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GENOMIC CHARACTERIZATION OF *Micromonospora* SP. B9E7 ISOLATED FROM A RICE FIELD IN THE BAFRA PLAIN: TAXONOMIC POSITION AND SECONDARY METABOLITE POTENTIAL

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Abstract: This study comprehensively examined the genetic, metabolic, and biotechnological potential of the *Micromonospora* sp. B9E7 isolate. Whole-genome sequencing and digital DNA-DNA hybridization (dDDH) analyses revealed that the isolate is genetically distinct from known species, suggesting it represents a novel species. Genome annotation identified 6,826 protein-coding genes and 297 functional subsystems; however, only 18% of the genome matched known functional categories, indicating many genes remain uncharacterized. Nineteen biosynthetic gene clusters associated with secondary metabolite production were detected, some showing similarity to known antibiotic and anticancer compounds. The isolate exhibited key plant growth-promoting traits, including phosphate solubilization, siderophore production, and high indole-3-acetic acid (IAA) synthesis. Conversely, it did not produce ammonia, fix nitrogen, or show antimicrobial activity against tested pathogens. These results suggest that B9E7 promotes plant growth primarily through direct mechanisms, such as nutrient solubilization and siderophore-mediated micronutrient acquisition, rather than indirect pathogen suppression. Overall, *Micromonospora* sp. B9E7 emerges as a promising candidate both as a taxonomically novel species and for biotechnological applications. Future research should focus on detailed phenotypic and biochemical characterization, and functional studies of its secondary metabolites. Additionally, combining B9E7 with other beneficial microorganisms or optimizing environmental conditions may enhance its efficacy in agricultural settings.

Keywords: Bafra plain, *Micromonospora*, Biotechnology, Whole-genome, Plant growth-promoting

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1. Introduction

The genus *Micromonospora* is classified under the family *Micromonosporaceae* (Parte et al., 2020). Members of this genus are Gram-positive bacteria that produce single spores on sporophores directly branching from substrate hyphae. Typically, *Micromonospora* species do not form aerial mycelium (Genilloud, 2012). These microorganisms have been recovered from a diverse array of habitats, such as soil, freshwater bodies, insects, the atmosphere, Antarctic terrains, marine environments, volcanic deposits, sponges, rhizosphere soils, and various plant-derived materials. However, soil remains the most common source for the isolation of *Micromonospora* species. Additionally, at least twelve species within the genus have been reported as endophytes, commonly isolated from different plant tissues. Endophytic *Micromonospora* strains are frequently obtained from root nodules of leguminous plants, particularly those belonging to the *Fabaceae* family (Trujillo et al., 2006, 2007; Garcia et al., 2010; Carro et al., 2012, 2016, 2018). Currently, over 150 species within the *Micromonospora* genus have been formally described. Species within the family *Micromonosporaceae* are recognized for their ability to synthesize a broad range of medically important secondary metabolites. These include actaplanin (Debono et al., 1984), abyssomycins (Riedlinger et al., 2004), arenimycin (Asolkar et al., 2010), daunorubicin (Cassinelli et al., 1980), gentamicin (Lancini and Lorenzetti, 1993), rifamycins (Huang et al., 2009; Kim et al., 2006), and teicoplanin (Coronelli et al., 1984). In particular, species within the *Micromonospora* genus are recognized as prolific producers of a diverse array of bioactive compounds, many of which exhibit antimicrobial and antitumor activities (Talukdar et al., 2016).

As of 2019, the Black Sea Region accounted for 22% of Türkiye's total rice production, with Samsun as the province with the highest production level (TUIK, 2019). Within Samsun, which ranks second nationally in rice production, the districts of Bafra, Alaçam, and Terme are among the leading areas. In the Bafra Plain alone, approximately 120,000 decares are devoted to rice cultivation. In this study, an isolate with the laboratory

code B9E7, obtained from a rice field in the Bafra Plain—a region of strategic importance for rice cultivation in Türkiye—was identified as belonging to the genus *Micromonospora* based on 16S rRNA gene sequence analysis. The taxonomic position and biotechnological potential of *Micromonospora* sp. B9E7 were comprehensively investigated through whole-genome analyses.

2. Materials and Methods

2.1. Habitat, Isolation, and Preservation of the Isolate

The *Micromonospora* sp. isolate designated as B9E7 was obtained from a soil sample collected in a rice field located within the Bafra Plain (coordinates: 41°41′42.69″ N, 35°55′27.12″ E). For the selective isolation of *Actinobacteria*, the soil sample (1 g) was suspended in 9 mL of Ringer's solution (Oxoid) to prepare a 10^{-1} dilution. This initial suspension was serially diluted to obtain 10^{-2} and 10^{-3} dilutions. The 10^{-3} dilution was subjected to heat treatment at 60 °C for 20 minutes to reduce the abundance of fast-growing non-target microorganisms. Subsequently, 200 µL of the treated suspension was spread onto Humic Acid-Vitamin (HV) agar medium (Hayakawa and Nonomura, 1987), which was supplemented with selective antibiotics — cycloheximide (50 µg/mL), nalidixic acid (10 µg/mL), rifampicin (5 µg/mL), and neomycin (4 µg/mL). The inoculated plates were incubated aerobically at 28 °C for up to four weeks to allow the development of actinobacterial colonies. Emerging colonies were screened, and a single colony was repeatedly subcultured on yeast extract-malt extract agar (ISP2; Shirling and Gottlieb, 1966) to obtain a pure culture. The purified isolate was designated as B9E7 and preserved as glycerol stocks (25%, v/v) at both –20 °C and –80 °C for long-term storage.

