



# *Microbacterium rhizophilus* sp. nov., an indole acetic acid-producing actinobacterium isolated from rhizosphere soil

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**Abstract** A novel gram-stain-positive, short rod, aerobic, non-motile and non-spore-forming actinobacterial strain, designated GXG1230<sup>T</sup> was isolated from the rhizosphere soil of a coastal mangrove forest in Beihai city, Guangxi Zhuang Autonomous Region, PR China. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain GXG1230<sup>T</sup> was affiliated with the genus *Microbacterium*. Additionally, it demonstrated a high degree of similarity to *Microbacterium paludicola* US15<sup>T</sup> (97.9%) and *Microbacterium marinilacus* YM11-607<sup>T</sup> (97.3%). Chemotaxonomic characteristics showed that the whole-cell sugars were glucose, xylose, rhamnose and galactose. Menaquinones MK-11 and MK-12 were detected as respiratory quinones. Lysine was found in the

peptidoglycan hydrolysate and the polar lipids were diphosphatidylglycerol, one phospholipid and two unidentified glycolipid. The major fatty acids were *anteiso*-C<sub>15:0</sub>, *iso*-C<sub>16:0</sub> and *anteiso*-C<sub>17:0</sub>. The strain GXG1230<sup>T</sup> exhibited a genomic DNA G+C content of 71.7%. Furthermore, the average nucleotide identity values of GXG1230<sup>T</sup> with the reference strains were 75.4% and 81.9%, respectively, while the digital DNA-DNA hybridization values were 20.1% and 25.0%. Based on physiological, chemotaxonomic and phylogenetic information, strain GXG1230<sup>T</sup> is considered to represent a novel species of the genus *Microbacterium*, for which the name *Microbacterium rhizophilus* sp.nov. is proposed, with GXG1230<sup>T</sup> (=MCCC 1K09302<sup>T</sup>=KCTC 59252<sup>T</sup>) as the type strain.

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## Introduction

The classification of the genus *Microbacterium* was initially proposed by Orla-Jensen et al. (1919), with reference to investigations conducted on the archetype *Microbacterium lacticum*. This taxonomy was subsequently refined by Takeuchi et al. (1998). This genus is Gram-stain-positive, and the members of the genus *Microbacterium* are distributed across a range of habitats, including swamps (Park et al.

2006), oceans (Kageyama et al. 2007), and cow dung (Zhang et al. 2021)<sup>0</sup>. The majority of bacteria in this genus are rod-shaped and non-spore-forming. The peptide subunit in this genus of peptidoglycan is composed of lysine or ornithine, which can be used as diagnostic amino acids. The guanine-cytosine content of genomic DNA in the genus *Microbacterium* typically ranges from 66 to 72%.

The advent of fertilizers and insecticides has led to a notable increase in crop yields. However, this has come at the expense of environmental and ecological unsustainability (Majeed and Muhammad 2018). In order to alter this situation, individuals have commenced focusing their attention on plant growth-promoting rhizobacteria (PGPR), which possess a multitude of advantages, including environmental protection, safety, and long-term efficacy. Indole-3-acetic acid (IAA) is a plant growth regulator secreted by PGPR, which plays a role in various physiological and biochemical processes and has a significant impact on plant growth (Sheng et al. 2008). Furthermore, PGPR exhibit biological activities that include the capacity to solubilize inorganic phosphorus and generate siderophores to promote growth, in addition to producing volatile organic compounds (Zhang et al. 2017). Secondary metabolites can attenuate root infections by specific plant pathogenic fungi (Barnett et al. 2006). These advantageous properties render PGPR strains the optimal choice for plant growth promotion and disease prevention (Kim et al. 2000). During the isolation and excavation of actinobacterial resources from the rhizosphere soil of plants near the coast of Beihai City, Guangxi, a novel strain of actinobacteria was isolated from the rhizosphere soil of a coastal mangrove forest. Preliminary studies have confirmed that this strain has been identified as a novel species of the genus *Microbacterium* that produces indole-3-acetic acid.

## Materials and methods

### Isolation, maintenance and cultural conditions

A bacterial strain, designated GXG1230<sup>T</sup>, was isolated from a rhizosphere soil collected from a coastal mangrove forest in Beihai City, Guangxi, China (21°56'30.14" N, 109°76'27.23" E). A

specified amount of soil sample was added to 30 mL of normal saline and incubated on a shaker for 2 h at 28 °C. The resulting suspension was then serially diluted to 10<sup>-6</sup> and 10<sup>-7</sup> with sterile saline and then 100 µL of each dilution was plated evenly on plates containing Reasoner's 2A (R<sub>2</sub>A) agar medium (comprising 0.5 g yeast extract, 0.45 g glucose, 0.3 g sodium 2-oxopropanoate, 0.5 g casein hydrolysate, 0.5 g soluble starch, 0.5 g peptone, 0.024 g MgSO<sub>4</sub>, 0.3 g K<sub>2</sub>HPO<sub>4</sub>, 16 g agar, 1L sterile water, pH=7.3). After approximately one week of cultivation at 28 °C, isolated colonies were transferred to trypticase soy broth (TSB) medium with the addition of agar and stored in a glycerol suspension (40%, v/v) at -80 °C. The type strains, *Microbacterium paludicola* US15<sup>T</sup> and *Microbacterium marinilacus* YM11-607<sup>T</sup>, were purchased from Marine Culture Collection of China (MCCC) and cultured under the same conditions for comparative analysis.

