackin, M. J., & Jones, D. (1993). *Computer-assisted classification*. In *Handbook of new bacterial systematics*, pp. 281-313. Edited by Goodfellow, M. & O'Donnell, A. G. Academic Press, London.
- Sands, D. C., Hankin, L., & Zucker, M. (1972). A selective medium for pectolytic fluorescent pseudomonads. *Phytopathology*, 62, 998-1000.
- Shirling, E.B. & Gottlieb, D. (1966) Methods for Characterization of Streptomyces Species. *International Journal of Systematic Bacteriology*, 16, 313-340. <http://dx.doi.org/10.1099/00207713-16-3-313>
- Sneath, P. H. A. & Johnson, R. (1972). The influence on numerical taxonomic similarities of errors in microbiological tests. *Journal of General Microbiology*, 72, 377-392. <https://doi.org/10.1099/00221287-72-2-377>
- Sokal, R. R., & Michener, C. D. (1958). A statistical method for evaluating systematic relationships. *Univ. Kansas Sci. Bull* 38, 1409–1438.
- Tamura, T., Hayakawa, M., & Hatano, K. (2001). A new genus of the order Actinomycetales, *Virgosporangium* gen. nov., with descriptions of *Virgosporangium ochraceum* sp. nov. and

- Virgosporangium aurantiacum* sp. nov. *Int. J. Syst. Evol. Microbiol.*, 51, 1809-1816. <https://doi.org/10.1099/00207713-51-5-1809>
- Tan, G. Y., Ward, A. C. & Goodfellow, M. (2006). Exploration of Amycolatopsis diversity in soil using genus-specific primers and novel selective media. *Syst. Appl. Microbiol.*, 29: 557–569. <https://doi.org/10.1016/j.syapm.2006.01.007>
- Thirsk, M. L. (1957). Hippurate hydrolysis in Klebsiella cloaca classification. *J. Gen. Microbiol.* 17: 390-395. <https://doi.org/10.1099/00221287-17-2-390>
- Trujillo, M. E., Bacigalupe, R., Pujic, P., Igarashi, Y., Benito, P., Riesco, R., & ... Normand, P. (2014). Genome features of the endophytic actinobacterium *Micromonospora lupini* strain Lupac 08: on the process of adaptation to an endophytic life style?. *PLoS One*, 9(9), e108522. <https://doi.org/10.1371/journal.pone.0108522>
- Trujillo, M. E., Kroppenstedt, R. M., Schumann, P., Carro, L., Martínez-Molina, E. (2006). *Micromonospora coriariae* sp. nov., isolated from root nodules of *Coriaria myrtifolia*. *International Journal of Systematic and Evolutionary Microbiology*, 56, 2381-2385. <https://doi.org/10.1099/ijs.0.64449-0>
- Valdés, M., Pérez, N. O., Estrada-de los Santos, P., Caballero-Mellado, J., PeñaCabriales, J.J., Normand, P., & Hirsch, A. M. (2005). Non-Frankia actinomycetes isolated from surface-sterilized roots of *Casuarina equisetifolia* fix nitrogen. *Applied and Environmental Microbiology*, 71, 460-466. <https://doi.org/10.1128/AEM.71.1.460-466.2005>
- Wagman, G. H., Gannon, R. D., & Weinstein, M. J. (1969). Production of vitamin B₁₂ by *Micromonospora*. *Applied Microbiology*, 17, 648-679.
- Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A. & Sackin, M. J. (1983). Numerical classification of Streptomyces and related genera. *Journal of General Microbiology*, 129: 1743-1813. <https://doi.org/10.1099/00221287-129-6-1743>
- Willoughby, L. G. (1969). A study of the aquatic actinomycetes of Blelham Tarn. *Hidrobiologia*, 34, 465-483.
- Woese, C. R. (1987). Bacterial evolution. *Microbiological reviews*, 51(2), 221-271.
- Zhao, H., Kassama, Y., Young, M., Kell, D. B., & Goodacre, R. (2004). Differentiation of *Micromonospora* isolates from a coastal sediment in Wales on the basis of Fourier transform infrared spectroscopy, 16S rRNA sequence analysis, and the amplified fragment length polymorphism technique. *Applied and environmental microbiology*, 70(11), 6619-6627. <https://doi.org/10.1128/AEM.70.11.6619-6627.2004>
- Zhi, X. Y., Tang, S. K., Li, W. J., Xu, L. H., & Jiang, C. L. (2006). New genus-specific primers for the PCR identification of novel isolates of the genus *Streptomonospora*. *FEMS Microbiol. Lett.*, 263, 48–53. <https://doi.org/10.1111/j.1574-6968.2006.00402.x>
- Ziegler, P., & Kutzner, H. J. (1973). Hippurate hydrolysis as a taxonomic criterion in the genus Streptomyces (order Actinomycetales). *Zeitschrift für allgemeine Mikrobiologie*, 13(3), 265-272. <https://doi.org/10.1002/jobm.19730130311>