2.2. Genomic DNA Isolation and Whole-Genome Sequencing

For genomic DNA extraction, the B9E7 isolate was cultured in liquid medium at its optimal growth temperature for approximately seven days. Following incubation, cells were harvested by centrifugation, and the resulting pellet was washed twice with sterile distilled water to remove residual medium components. DNA isolation from the *Actinobacteria* isolate was performed using a commercial genomic DNA extraction kit, following the manufacturer's instructions. The purity and concentration of the extracted DNA were assessed spectrophotometrically using a Nanodrop instrument. To meet the quality requirements for whole-genome sequencing, DNA samples were adjusted to have 260/280 nm absorbance ratios between 1.7 and 2.0, with concentrations of at least 10 ng/µL and total volumes ranging from 30 µL to 100 µL.

Purified genomic DNA samples that met the specified criteria were labeled with barcodes provided by the sequencing service and shipped in stock tubes to MicrobesNG (United Kingdom) for sequencing. At MicrobesNG, genomic libraries were constructed with the Nextera XT Library Preparation Kit (Illumina, San Diego, CA, USA) following the manufacturer's guidelines with slight adjustments. Sequencing was performed on an Illumina HiSeq 2500 system, producing 250 bp paired-end reads with a minimum coverage depth of 30X.

2.3. Assembly of Whole-Genome Data of Actinobacterial Isolates

Following next-generation sequencing, the raw genomic data of the actinobacterial isolates were obtained as paired-end fastq files. These raw reads were assembled into draft genomes using the PATRIC server (Wattam et al., 2017). Genome assembly was performed with SPAdes version 3.13.0 (Bankevich et al., 2012) integrated within the platform, applying quality filtering criteria by removing contigs shorter than 500 bp and those with coverage lower than 5X. The resulting genome assemblies, consisting of contigs merged into draft genome sequences, were prepared for deposition in the National Center for Biotechnology Information (NCBI) database to obtain accession numbers.

2.4. Genome-Based Bioinformatic Analyses of the Isolates Genome Annotation

Functional annotation of the draft genomes was carried out using the Rapid Annotation using Subsystem Technology (RAST) server (Brettin et al., 2015), which provided comprehensive information on genomic features such as genome size, number of coding sequences, RNA genes, number of contigs, G+C content, N50 and L50 statistics, and overall coverage depth (Chun et al., 2018). Additionally, individual gene sequences and annotations were retrieved from open-access databases for further analyses. To identify phylogenetically close type strains, genome sequences of related taxa were obtained from public repositories such as the Joint Genome Institute Genome Portal, NCBI Genomes, and PATRIC.

2.5. Identification of Biosynthetic Gene Clusters

To assess the biosynthetic potential of the isolates, the assembled genomes were analyzed using the antiSMASH bacterial version 5.0 platform (Blin et al., 2019). This in silico analysis enabled the identification and characterization of biosynthetic gene clusters (BGCs), providing data on the number, type (e.g., NRPS, PKS, terpenes), and size of BGCs, as well as their similarity to known clusters encoding secondary metabolites. In addition, the annotated genomes from RAST and NCBI were screened for genes encoding metabolites of biotechnological interest, including polyketides, non-ribosomally synthesized peptides, ribosomally synthesized and post-translationally modified peptides, terpenes, and siderophores, which are relevant to applications in medicine, agriculture, and the food industry.

2.6. Phylogenomic Analyses of the Isolates

Phylogenomic trees were constructed based on the draft genome sequences using the Type Strain Genome Server (TYGS; Meier-Kolthoff and Göker, 2019) provided by the German Collection of Microorganisms and Cell Cultures (DSMZ). Digital DNA–DNA hybridization (dDDH) analyses were also conducted through the TYGS platform to

determine the taxonomic position of each isolate, and values derived from all recommended formulas were recorded to support species delineation.

2.7. Assessment of Biotechnological Traits Related to Plant Growth Promotion

Indole-3-Acetic Acid (IAA) Production; The ability of *Micromonospora* sp. B9E7 to produce indole-3-acetic acid (IAA) was assessed following the method described by Ali et al. (2009). For this purpose, the isolate was cultured on an appropriate growth medium, and IAA production was qualitatively evaluated.

Siderophore Production; Siderophore production by the isolate was tested on Chrome Azurol S (CAS) agar, as outlined by Alexander and Zuberer (1991). The presence of siderophore activity was indicated by a color change in the agar medium surrounding the colonies.

Nitrogen Fixation Ability; The capacity of the isolate to fix atmospheric nitrogen was evaluated by inoculating it onto two nitrogen-free media. Growth of the isolate on NFC was considered indicative of nitrogen fixation capability.

Phosphate Solubilization Ability; The phosphate-solubilizing potential of the isolate was tested qualitatively using Pikovskaya agar plates (Gaur, 1990). The formation of clear halos around the colonies indicated the solubilization of insoluble phosphate.

Phosphate Solubilization Ability; Ammonia production by *Micromonospora* sp. B9E7 was assessed according to the method described by Cappuccino and Sherman (2002). The accumulation of ammonia in the culture supernatant was detected and recorded as evidence of production.

2.8. Antimicrobial Activity Assays

In this study, the antimicrobial potential of the isolated actinobacterial strain B9E7 was assessed using the overlay technique originally described by Williams et al. (1983), which has since become a standard method in the literature (Veyisoğlu and Tatar, 2021). The strain's ability to inhibit the growth of 10 different pathogenic microorganisms was evaluated. B9E7 was first cultivated on GYM Agar, and the resulting colonies were aseptically transferred into glass vials containing 2 mL of Ringer's solution. 7 µL aliquots were inoculated from this suspension onto antibiotic-free, modified Bennett's Agar medium. After visible colony development, the agar surfaces were overlaid with chloroform to inactivate the colonies, and the Petri dishes were left partially open for approximately 40 minutes to allow for complete chloroform evaporation.

Following inactivation, test pathogens previously cultured in Nutrient Agar supplemented with 0.5% agar were inoculated onto the plates using the spread plate method. The plates were then incubated at 37°C for 48 hours to promote the growth of both clinical and phytopathogenic test organisms. After incubation, the diameters of inhibition zones surrounding the inactivated colonies were measured to determine the antimicrobial activity levels of the B9E7 isolate.