### Determination of 16S rRNA gene sequences and phylogenetic relationships

The genomic DNA derived from the isolate denoted as GXG1230<sup>T</sup> was isolated, followed by the amplification of the 16S rRNA gene sequence using the PCR. Following amplification, the purified product was purified and cloned into the pMD19-T vector. The clones were then submitted to Sangon Biotech (Guangzhou, China) for sequencing, and the resulting sequences were compared in EzBioCloud (Yoon et al. 2017). Phylogenetic trees were generated using the MEGA11 software tool (Tamura et al. 2021) with neighbour-joining (NJ) (Saitou and Nei 1987) maximum-likelihood (ML) (Felsenstein 1981) and maximum-parsimony (MP) (Fitch 1971) method, and topology stability was assessed by bootstrap method 1000 times. Evolutionary distances were calculated in accordance with the methodology of Kimura (1980).

### Genomic characterization

The whole genome sequencing of strain GXG1230<sup>T</sup> was performed by Shanghai Biozeron Biotechnology Co., Ltd. (Shanghai, China.). Based on the core genome, the phylogenomic tree of the genome was constructed using a high-resolution phylogenetic pipeline tool (UBCG) (Na et al. 2018). The NCBI species database provided the draft genomes for

19 strains of the genus *Microbacterium*, which were used to construct the phylogenomic tree. *Pseudoclaudibacter helvolus* DSM 20419<sup>T</sup> (JACHWJ000000000) was employed as an outgroup. The dDDH value between strain GXG1230<sup>T</sup> and closely related members was computed using genome-genome distance calculator (<http://ggdc.dsmz.de/>) (Meier-Kolthof et al. 2013). The ANI calculator (<https://www.ezbiocloud.net/tools/ani>) and AAI calculator (<https://enve-omics.gatech.edu/>) provided by CJ Bioscience and Enveomics Lab were employed to calculate the ANI and AAI of strains that are closely related to GXG1230<sup>T</sup>. The features of the genome were analysed by RAST Server (<https://rast.nmpdr.org/>) (Aziz and Bartels 2008) and the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (<https://www.ncbi.nlm.nih.gov/genome/annotation.prok/>) (Tatusova et al. 2016). The orthology analysis Venn diagram among the three genomes were generated using Ortho Venn3 (<https://orthovenn3.Bioinfotoolkits.net/home>) (Sun et al. 2023). The secondary metabolite biosynthesis gene cluster in the genome was rapidly analysed using the antiSMASH v.7.1 analysis tool (<https://antismash.secondarymetabolites.org/>) (Blin and Shaw 2021). Rapid analysis of annotated genomic functional gene clusters and metabolic pathways using COG (<http://www.ncbi.nlm.nih.gov/COG/>) and KEGG (<http://www.genome.jp/kegg/>) annotation tools (Cantalapiedra et al. 2021).

#### Physiological, biochemical and phenotypic characterization

Morphological features were examined using the Hitachi HT-7700 transmission electron microscopy. The strains to be tested were stained in accordance with the protocol of the Solarbio (Beijing, China) Gram staining kit. The growth of strain GXG1230<sup>T</sup> at different temperatures (0–48 °C, with increments of 6 °C) and at different pH range (pH 4–11 at 1 pH unit intervals) was quantified after 1 week of incubation in LB medium. The NaCl tolerance of strain GXG1230<sup>T</sup> was quantified in LB medium at 28 °C for 1 week, with NaCl concentrations ranging from 0 to 14% (w/v) at 2% (w/v) intervals. The motility of the strain was quantified in LB medium containing 0.4% agar (Cai et al. 2021). Catalase activity was assessed using medium with 3.5%

(v/v) hydrogen peroxide content. To ascertain the sensitivity of the strains to antibiotics, a sensitivity test was conducted using test pieces containing antibiotics (Zhao et al. 2024). The production of siderophores and the utilization of inorganic phosphorus were quantified using a CAS detection medium and an inorganic phosphorus medium. The physiological characteristics of the strains, including acid production and enzyme activity, were evaluated using API 20NE, API ZYM, API 50CH, and Biolog GEN III test strips (bioMerieux). Strain GXG1230<sup>T</sup> was incubated in LB medium containing 20 mg/L tryptophan at 28 °C for 24 h. The presence of IAA in the supernatant of the culture after centrifugation was determined spectrophotometrically at 535 nm. The concentration of IAA was determined by linear regression analysis, using the calibration curve of pure IAA as a standard (Zhang et al. 2021). The effect of strain GXG1230<sup>T</sup> on the growth of *Nicotiana benthamiana* was investigated. The seeds were disinfected in 75% (v/v) ethanol for 1 min and then in 3% NaClO (v/v) ethanol for 5 min. Subsequently, the seeds were rinsed three times with sterile distilled water and placed on MS medium at 25 °C for germination. Meanwhile, strain GXG1230 was inoculated on MS medium in accordance with the methodology described by Yang et al. (2023).

#### Chemotaxonomic characteristics

After 72 h of incubation on TSB agar at 30 °C, cells were harvested for chemical analysis. Fatty acid samples were then meticulously prepared and analysed in strict accordance with the Sherlock Microbial Identification System (MIDI, version 6.0) operating guidelines (Sasser et al. 2005). Polar lipids were extracted according to the comprehensive procedure described by Minnikin and Odonnell (1984). Methylnaphthoquinone extraction followed the methodology described by Collins et al. (1977) and was identified by HPLC (High Performance Liquid Chromatography) (Kroppenstedt 1982). The peptidoglycan preparation was performed according to the method described by Komagata and Suzuki (1988). A whole-cell sugar analysis was conducted in accordance with the methodology of Lechevalier and Bievre (1980).