3. Results

In this study, genomic DNA was extracted from the *Micromonospora* sp. B9E7 isolate with the aim of evaluating its biotechnological potential at the genomic level. The DNA sample was submitted to MicrobesNG for whole-genome sequencing. Sequencing was performed using the Illumina HiSeq 2500 next-generation sequencing platform, and the resulting raw reads were delivered in FASTQ format. The genome sequences of *Micromonospora* sp. B9E7 were then assembled using the SPAdes v3.13.0 algorithm via the Bacterial and Viral Bioinformatics Resource Center platform (Bankevich et al., 2012; Wattam et al., 2017).

The complete genome sequence of the *Micromonospora* sp. B9E7 isolate has been deposited in the NCBI GenBank database under the accession number GCA_039871815.1. To accurately and definitively determine the taxonomic position of the isolate, its whole-genome sequence was compared with those of established reference type strains. These comprehensive phylogenetic analyses were conducted using the Type (Strain) Genome Server (TYGS), a platform that facilitates genome-based taxonomic classification and phylogenetic tree construction (Meier-Kolthoff and Göker, 2019; Meier-Kolthoff et al., 2022).

The taxonomic position of *Micromonospora* sp. B9E7 was assessed using a phylogenomic tree constructed based on digital DNA-DNA hybridization (dDDH) values through the Genomic BLAST Distance Phylogeny (GBDP) approach. The phylogenetic reconstruction was performed using the FastME 2.1.6.1 algorithm. GBDP is a distance-based method designed to infer phylogenetic trees from fully or partially sequenced genomes. In this study, the d4 distance formula provided by GBDP was applied to evaluate phylogenetic relationships. The resulting phylogenomic tree includes information on the underlying genomic data, the distance formula used, and the computational algorithm applied. Additionally, the right panel of the tree displays clustering at the species and subspecies levels, as well as relevant genomic features such as GC content (%), δ values (with lower δ values indicating higher accuracy), total sequence length (in base pairs), number of encoded proteins, and an indicator specifying whether the genomic data were submitted by the user or retrieved from the system database.

Whole-genome-based phylogenetic analysis of *Micromonospora* sp. B9E7 isolate was initiated by examining the 16S rRNA gene region, which is 1,522 base pairs in length and was obtained via the RAST (Rapid Annotation using Subsystem Technology) platform. Analysis of this sequence using the EzTaxon server of the EzTaxon server of the EzBioCloud platform (Kim et al., 2012) revealed 100% similarity with the type of strain *Micromonospora taraxaci* DSM 45885^T. No nucleotide differences were observed over an aligned region of 1,437 base pairs between the isolate and the reference strain (Figure 1).

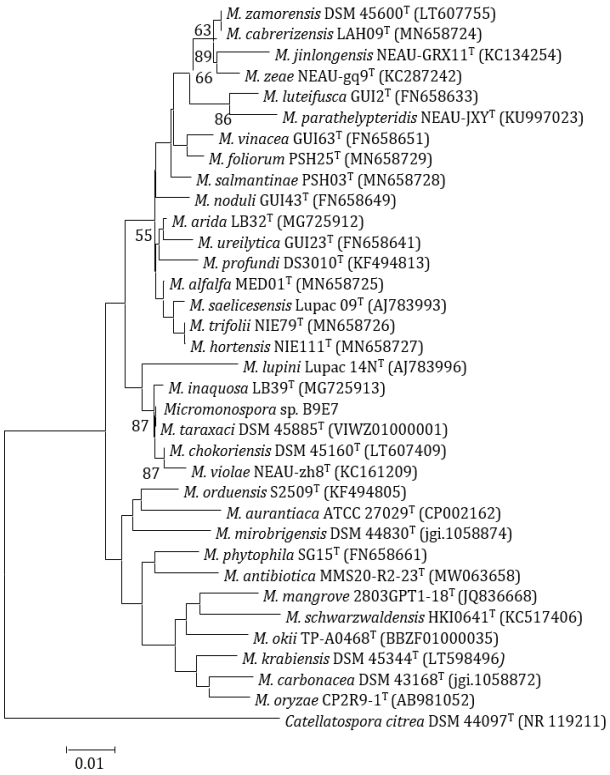

Figure 1. The phylogenetic analysis of the *Micromonospora* sp. B9E7 strain was performed using the Neighbor-Joining method based on 16S rRNA sequences with 1000 bootstrap replicates.

To enable a more robust taxonomic classification, the complete genome sequence of the isolate was uploaded to the Type (Strain) Genome Server (TYGS), and a genome-scale phylogenetic tree was constructed using the FastME 2.1.6.1 algorithm. As a result of the analysis, *Micromonospora* sp. B9E7 was clustered within a group comprising 17 different species. The GC content difference between the isolate and the closest related type of strain, *M. taraxaci* DSM 45885^T, was calculated to be 0.1%, indicating high genomic similarity since values closer to 1% imply greater divergence. Delta (δ) values ranging from 0.063 to 0.209 further support the high accuracy and reliability of the generated phylogenetic tree.

According to the widely accepted threshold for species delineation, a DNA-DNA hybridization (DDH) value of ≥70% is required to classify two organisms within the same species (Meier-Kolthoff et al., 2013). However, the digital DDH (dDDH) value calculated between *Micromonospora* sp. B9E7 and *M. taraxaci* DSM 45885^T was found to be 51.8% (Table 1).

Table 1. Digital DNA-DNA hybridization (dDDH) percentages between *Micromonospora* sp. B9E7 and closely related *Micromonospora* species.

	Type of Comparison	dDDH (%)
Micromonospora sp. B9E7	*Micromonospora taraxaci* DSM 45885^T (VIWZ01000001)	51.3
Micromonospora sp. B9E7	*Micromonospora chokoriensis* DSM 45160^T (LT607409)	48.7
Micromonospora sp. B9E7	*Micromonospora jinlongensis* DSM 45876 ^{T}(KC134254)	43.5
Micromonospora sp. B9E7	*Micromonospora cabrerizensis* LAH09 ^{T} (MN658724)	43.3
Micromonospora sp. B9E7	*Micromonospora zamorensis* DSM 45600^T (LT607755)	42.8
Micromonospora sp. B9E7	*Micromonospora arida* LB32^T (MG725912)	41
Micromonospora sp. B9E7	*Micromonospora vinacea* DSM 101695^T (FN658651)	41
Micromonospora sp. B9E7	*Micromonospora saelicesensis* DSM 44871^T (AJ783993)	40.9
Micromonospora sp. B9E7	*Micromonospora salmantinae* PSH03^T (AJ783993)	40.9
Micromonospora sp. B9E7	*Micromonospora ureilytica* DSM 101692^T (FN658641)	40.8
Micromonospora sp. B9E7	*Micromonospora noduli* DSM 101694^T (FN658649)	40.8
Micromonospora sp. B9E7	*Micromonospora inaquosa* LB39^T (MG725913)	40.6
Micromonospora sp. B9E7	*Micromonospora trifolii* NIE79^T (MN658726)	40.5
Micromonospora sp. B9E7	*Micromonospora hortensis* NIE111^T (MN658727)	40.1
Micromonospora sp. B9E7	*Micromonospora alfalfae* MED01^T (MN658725)	39.8
Micromonospora sp. B9E7	*Micromonospora violae* DSM 45888^T (KC161209)	39.2

This result, along with the isolate's position on the phylogenetic tree (Figure 2), suggests that *Micromonospora* sp. B9E7 may represent a novel species. Nonetheless, to confirm its novelty, further comparative genomic analysis is needed to assess its divergence from the closest related type strains.

Based on the genome annotation performed using the RAST (Rapid Annotation using Subsystem Technology) platform, approximately 18% of the sequences of the *Micromonospora* sp. B9E7 isolate were assigned to defined functional subsystems, whereas the remaining 82% could not be associated with any known functional category (Figure 3). According to the RAST annotation report, the genome of the isolate consists of 54 contigs, with a total length of 7,354,903 base pairs and a GC content of 71.10%. Assembly quality metrics revealed N50 and L50 values of 291,871 and 9, respectively. Additionally, the report identified 6,826 protein-coding sequences, 55 RNA genes, and the presence of 297 functional subsystems. The annotation results obtained via RAST were found to be largely consistent with those generated by the BV-BRC database.

A comprehensive analysis of functional gene categories revealed that 1,748 gene groups identified in the

Micromonospora sp. B9E7 genome are associated with a variety of metabolic and biological processes (Figure 3). These include genes involved in phages, prophages, transposable elements, and plasmids (2); carbohydrate metabolism (288); membrane transport systems (47); metabolism of aromatic compounds (39); potassium metabolism (7); functions related to DNA (90), RNA (49), and proteins (201); amino acid and derivative metabolism (301); nucleoside and nucleotide metabolism (79); biosynthesis of fatty acids, lipids, and isoprenoids (100); stress response mechanisms (43); secondary metabolite biosynthesis (17); dormancy and sporulation (8); pathways involving cofactors, vitamins, prosthetic groups, and pigments (161); virulence, disease, and defense mechanisms (43); iron acquisition and metabolism (20); cellular regulation and signal transduction (20); phosphorus metabolism (28); sulfur metabolism (12); respiratory processes (112); and biosynthesis of the cell wall and capsule (37).