## Results and discussion

### Phylogenetic characterization

The complete 16S gene rRNA sequence of strain GXG1230<sup>T</sup> was obtained at a length of 1459 bp (PP528786) and compared in the EzBioCloud's database. This analysis revealed that *M. paludicola* US15<sup>T</sup> (97.9%) exhibited the highest degree of similarity to strain GXG1230<sup>T</sup>. Based on 16S rRNA gene sequences, the Neighbor-Joining tree demonstrated that strain GXG1230<sup>T</sup> formed a stable clade with *M. paludicola* US15<sup>T</sup> and *M. marinilacus* YM11-607<sup>T</sup> (Fig. 1), with similarities of 97.9% and 97.3%, respectively (Table 1). This relationship was also observed in the the maximum-likelihood tree and maximum-parsimony tree (Figs S1 and S2). Based on the above results, *M. paludicola* US15<sup>T</sup> and *M. marinilacus* YM11-607<sup>T</sup> were selected as the reference strains for comparative analysis. The construction of a whole genome phylogenomic tree based on core genes indicates that strain GXG1230<sup>T</sup> belongs to the genus *Microbacterium* and is associated with strain *M. paludicola* US15<sup>T</sup> and *M. Marinilacus* YM11-607<sup>T</sup>, which forms a stable branch (Fig. S3). The aforementioned outcomes lend support to the hypothesis that strain GXG1230<sup>T</sup> represents a novel species.

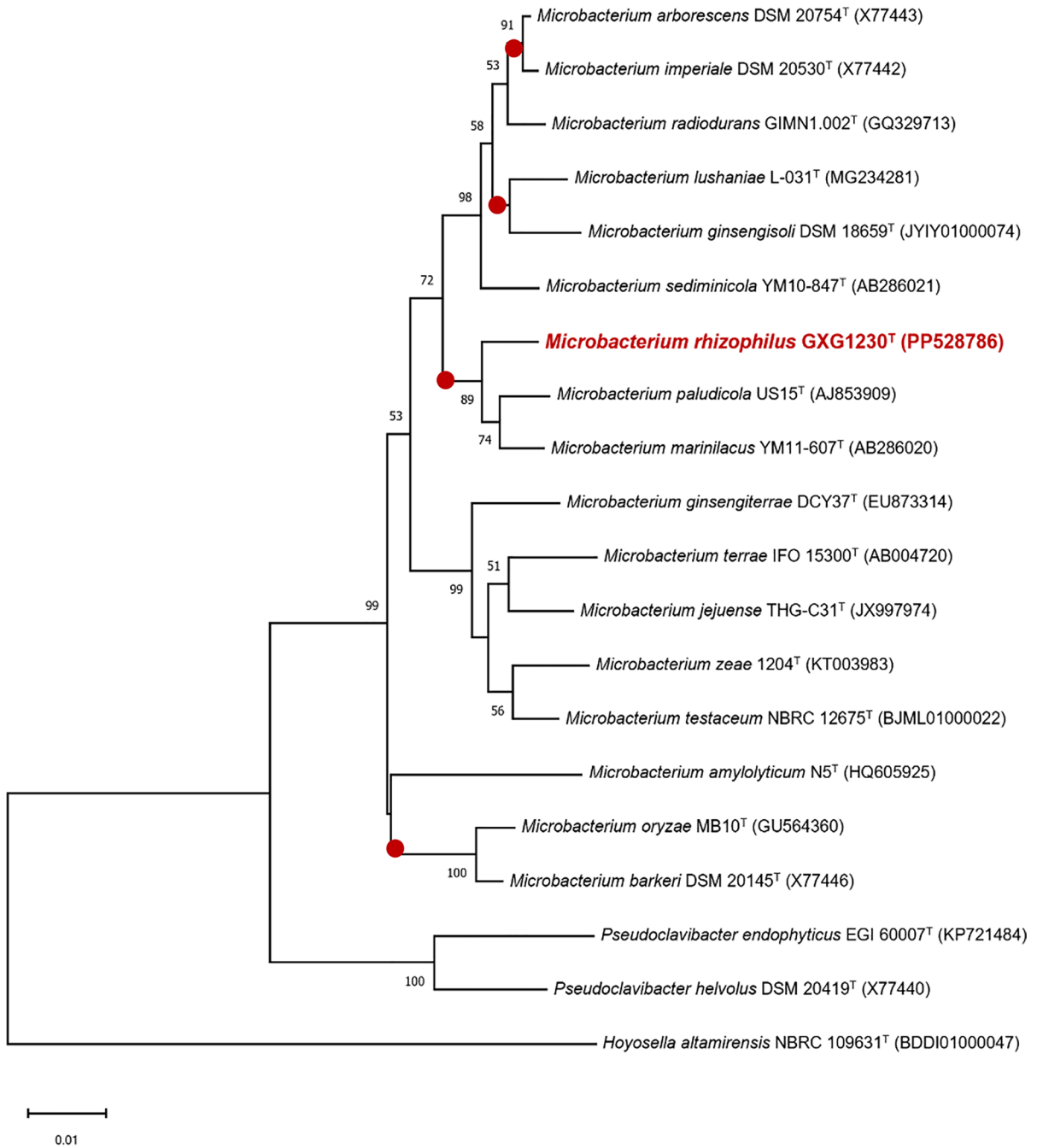
### Genomic analysis

The draft genome sequence analysis revealed that strain GXG1230<sup>T</sup> has a DNA G+C content of 71.7% and the full genome length of was approximately 3,242,536 bp (GenBank accession number JBCNUN000000000). The genome was comprised of 14 overlapping groups, with the largest and smallest sequences measuring 791,416 bp and 1396 bp, respectively. The L50 value was 3, with an N50 of 273,096 bp, with an average genome coverage 167.2×. The genomic analysis enabled the differentiation of strain GXG1230<sup>T</sup> from closely related species of the genus *Microbacterium* (Table 2). The dDDH calculation results demonstrated that the hybridization values of GXG1230<sup>T</sup> with *M. paludicola* US15<sup>T</sup> and *M. marinilacus* YM11-607<sup>T</sup> were 20.1% and 25.0%, respectively, which is below the 70% cutoff for species differentiation. Similarly, strains GXG1230<sup>T</sup>

with *M. paludicola* US15<sup>T</sup> and *M. marinilacus* YM11-607<sup>T</sup> exhibited ANI and AAI values of 75.4%, 81.9%, and 84.3%, 77.9%, respectively. These values were also below the 95–96% species delimitation threshold typically employed to distinguish prokaryotic species (Table 1). In RAST annotation (Table S1) and COG analysis (Table S2), the genomic characterization of strain GXG1230<sup>T</sup> was found to be distinct from that of closely related species within the genus *Microbacterium*.

Pan-genomic comparison revealed that strain GXG1230<sup>T</sup> contained 2263 gene clusters, while the other two bacteria had 2102 and 2477 gene clusters, respectively. The highest degree of similarity between gene clusters was observed in the case of strain GXG1230<sup>T</sup> and *M. marinilacus* YM11-607<sup>T</sup>, with 2121 gene clusters. The analysis of three strains revealed the presence of a "core" genome, comprising 1647 homologous gene clusters. The majority of these gene clusters encoded proteins related to cellular metabolism, colonization, and exchange systems. We identified 19 gene clusters were identified as unique to strain GXG1230<sup>T</sup> (Fig. S4).

The gene annotation results indicate that the draft genome of this strain contains 3180 features and 3131 coding sequences. The genome sequences were subjected to annotation and analysis using the RAST and the NCBI Prokaryotic Genome Annotation Pipeline (Table S3), which revealed the presence of auxin biosynthesis in GXG1230<sup>T</sup>, such as the gene encoding indole-3-glycerol phosphate synthase (WP\_345750730.1), sensor histidine kinase (WP\_345752964.1), anthranilate phosphoribosyltransferase (WP\_345750583.1), tryptophan synthase subunit alpha and subunit beta (WP\_345750234.1, WP\_345750731.1). These genes are recognized as pivotal elements within the IAA synthesis pathway (Bartel 1997; Yang et al. 2023). Based on the KEGG database, *iaaM* gene tryptophan metabolism (PATH: ko00380), which is the key gene to synthesize indole acetic acid in the indole acetamide pathway were annotated. Furthermore, genes associated with gibberellin synthesis (K01775), phosphatase activity (K01126), and polyphosphate kinase (K00937) have also been identified in the genome of strain GXG1230<sup>T</sup>. However, this process was not observed in *M. paludicola* US15<sup>T</sup> and *M. marinilacus* YM11-607<sup>T</sup>.



**Fig. 1** The Neighbor-Joining tree, based on the 16S rRNA gene sequences illustrates the phylogenetic relationship of GXG1230<sup>T</sup> with related taxa. The evolutionary distances were calculated using the Kimura 2-parameter method. The bootstrap support values were calculated from 1000 replicates.

The red filled circles indicate that the corresponding nodes were recovered from the trees generated with the maximum-likelihood and maximum-parsimony algorithms. The bar represents 0.02 substitutions per nucleotide position



**Table 1** ANI, AAI and dDDH values between strain GXG1230<sup>T</sup> and other closely related strains

Strain 1	Strain 2	ANI (%)	AAI(%)	dDDH (%)	16S rRNA gene identity (%)
GXG1230 <sup>T</sup>	<i>M. paludicola</i> US15 <sup>T</sup>	75.4	84.3	20.1	97.9
	<i>M. marinilacus</i> YM11-607 <sup>T</sup>	81.9	77.9	25.0	97.3

**Table 2** Comparison genomic characteristics of GXG1230<sup>T</sup> and related members of the genus *Microbacterium*

Genes putatively encoding	GXG1230 <sup>T</sup>	<i>M. paludicola</i> US15 <sup>T</sup>	<i>M. marinilacus</i> YM11-607 <sup>T</sup>
GenBank accession number	JBCNUN000000000	SPQB000000000	JAIJZW000000000
Genome size (bp)	3,242,536	3,410,829	3,888,012
DNA G+C content (%)	71.7	70.1	71.7
N50 value (bp)	273,096	2,793,096	216,039
L50 value (bp)	3	1	5
Number of coding sequences	3131	3256	3748
Features in the draft genome	3180	3309	3796
Number of rRNAs	49	53	48
Number of Subsystems	242	250	252

Furthermore, the gene annotation results of strain GXG1230<sup>T</sup> identified genes associated with Ammonia assimilation and salt tolerance (Table S3), including the ammonium transporter (WP\_345750172.1), glutamine amidotransferase (WP\_345752161.1) and Na<sup>+</sup>/H<sup>+</sup> reverse transporter proteins NhaD (WP\_345751804.1) and NhaA (WP\_345751033.1). The organonitrogen compound present in soil are primarily proteins, nucleic acids, peptidoglycans, chitin, and other similar substances. Additionally, there are minor quantities of water-soluble organonitrogen compound, including amino acids and urea. With the exception of soluble amino acids, these substances cannot be directly absorbed by plants and must be decomposed by microorganisms to release ammonia for plant utilization.

The capacity of strain GXG1230<sup>T</sup> to produce secondary metabolites was assessed using antiSMASH software indicated that GXG1230<sup>T</sup>, *M. paludicola* US15<sup>T</sup> and *M. marinilacus* YM11-607<sup>T</sup> contain terpene and betalactone genes, and that the Non-alpha poly-amino acids (NAPAA) gene is present only in GXG1230<sup>T</sup> and *M. marinilacus* YM11-607<sup>T</sup> (Table S4). The strain GXG1230<sup>T</sup> exhibited a terpene gene cluster that shares 35% similarity with the carotenoid. This finding is consistent with the phenotypic characteristics of GXG1230<sup>T</sup>, which

displays a yellow colony color. Furthermore, strain GXG1230<sup>T</sup> contained an NAPAA gene cluster with 100% similarity to ε-Poly-L-lysine, a bioactive metabolites that protects host plants against biotic and abiotic stresses. It has been shown to inhibit spore germination of plant pathogens, such as *Phytophthora infestans*, *Botrytis cinerea* and *Drechslera erythrospila* (Purev et al. 2020).