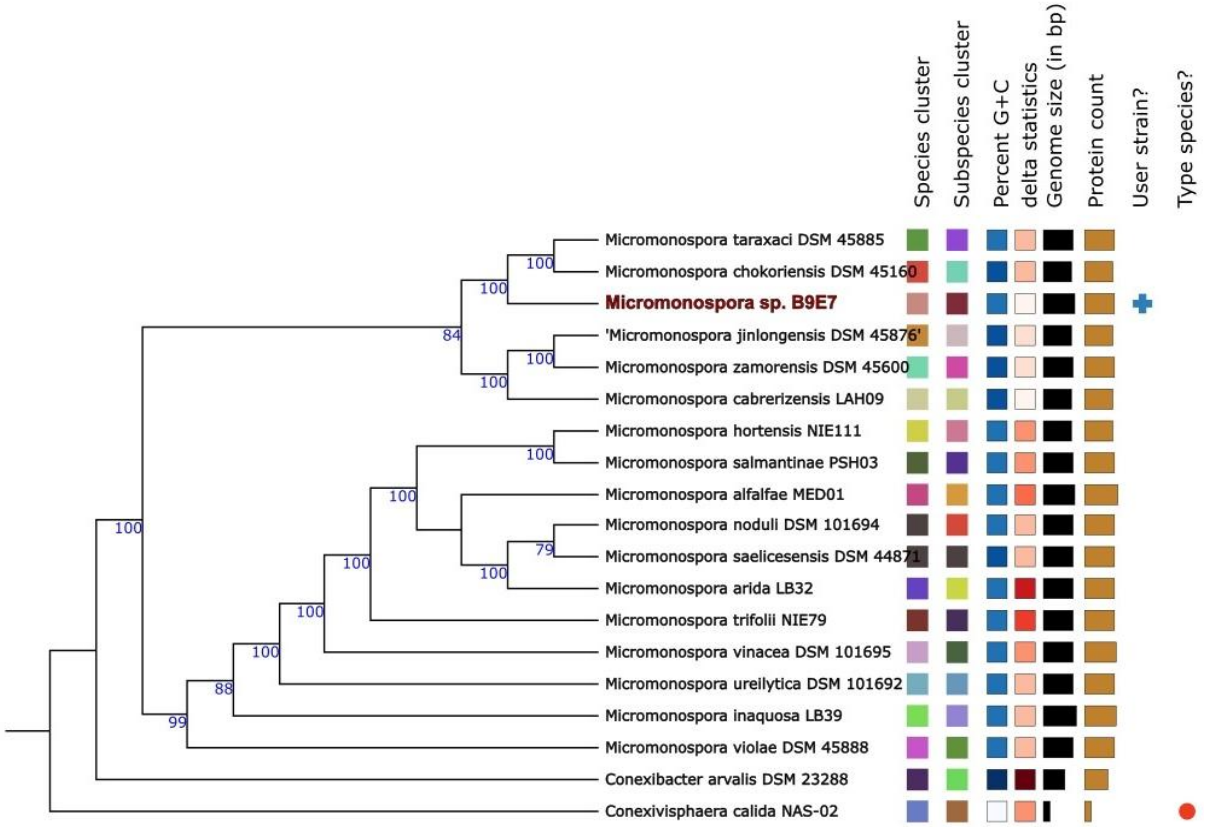

Figure 2. Phylogenetic tree showing the taxonomic relationship of *Micromonospora* sp. B9E7 isolate to *Micromonospora* species based on Digital DNA-DNA hybridization (dDDH) values generated on the TYGS server.

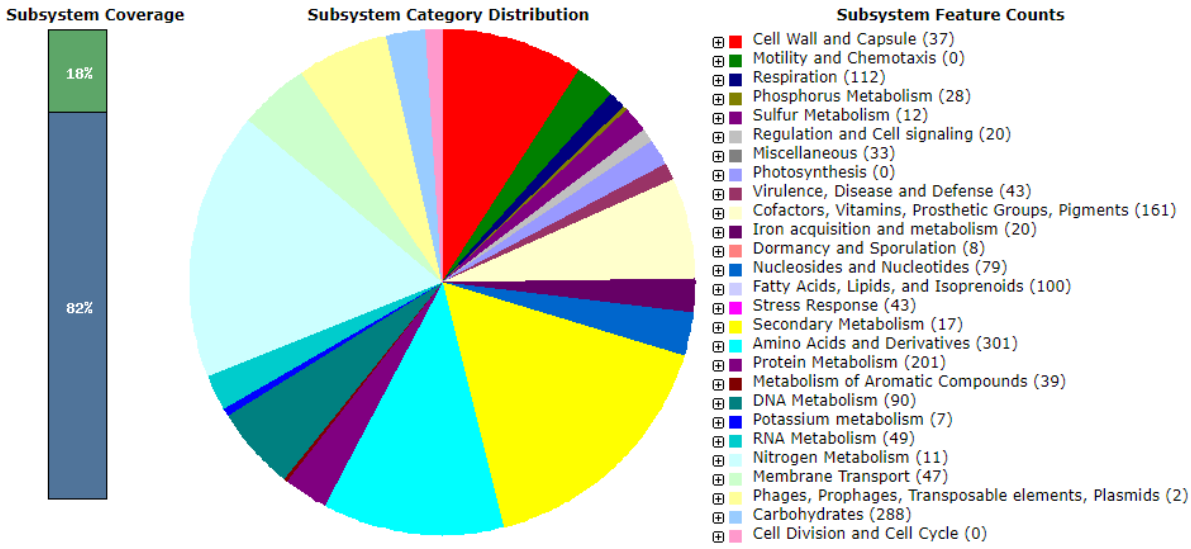

Figure 3. Functional subsystem distribution of *Micromonospora* sp. B9E7 strain according to genome annotation (RAST server analysis).

Table 2. Bioactive secondary metabolite-encoding gene clusters detected in the antiSMASH server of *Micromonospora* sp. B9E7 isolate

Gene Region No.	Type	Similar metabolite	Similarity ratio
Region 1.1	NI-siderophore	FW0622	87%
Region 1.2	thiopeptide,LAP		
Region 2.1	terpene	tetrachlorizine	13%
Region 4.1	terpene	formicamycins A-M	4%
Region 4.2	T1PKS	selvamicin	24%
Region 5.1	lanthipeptide-class-iii		
Region 6.1	lanthipeptide-class-iii	SapB	100%
Region 8.1	NRPS	clipibicyclene/azabicyclene B/azabicyclene C/azabicyclene D	11%
Region 10.1	T3PKS	loseolamycin A1/loseolamycin A2	92%
Region 12.1	NRPS,T1PKS,T3PKS,RiPP-like	sporolide A/sporolide B	48%
Region 12.2	thiopeptide,LAP		
Region 14.1	T1PKS,betalactone	quinolidomicin A	23%
Region 19.1	terpene	phosphonoglycans	3%
Region 21.1	NI-siderophore	peucechelin	10%
Region 26.1	terpene	meridamycin	10%
Region 29.1	T2PKS	xantholipin	14%
Region 31.1	RiPP-like	lymphostin/neolymphostinol B/lymphostinol/neolymphostin B	33%
Region 36.1	NAPAA	ε-Poly-L-lysine	100%

The biosynthetic potential of bioactive secondary metabolites in the *Micromonospora* sp. B9E7 isolate was analyzed using the antiSMASH (antibiotics & Secondary Metabolite Analysis Shell) platform. This analysis identified a total of 19 biosynthetic gene clusters (BGCs) within the genome of the isolate, each associated with the production of various metabolites (Table 2). The detected clusters include NI-siderophore, thiopeptide, LAP (linear azole-containing peptides), terpene, Type I PKS (polyketide synthase), lanthipeptide-class-III, NRPS (nonribosomal peptide synthetase), Type III PKS, betalactone, Type II PKS, RiPP-like (ribosomally synthesized and post-translationally modified peptides), and NAPAA (non-alpha poly-amino acids).

Some of these gene clusters showed over 50% similarity to known biosynthetic pathways, with particularly high similarity observed for FW0622, SapB, loseolamycin A1/A2, and ε-Poly-L-lysine clusters. Additionally, gene clusters associated with metabolites such as quinolidomicin A, phosphonoglycans, xantholipin, meridamycin, and lymphostin—despite having lower similarity percentages—were also detected, suggesting their potential antimicrobial activity. These findings indicate that *Micromonospora* sp. B9E7 possesses a broad capacity for the biosynthesis of diverse secondary metabolites.