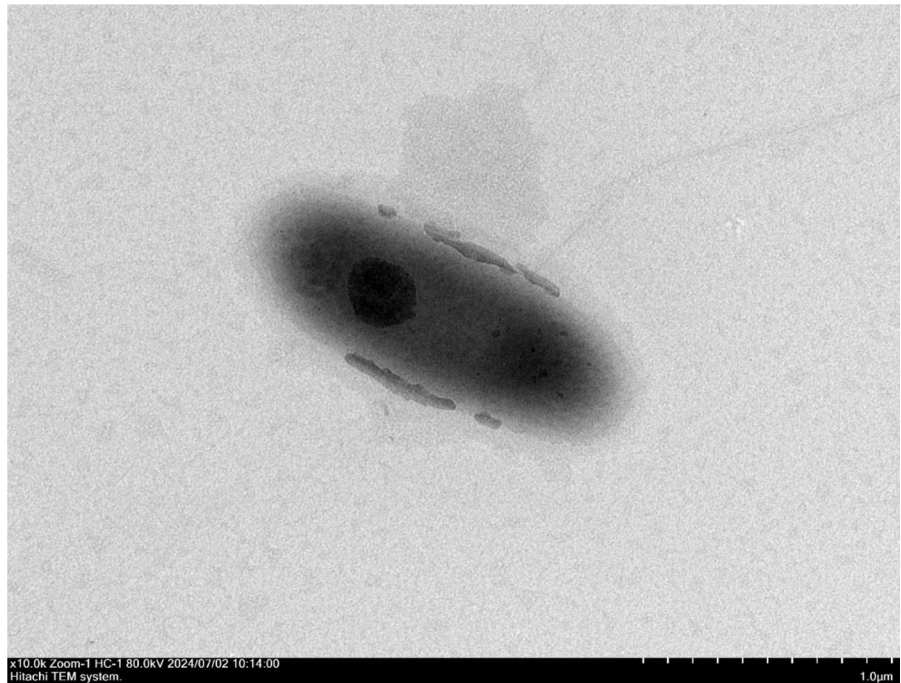
The identification of these genes and secondary metabolites demonstrated that strain GXG1230<sup>T</sup> is capable of thriving in the rhizosphere soil environment of coastal plants. Furthermore, the secondary metabolites produced by strain GXG1230<sup>T</sup> are utilized by plants to promote growth or protect the host from plant pathogens.

In addition, Genetic annotation revealed the presence of genes for Type IV pilus, but not for motility and chemotaxis. This finding is consistent with the results of our previous physiological experiment, in which we observed the presence of flagella in transmission electron microscope but not motility.

Morphological, physiological, and biochemical characteristics

Strain GXG1230<sup>T</sup> was a Gram-stain-positive, non-motile, aerobic strain. Cells exhibited a short rod-like

**Fig. 2** Transmission electron micrographs of the cells of strain GXG1230<sup>T</sup>. Cells are cultured on LB medium at 30 °C for 2–3 days. Bar, 1.0 μm



morphology (Fig. 2) with a length of approximately 1.5–1.8 μm and a width of 0.4–0.7 μm. The colonies of the strain GXG1230<sup>T</sup> exhibited a yellow, rounded, raised morphology, characteristics with a diameter of 1.5–2.5 mm and no diffuse pigmentation. The strain was subjected to a series of growth conditions with GXG1230<sup>T</sup> demonstrating optimal growth at 28 °C. The strain demonstrated a sodium chloride tolerance concentration range of 0–10% (optimal 3.5%). The strain GXG1230<sup>T</sup> was found to be catalase negative. The strain grew in a pH range of 6–8, with an optimal pH 7.5. The differential enzyme activities, carbon source utilization, and other physiological characteristics of strain GXG1230<sup>T</sup> and closely related species of the genus *Microbacterium* are provided in Table 3. Chemotaxonomic analysis revealed that strain GXG1230<sup>T</sup> exhibited the typical chemotaxonomic characteristics of members of the genus *Microbacterium*. The whole cell sugars contain glucose, xylose, rhamnose and galactose. Lysine was identified in the peptidoglycan hydrolysate. Polar lipids of strain GXG1230<sup>T</sup> were composed of diphosphatidylglycerol, one phospholipid and two unidentified glycolipid. In contrast to GXG1230<sup>T</sup>, polar lipids of *M. paludicola* US15<sup>T</sup> were diphosphatidylglycerol, phosphatidylglycerol

and unidentified glycolipid (Fig. S5). The major isoprenoid quinones were designated as MK-11 and MK-12, consistent with the composition of the reference strains. The main fatty acids (> 10%) of strain GXG1230<sup>T</sup> were identified as *anteiso*-C<sub>15:0</sub> (36.9%), *iso*-C<sub>16:0</sub> (28.9%) and *anteiso*-C<sub>17:0</sub> (12.1%) (Table S5), respectively. The main fatty acids (> 10%) of strain *M. paludicola* US15<sup>T</sup> were identified as *anteiso*-C<sub>15:0</sub> (63.9%), *anteiso*-C<sub>17:0</sub> (14.4%) and *iso*-C<sub>16:0</sub> (11.7%). The main fatty acids (> 10%) of strain *M. marinilacus* YM11-607<sup>T</sup> were identified as *anteiso*-C<sub>15:0</sub> (59.1%), *anteiso*-C<sub>17:0</sub> (20.9%) and *iso*-C<sub>16:0</sub> (14.4%). The main fatty acids content of strain GXG1230<sup>T</sup> were different from those of *M. paludicola* US15<sup>T</sup> and *M. marinilacus* YM11-607<sup>T</sup>. Strain GXG1230<sup>T</sup> demonstrated resistance to erythromycin and amoxicillin. In strain GXG1230<sup>T</sup>, the production of indole-3-acetic acid (IAA) was identified by the Salkowski colorimetric method. Qualitative experiments indicated that the production of IAA was evidenced by a reddening of the solution (Fig. S6), with an approximate yield of 25.0 ± 0.6 mg/L. The germination test showed that the strain GXG1230<sup>T</sup> with appropriate cell concentration (10<sup>7</sup> CFU/mL) can promote the germination of *Nicotiana benthamiana* seeds and root growth (Fig. 3).