Analyses conducted to assess the plant growth-promoting traits of the *Micromonospora* sp. B9E7 isolate revealed that it exhibited positive activity in phosphate solubilization, indole-3-acetic acid (IAA) production, and siderophore synthesis, with particularly high levels of IAA observed. In contrast, the isolate tested negative for both ammonia production and nitrogen fixation ability (Table 3). Moreover, antimicrobial assays performed against ten distinct pathogenic microorganisms showed no inhibitory effect, indicating that the isolate lacks detectable antimicrobial activity.

Table 3. The plant growth-promoting traits of *Micromonospora* sp. B9E7 strain and its antimicrobial activity against selected phytopathogens and clinically relevant pathogens are presented

	Micromonospora sp. B9E7*
Ammonia Production	-
Phosphate Solubilization Ability	++
Siderophore Production	++
Indole-3-Acetic Acid (IAA) Production	+++
Nitrogen Fixation Ability	-
Pathogens	
Alternaria alternata	-
Fusarium moniliforme	-
Bipolaris sorokiniana	-
Fusarium graminearum	-
Klebsiella pneumoniae ATCC 700603	-
Pseudomonas aeruginosa ATCC 27853	-
Escherichia coli ATCC 25922	-
Candida albicans ATCC 10231	-
Bacillus subtilis ATCC 14579	-
Staphylococcus aureus ATCC 25923	-

*****The "+" symbols in the table indicate the level of the respective trait or activity; three plus signs (+++) denote a high level of activity, two plus signs (++) indicate moderate activity, one plus sign (+) represents low activity, whereas a minus sign (–) signifies the absence of activity.

4. Discussion

Molecular-level taxonomic analyses are among the most reliable approaches for species-level classification of bacterial isolates. In this study, the whole-genome sequence of the *Micromonospora* sp. B9E7 isolate was compared with those of recognized type strains, and its phylogenetic position was assessed based on digital DNA–DNA hybridization (dDDH) analysis. According to widely accepted standards, two prokaryotic organisms can be considered to belong to the same species if their dDDH similarity is at least 70% (Meier-Kolthoff et al., 2013; Chun et al., 2018). However, the highest dDDH value obtained in this study was only 51.3%, observed between the B9E7 isolate and *Micromonospora taraxaci* DSM 45885. This value indicates that the isolate is genetically distinct from all currently described species and suggests that it may represent a potential novel species.

Similarly, dDDH values between the B9E7 isolate and other reference strains ranged from 39.2% to 48.7%, all of which fall significantly below the species delineation threshold. For instance, *Micromonospora chokoriensis* DSM 45160 and *Micromonospora jinlongensis* DSM 45876 showed dDDH values of 48.7% and 43.5%, respectively. While these results indicate phylogenetic proximity, the genetic distances remain too large to classify B9E7 within the same species as these strains.

The findings of this study underscore the reliability of genome-based taxonomic tools such as dDDH analysis, which provides high-resolution discrimination at the species level. These results support the hypothesis that *Micromonospora* sp. B9E7 represents a previously undescribed species. Nevertheless, species delineation should not rely solely on genomic data; a valid taxonomic proposal requires integration of phenotypic, morphological, and biochemical characteristics (Rosselló-Móra and Amann, 2015). In this context, the unique genomic profile and distinct phylogenetic position of the B9E7 isolate position it as a strong candidate for description as a novel *Micromonospora* species, potentially contributing new insights to the taxonomy of the genus.

Functional genome annotation is a crucial step in revealing the metabolic capacity, ecological adaptability, and biotechnological potential of microorganisms. In this study, the genome of *Micromonospora* sp. B9E7 was analyzed using the RAST (Rapid Annotation using Subsystem Technology) platform, which identified 6,826 protein-coding genes and 55 RNA genes. The annotation revealed the presence of 297 functional subsystems, highlighting the isolate's considerable genetic diversity and functional versatility.

Interestingly, only 18% of the genome could be assigned to defined functional subsystems, whereas the remaining 82% lacked association with any known categories. This observation suggests that the B9E7 genome harbors a significant number of uncharacterized or potentially novel genes, underscoring its value as a candidate for further exploration in the context of natural product discovery and microbial biotechnology (Overbeek et al., 2014).

A detailed examination of functional gene categories demonstrated that the isolate possesses a broad spectrum of core metabolic processes. Notably, the presence of numerous genes involved in amino acid metabolism (301 genes), carbohydrate metabolism (288 genes), protein metabolism (201 genes), DNA (90 genes), and RNA metabolism (49 genes) suggests a robust biochemical repertoire. Additionally, the identification of 100 genes related to fatty acid, lipid, and isoprenoid biosynthesis points to a well-developed capacity for membrane integrity and secondary metabolite production (Katz and Baltz, 2016).

Of particular biotechnological interest is the presence of 17 genes associated with secondary metabolism. Members of the genus *Micromonospora* are known producers of bioactive compounds, including antibiotics, and the genomic profile of B9E7 indicates that it may likewise possess the capacity to synthesize novel antimicrobial or anticancer agents (Subramani and Aalbersberg, 2012; Genilloud, 2017). The genome also contains genes involved in membrane transport systems (47), aromatic compound metabolism (39), and phosphate/sulfur metabolism, suggesting an ability to survive under various environmental stresses and utilize diverse substrates.

Notably, only two genes were associated with phages, prophages, and plasmids, which may indicate a relatively low potential for horizontal gene transfer. This genomic stability could be advantageous in industrial applications where genetic consistency is critical (Thomas and Nielsen, 2005).

The genome spans 7.35 Mb with a GC content of 71.10%, a characteristic feature of the phylum *Actinobacteria*, known for its genomic stability and resilience to environmental pressures (Ventura et al., 2007). The assembly yielded 54 contigs, with an N50 value of 291,871 bp and an L50 value of 9, reflecting high assembly quality. Despite the whole-genome data suggesting that B9E7 is distinct from previously described species, 16S rRNA-based analysis (1,522 bp) revealed 100% identity with the type strain *Micromonospora taraxaci* DSM 45885^T. However, no nucleotide differences were observed over a 1,437 bp aligned region, illustrating the limitations of 16S rRNA gene analysis in resolving species boundaries. In contrast, whole-genome-based metrics such as dDDH (<70%) provided clear evidence that the isolate is genetically distinct, reinforcing the need for high-resolution genomic methods in modern taxonomy (Chun et al., 2018; Meier-Kolthoff et al., 2013).

In conclusion, *Micromonospora* sp. B9E7 exhibits substantial genomic diversity, broad metabolic capabilities, and promising biosynthetic potential. These findings suggest that it may not only represent a novel species within the genus *Micromonospora* but also serve as a valuable candidate for future biotechnological applications.

The *Micromonospora* sp. B9E7 isolate exhibits several key biological traits that directly contribute to plant growth promotion. Specifically, it demonstrated significant capabilities in indole-3-acetic acid (IAA) production, phosphate solubilization, and siderophore synthesis. Collectively, these traits suggest a strong potential for enhancing plant development through improved nutrient availability and hormonal stimulation. IAA is a well-known phytohormone that facilitates root elongation and cell expansion (Spaepen and Vanderleyden, 2011), while phosphate solubilization increases access to phosphorus, an essential macronutrient for plant metabolism (Rodríguez and Fraga, 1999). Additionally, the ability to produce siderophores may improve iron acquisition under nutrient-limited conditions, further supporting plant growth (Rajkumar et al., 2010).

In contrast, the B9E7 isolate tested negative for ammonia production and nitrogen fixation. It also lacked antimicrobial activity against a range of tested phytopathogens, indicating that its contribution to plant health is primarily through direct growth-promotion mechanisms rather than pathogen suppression (Compant et al., 2005).

In summary, *Micromonospora* sp. B9E7 holds promise as a plant growth-promoting agent due to its IAA synthesis, phosphate-solubilizing ability, and siderophore production. However, its limited indirect benefits highlight the potential advantage of combining it with other beneficial microorganisms to maximize its effectiveness in agricultural applications.

5. Conclusion

In this study, the genetic, metabolic, and biotechnological potential of the *Micromonospora* sp. B9E7 isolate was comprehensively investigated. Digital DNA–DNA hybridization (dDDH) analyses demonstrated that the isolate is genetically distinct from currently described species, indicating that it may represent a novel species. Functional genome annotation combined with antiSMASH analyses revealed the presence of multiple gene clusters responsible for secondary metabolite biosynthesis. Notably, the siderophore production capacity was found to be consistent and complementary with other plant growth-promoting biological activities.

The isolate exhibited key direct plant growth-promoting traits, including phosphate solubilization, siderophore synthesis, and high-level indole-3-acetic acid (IAA) production. Conversely, it tested negative for ammonia production, nitrogen fixation, and antimicrobial activity, suggesting that its contribution to plant health predominantly relies on direct growth promotion mechanisms. Siderophore production plays a critical role in facilitating micronutrient uptake under nutrient-limited conditions, thereby enhancing plant growth.

In conclusion, *Micromonospora* sp. B9E7 stands out as a taxonomically novel isolate with promising biotechnological potential for plant growth promotion, supported by antiSMASH data. Future research should aim to elucidate the isolate's detailed phenotypic and biochemical characteristics and conduct in-depth functional analyses of its secondary metabolite gene clusters.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	A.T.	K.I.
C	50	50
D	50	50
S	50	50
DCP	50	50
DAI	50	50
L	50	50
W	50	50
CR	50	50
SR	50	50
PM	50	50
FA	50	50

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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References

Alexander DB, Zuberer DA. 1991. Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. Biol Fertil Soils, 12(1): 39-45.

Ali B, Sabri AN, Ljung K, Hasnain S. 2009. Auxin production by plant associated bacteria: impact on endogenous IAA content and growth of *Triticum aestivum* L. Lett Appl Microbiol, 48(5): 542-547.

Asolkar RN, Kirkland TN, Jensen PR, Fenical W. 2010. Arenimycin, an antibiotic effective against rifampin-and methicillin-resistant *Staphylococcus aureus* from the marine actinomycete *Salinispora arenicola*. J Antibiot, 63(1): 37-39.

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol, 19(5): 455-477.

Blin K, Shaw S, Steinke K, Villebro R, Ziemert N, Lee S, Weber T.

2019. antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. Nucleic Acids Res, 47(W1): W81-W87.

Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Xia F. 2015. RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci Rep, 5(1): 8365.

Cappuccino JG, Sherman N. 2002. Microbiology: A laboratory manual. San Francisco, CA: Pearson Education, Inc. 6th ed., pp: 15-224.

Carro L, Pukall R, Spröer C, Kroppenstedt RM, Trujillo ME. 2012. *Micromonospora cremea* sp. nov. and *Micromonospora zamorensis* sp. nov., isolated from the rhizosphere of Pisum sativum. Int J Syst Evol Microbiol, 62 (12): 2971-2977.

Carro L, Riesco R, Spröer C, Trujillo ME. 2016. *Micromonospora luteifusca* sp. nov. isolated from cultivated Pisum sativum. Syst Appl Microbiol, 39(4): 237-242.

Carro L, Veyisoglu A, Riesco R, Spröer C, Klenk HP, Sahin N, Trujillo ME. 2018. *Micromonospora phytophila* sp. nov. And *Micromonospora luteiviridis* sp. nov., isolated as natural inhabitants of plant nodules. Int J Syst Evol Microbiol, 68(1): 248-253.

Cassinelli G, Di Matteo FR, Forenza S, Ripamonti MC, Rivola G, Arcamone, F, Pratesi G. 1980. New Anthracycline Glycosides from *Micromonospora* Ii. Isolation, Characterization and Biological Properties. J Antibiot, 33(12): 1468-1473.

Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, da Costa M, Trujillo ME. 2018. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol, 68(1): 461-466.