**Table 3** Differential physiological characteristics of GXG1230<sup>T</sup> from the closely related species *M. paludicola* US15<sup>T</sup> and *M. marinilacus* YM11-607<sup>T</sup>

	GXG1230 <sup>T</sup>	<i>M. paludicola</i> US15 <sup>T</sup>	<i>M. marinilacus</i> YM11-607 <sup>T</sup>
Colour of colonies	Yellow	Lemon yellow	Lemon yellow
pH (optimum)	6–8	6–9	6–11
Temperature (optimum) (°C)	4–40	5–45	4–37
NaCl (optimum) (% w/v)	0–10	1–9	0–7
Assimilation of (API 20NE)			
Indole production	+	–	–
Oxidase	–	+	+
Trisodium citrate	–	+	+
Potassium gluconate	–	+	+
Acid production from (API 50CH)			
LRhamnose	+	+	–
D-Melezitose	+	ND	–
D-Saccharose	+	–	ND
Utilization as sole carbon source			
D-Fructose	–	+	+
L-Rhamnose	–	+	+
Sucrose	+	+	–
Utilization as sole nitrogen source			
L-Threonine	+	–	–
Enzymology (API ZYM)			
Esterase (C4)	+	–	+
Esterase lipase (C8)	+	–	+
Leucine arylamidase	+	–	+
Valine arylamidase	+	–	+
Cystine arylamidase	+	–	+
Naphthol-as-bi-phosphohydrolase	+	–	–
α-Mannosidase	+	–	+
α-Glucosidase	–	+	+

All data presented are from this study. +, positive; –, negative; ND, not determined

### Description of microbacterium rhizophilus sp. nov

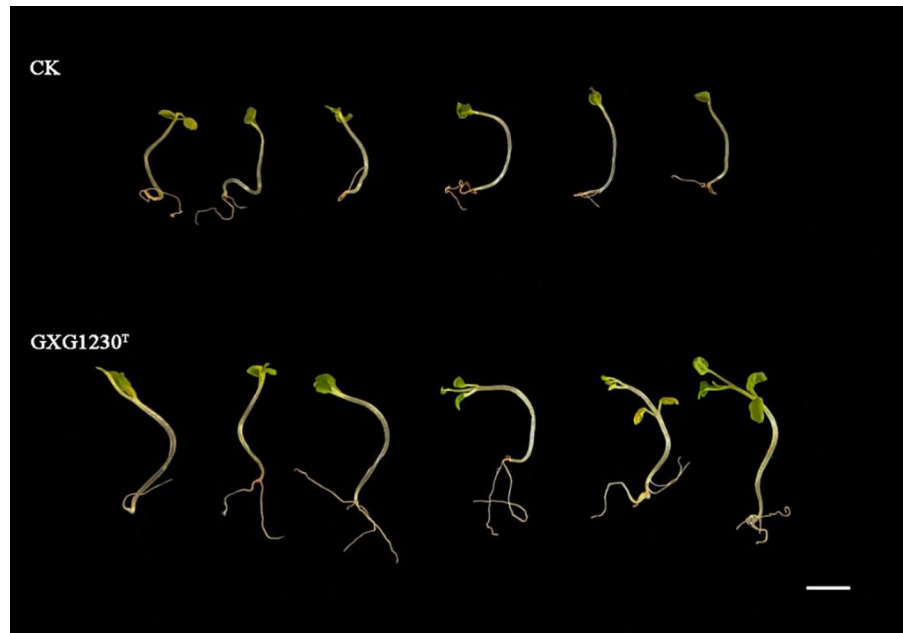
*Microbacterium rhizophilus* (rhi.zo'phi.lus. Gr. fem. n. *rhiza*, root; N.L. masc. adj. suff.—*philus* (from Gr. masc. adj. *philos*), loving; N.L. masc. adj. *rhizophilus* root-loving).

The strain is Gram-stain-positive, short rod, aerobic, no spore formation, with a length of approximately 1.5–1.8 μm and a width 0.4–0.7 μm. After 2–4 days of incubation at 28 °C on LB medium, colonies exhibited a smooth, round, and yellow appearance. The strains can be cultivated at temperatures spanning from 4 to 40 °C (with an optimal range of 28 °C) and at a pH level ranging from 6 to 8 (with an optimal pH level of 7.5). The strain

is tolerant to up to 10% (w/v) NaCl (optimum 4%) in the medium. It is catalase negative. This strain shows production of indole, production of siderophores, and degradation of inorganic phosphorus. The strain utilizes D-mannose, D-ribose, maltose, lactose, D-sorbitol, D-glucose, D-galactose, and sucrose as the sole carbon source. Glycine, L-serine, L-proline, L-threonine, sarcosine, L-aspartic acid, L-tyrosine, and L-arginine as the sole nitrogen sources. The production of acid from D-xylose, D-glucose, D-galactose, esculin, D-maltose, D-saccharose, D-cellobiose, D-melezitose, D-fructose, D-turanose, and D-mannose is observed in API 50CH test strips. In API 20NE test strips, positive results are obtained for indole production,