Compant S, Duffy B, Nowak J, Clément C, Barka EA. 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action, and future prospects. Appl Environ Microbiol, 71(9): 4951-4959.

Coronelli C, Bardone MR, Depaoli A, Ferrari P, Tuan G, Gallo GG. 1984. Teicoplanin, antibiotics from *Actinoplanes teichomyceticus* sp. nov. J Antibiot, 37(6): 621-626.

Debono M, Merkel KE, Molloy RM, Barnhart M, Presti E, Hunt AH, Hamill RL. 1984. Actaplanin, new glycopeptide antibiotics produced by *Actinoplanes missouriensis*. J Antibiot 37(1): 85-95.

Garcia LC, Martínez ME, Trujillo ME. 2010. *Micromonospora pisi* sp. nov., isolated from root nodules of *Pisum sativum*. Int J Syst Evol Microbiol 60(2): 331-337.

Gaur AC. 1990. Phosphate solubilizing microorganisms as biofertilizers. Omega Scientific, New Delhi, India

Genilloud O. 2012. Genus micromonospora. The actinobacteria. In: Goodfellow M et al (eds) Bergey's manual of systematic bacteriology, 2nd edn. Springer, New York, pp: 1039-1057.

Genilloud O. 2017. Actinomycetes: still a source of novel antibiotics. Nat Prod Rep, 34(10): 1203-1232.

Hayakawa M, Nonomura H. 1987. Humic acid-vitamin agar, a new medium for the selective isolation of soil *Actinomycetes*. J Ferment Technol, 65(5): 501-509.

Huang H, Wu X, Yi S, Zhou Z, Zhu J, Fang Z, Bao S. 2009. Rifamycin S and its geometric isomer produced by a newly found Actinomycete, *Micromonospora rifamycinica*. Antonie Van Leeuwenhoek, 95(2):143-148.

Katz L, Baltz RH. 2016. Natural product discovery: past, present, and future. J Ind Microbiol Biotechnol, 43(2-3): 155-176.

Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Chun J. 2012. Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int. J Syst Evol Microbiol, 62(Pt 3): 716-721.

Kim TK, Hewavitharana AK, Shaw PN, Fuerst JA. 2006. Discovery of a new source of rifamycin antibiotics in marine sponge actinobacteria by phylogenetic prediction. Appl Environ Microb, 72(3): 2118-2125.

Lancini G, Lorenzetti R. 1993. Biosynthesis of secondary metabolites. Biotechnology of antibiotics and other bioactive microbial metabolites. Springer, Berlin, pp 95-132.

Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. 2013. Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics, 14(1): 60.

Meier-Kolthoff JP, Carbasse JS, Peinado-Olarte RL, Göker, M. 2022. TYGS and LPSN: a database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. Nucleic Acids Res, 50(D1): D801-D807.

Meier-Kolthoff JP, Göker M. 2019. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun, 10(1): 2182.

Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Stevens R. 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res, 42(D1): D206-D214.

Parte AC, Sardà CJ, Meier-Kolthoff JP, Reimer LC, Göker M 2020. List of Prokaryotic names with Standing in Nomenclature (LPSN) moves to the DSMZ. Int J Syst Evol Microbiol, 70(11): 5607-5612.

Rajkumar M, Ae N, Prasad MNV, Freitas H. 2010. Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol, 28(3): 142-149.

Riedlinger J. 2004. Abyssomicins, inhibitors of the paraaminobenzoic acid pathway produced by the marine Verrucosispora strain AB-18-032. J Antibiot, 57(3): 271-279.

Rodríguez H, Fraga R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv, 17(4-5): 319-339.

Rosselló-Móra R, Amann R. 2015. Past and future species definitions for *Bacteria* and *Archaea*. Syst Appl Microbiol, 38(4): 209-216.

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

Spaepen S, Vanderleyden J. 2011. Auxin and plant-microbe interactions. Cold Spring Harb Perspect Biol, 3(4): a001438.

Subramani R, Aalbersberg W. 2012. Marine actinomycetes: an ongoing source of novel bioactive metabolites. Microbiol Res, 167(10): 571-580.

Talukdar M, Bora TC, Jha DK. 2016. *Micromonospora*: a potential source of antibiotic. In Bioprospecting of Indigenous Bioresources of North-East India. Singapore, Springer Singapore, pp. 195-213.

Thomas CM, Nielsen KM. 2005. Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nature Rev Microbiol, 3(9): 711-721.

Trujillo ME, Kroppenstedt RM, Fernádez-Molinero C, Schumann P, Martínez Molina E. 2007. Micromonospora lupini sp. nov. and Micromonospora saelicesensis sp. Nov., isolated from root nodules of Lupinus angustifolius. Int J Syst Evol Microbiol, 57(12): 2799-2804.

Trujillo ME, Kroppenstedt RM, Schumann P, Carro L, Martínez-Molina E. 2006. *Micromonospora coriariae* sp. nov., isolated from root nodules of *Coriaria myrtifolia*. Int J Syst Evol Microbiol, 56(10): 2381-2385.

Ventura M, Canchaya C, Tauch A, Chandra G, Fitzgerald GF, Chater KF, van Sinderen D. 2007. Genomics of *Actinobacteria*: tracing the evolutionary history of an ancient phylum. Microbiol Mol Biol Rev, 71(3): 495-548.

Veyisoğlu A, Tatar D. 2021. Screening of acidophilic

actinobacteria that show activity against paddy pest fungi. Int J Agric Environ Food Sci, 5(3): 425-432.

Wattam AR, Davis JJ, Assaf R, Boisvert S, Brettin T, Bun C, Stevens RL. 2017. Improvements to PATRIC, the all-bacterial bioinformatics database and analysis resource center. Nucleic Acids Res, 45(D1): D535-D542.

Williams ST, Goodfellow M, Wellington EMH, Vickers JC, Alderson G, Sneath PHA, Mortimer AM. 1983. A probability matrix for identification of some streptomycetes. Microbiology, 129(6): 1815-1830.