**Fig. 3** Growth promoting effects of GXG1230<sup>T</sup> on *Nicotiana benthamiana* seedlings. Growth phenotype of seedlings in MS medium without inoculation and inoculated with strain GXG1230<sup>T</sup> after 10 days. CK: 1 mL of LB liquid medium added to MS medium; experimental group: LB culture medium of strain GXG1230<sup>T</sup> with a cell concentration of 10<sup>7</sup> CFU/mL was added to MS medium. Bar = 1 cm



urease, nitrate, D-mannose, maltose, D-mannitol, D-glucose, glucose fermentation, but negative for Larabinose, oxidase, hydrolysis of aesculin, adipic acid, arginine dihydrolase, capric acid, gelatin hydrolysis, malic acid,  $\beta$ -galactosidase, *N*-acetylglucosamine, potassium gluconate, phenylacetic acid and trisodium citrate. In API ZYM tests, positive results for cystine arylamidase, trypsin, valine arylamidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, esterase lipase (C8), esterase (C4), and  $\alpha$ -mannosidase, but negative results in tests for *N*-acetyl- $\beta$ -glucosaminidase,  $\alpha$ -galactosidase,  $\beta$ -galactosidase,  $\alpha$ -glucosidase,  $\beta$ -glucosidase,  $\beta$ -glucuronidase, lipase (C14), acid phosphatase, alkaline phosphatase,  $\alpha$ -chymotrypsin and  $\alpha$ -fucosidase.

The phospholipid profile includes diphosphatidylglycerol, one phospholipid and two unidentified glycolipid. The whole cell sugars contain glucose, xylose, rhamnose and galactose. Lysine was identified in the peptidoglycan hydrolysate. The major cellular fatty acids were *anteiso*-C<sub>15:0</sub>, *iso*-C<sub>16:0</sub> and *anteiso*-C<sub>17:0</sub>. The major isoprenoid quinones were identified as MK-11 and MK-12. The genomic DNA G + C content of the type strain was determined to be 71.7%.

The type strain is GXG1230<sup>T</sup> (=MCCC 1K09302<sup>T</sup>=KCTC 59252<sup>T</sup>), isolated from the

rhizosphere soil of a coastal mangrove forest in Beihai city, Guangxi Zhuang Autonomous Region, China.

**Author's Contribution** Mingguo Jiang, conceptualisation and project administration. Contributed to the conception of the study, formulation or evolution of overarching research goals and aims; Haifei Liu, investigation and writing, original draft preparation, performing all of the experiments; Quan Yang, review and editing, performing part of the experiments. Jiawei Li, performed research; Lifang Yang, resources. Provision of some study materials; Aolin Zhao, performed research; Ying Huang, analysed data; Hongcun Liu, performed research; Shujing Wu, performed research.

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**Data availability** The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and the draft genome sequences of strain GXG1230<sup>T</sup> are PP528786 and JBCNUN000000000, respectively.

#### Declarations

**Conflict of interest** All authors confirm that no competing interests, both financial and personal, are with this manuscript.

## References

- Aziz RK, Bartels D (2008) The RAST server: rapid annotations using subsystems technology. *BMC Genom* 9:75. <https://doi.org/10.1186/1471-2164-9-75>
- Barnett SJ, Roget DK, Ryder MH (2006) Suppression of *Rhizoctonia solani* AG-8 induced disease on wheat by the interaction between *Pantoea*, *Exiguobacterium*, and *Microbacteria*. *Soil Res* 44:331–342. <https://doi.org/10.1071/SR05113>
- Bartel B (1997) Auxin biosynthesis. *Annu Rev Plant Physiol Plant Mol Biol* 48:51–66. <https://doi.org/10.1146/annurev.arplant.48.1.51>
- Blin K, Shaw S (2021) antiSMASH 6.0: improving cluster detection and comparison capabilities. *Nucl Acids Res* 49(W1):W29–W35. <https://doi.org/10.1093/nar/gkab335>
- Cai H, Shi S, Wu J et al (2021) *Flavimobilis rhizosphaerae* sp. nov., isolated from rhizosphere soil of *Spartina alterniflora*. *Int J Syst Evol Microbiol*. <https://doi.org/10.1099/ijsem.0.004829>
- Cantalapiedra CP, Hernandez-Plaza A, Ivica L et al (2021) eggNOG-mapper v2: functional annotation, orthology assignments, and domain prediction at the metagenomic scale. *Mol Biol Evol* 38(12):5825–5829. <https://doi.org/10.1093/molbev/msab293>
- Collins MD, Pirouz T, Goodfellow M, Minnikin DE (1977) Distribution of menaquinones in Actinomycetes and Corynebacteria. *J Gen Microbiol* 100:221–230. <https://doi.org/10.1099/00221287-100-2-221>
- Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 17:368–376. <https://doi.org/10.1007/BF01734359>
- Fitch WM (1971) Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* 20:406–416. <https://doi.org/10.1093/sysbio/20.4.406>
- Kageyama A, Takahashi Y, Matsuo Y et al (2007) *Microbacterium sediminicola* sp. nov. and *Microbacterium marinilacus* sp. nov., isolated from marine environments. *Int J Syst Evol Microbiol* 57(Pt 10):2355–2359. <https://doi.org/10.1099/ijms.0.65038-0>
- Kim SB, Brown R, Oldfield R et al (2000) *Gordonia amicalis* sp. nov., a novel dibenzothiophene-desulphurizing actinomycete. *Int J Syst Evol Microbiol* 50(Pt 6):2031–2036. <https://doi.org/10.1099/00207713-50-6-2031>
- Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16:111–120. <https://doi.org/10.1007/BF01731581>
- Kroppenstedt RM (1982) Separation of bacterial menaquinones by HPLC using reverse phase (RP18) and a silver loaded ion exchanger as stationary phases. *J Liq Chromatogr* 5:2359–2367. <https://doi.org/10.1080/01483918208067640>
- Komagata K, Suzuki KI (1988) Lipid and cell-wall analysis in bacterial systematics. *Method Microbiol* 19:161–207. [https://doi.org/10.1016/S0580-9517\(08\)70410-0](https://doi.org/10.1016/S0580-9517(08)70410-0)
- Lechevalier MP, Bievre CD (1980) Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Syst Ecol*. [https://doi.org/10.1016/0305-1978\(77\)90021-7](https://doi.org/10.1016/0305-1978(77)90021-7)
- Li R, Li Y, Wang J et al (2008) SOAP: short oligonucleotide alignment program. *Bioinformatics* 24:713–714. <https://doi.org/10.1093/bioinformatics/btn025>
- Majeed A, Muhammad Z (2018) Plant growth promoting bacteria: role in soil improvement, abiotic and biotic stress management of crops. *Plant Cell Rep* 37(12):1599–1609. <https://doi.org/10.1007/s00299-018-2341-2>
- Meier-Kolthoff JP, Auch AF, Klenk HP et al (2013) Genome sequence based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 14:60. <https://doi.org/10.1186/1471-2105-14-60>
- Minnikin DE, Odonnell AG (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* 2:233–241. [https://doi.org/10.1016/0167-7012\(84\)90018-6](https://doi.org/10.1016/0167-7012(84)90018-6)
- Na SI, Kim YO, Hans-Peter K et al (2018) UBCG: Up-to-date bacterial core gene set and pipeline for phylogenomic tree reconstruction. *J Microbiol* (Seoul, Korea) 56(4):280–285. <https://doi.org/10.1007/s12275-018-8014-6>
- Orla-Jensen S (1919) The lactic acid bacteria. Host & Sons, Copenhagen. <https://doi.org/10.4236/fns.2014.518190>
- Park HY, Kim KK, Long J et al (2006) *Microbacterium paludicola* sp. nov., a novel xylanolytic bacterium isolated from swamp forest. *Int J Syst Evol Microbiol* 56(Pt 3):535–539. <https://doi.org/10.1099/ijms.0.63945-0>
- Purev E, Kondo T, Daigo T et al (2020) Identification of  $\epsilon$ -Poly-L-lysine as an antimicrobial product from an *Epichloë* endophyte and isolation of fungal  $\epsilon$ -PL synthetase gene. *Molecules* (Basel, Switzerland) 25(5):1032. <https://doi.org/10.3390/molecules25051032>
- Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425. <https://doi.org/10.1093/oxfordjournals.molbev.a040454>
- Sasser M, Kunitsky C, Gary J et al (2005) Identification of *Bacillus anthracis* from culture using gas chromatographic analysis of fatty acid methyl esters. *J AOAC Int* 88:178–181. <https://doi.org/10.1093/jaoac/88.1.178>
- Sheng X, He L et al (2008) Effects of inoculation of biosurfactant-producing *Bacillus* sp. J119 on plant growth and cadmium uptake in a cadmium-amended soil. *J Hazardous Mater* 155(1–2):17–22. <https://doi.org/10.1016/j.jhazmat.2007.10.107>
- Sun J, Lu F, Luo Y et al (2023) OrthoVenn3: an integrated platform for exploring and visualizing orthologous data across genomes. *Nucleic Acids Res* 51(W1):W397–W403. <https://doi.org/10.1093/nar/gkad313>
- Takeuchi M, Hatano K (1998) Union of the genera *Microbacterium* Orla-Jensen and *Aureobacterium* Collins et al. in a redefined genus *Microbacterium*. *Int J Syst Bacteriol* 48:739–747. <https://doi.org/10.1099/00207713-48-3-739>
- Tamura K, Stecher G, Sudhir K (2021) MEGA11: molecular evolutionary genetics analysis version 11. *Mol Biol Evol* 38(7):3022–3027. <https://doi.org/10.1093/molbev/msab120>
- Tamura K, Stecher G, Peterson D et al (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30:2725–2729. <https://doi.org/10.1093/molbev/mst197>

- Tatusova T, DiCuccio M, Badretdin A et al (2016) NCBI prokaryotic genome annotation pipeline. *Nucl Acids Res* 44:6614–6624. <https://doi.org/10.1093/nar/gkw569>
- Yang N, Zhang W, Wang D et al (2023) A novel endophytic fungus strain of *Cladosporium*: its identification, genomic analysis, and effects on plant growth. *Front Microbiol* 14:1287582. <https://doi.org/10.3389/fmicb.2023.1287582>
- Yoon SH, Ha SM, Soonjae K et al (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 67:1613–1617. <https://doi.org/10.1099/ijsem.0.001755>
- Zhang BH, Salam N, Cheng J et al (2017) *Microbacterium lacusdiani* sp. nov., a phosphate-solubilizing novel actinobacterium isolated from mucilaginous sheath of *Microcystis*. *J Antibiot* 70(2):147–151. <https://doi.org/10.1038/ja.2016.125>
- Zhang L, Jiao Y, Ling L et al (2021) *Microbacterium stercoris* sp. nov., an indole acetic acid-producing actinobacterium isolated from cow dung. *Int J Syst Evol Microbiol*. <https://doi.org/10.1099/ijsem.0.005099>
- Zhao A, Cai H, Huang Y et al (2024) *Nesterenkonia marinintestina* sp. nov., isolated from the fish intestine. *Arch Microbiol* 206(3):110. <https://doi.org/10.1007/s00203-023-03825-0>

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