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TAXONOMIC DESCRIPTION
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Nocardioides malaquae sp. nov., a novel actinobacterium isolated from sewage sludge of a fisheries processing factory

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Abstract

A Gram-stain-positive, rod-shaped, strictly aerobic bacterial strain (Y6^T) was isolated from a sewage sludge sample collected from a fisheries processing factory in Zhoushan, Zhejiang Province, PR China. The growth range of NaCl concentration was 0–6.0% (w/v), with an optimum at 3.0% (w/v). The temperature range for growth was 10–42 °C, with an optimum at 37 °C. The pH range for growth was pH 7.0–10.0, with an optimum at pH 9.0. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain Y6^T belonged to the genus *Nocardioides* and showed the highest sequence similarity of 97.8% to *Nocardioides jishulii* dk3136^T. The average nucleotide identity and *in silico* DNA–DNA hybridization values between strain Y6^T and the reference strains were 76.9–81.2% and 20.6–23.6%, respectively. Chemotaxonomic analysis indicated that the sole respiratory quinone was MK-8(H_4) and the predominant cellular fatty acids were iso-$C_{16:0}$, 10-methyl-$C_{17:0}$ and $C_{18:1}$ ω9*c*. The polar lipid profile was composed of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, four unidentified phospholipids, three unidentified aminolipids and five unidentified lipids. The peptidoglycan was LL-2,6-diaminopimelic acid. On the basis of the phenotypic, genotypic, phylogenetic and chemotaxonomic features, strain Y6^T is considered to represent a novel species, for which the name *Nocardioides malaquae* sp. nov. is proposed. The type strain is Y6^T (=KCTC 49504^T=MCCC 1K04765^T).

INTRODUCTION

The genus *Nocardioides*, belonging to the family *Nocardioidaceae*, was proposed in 1976 by Prauser *et al.* [1]. At the time of writing, 124 species have been published with valid names according to the LPSN (List of Prokaryotic Names with Standing in Nomenclature; https://lpsn.dsmz.de/genus/nocardioides) [2]. *Nocardioides* strains have been isolated from many different environments, including various types of environmental soil [3–5], marine organisms and habitats [6–9], sewage water and sludge [10, 11], and wild animals faeces [12, 13]. In general, members of the genus *Nocardioides* are Gram-stain-positive, aerobic, non-spore-forming, non-motile and rod- or coccoid-shaped. The major or sole respiratory quinone is MK-8(H_4), and most *Nocardioides* members contain LL-2,6-diaminopimeric acid in the peptidoglycan and iso-$C_{16:0}$ as the major fatty acid. The G+C contents of most members are above 69.0mol%.

In this study, a novel Gram-stain-positive, rod-shaped bacterium was isolated from a sewage sludge sample collected from a fisheries processing factory. We described the polyphasic characterizations by polyphasic taxonomical approaches, including phenotypic, chemotaxonomic and genotypic analyses. Based on the data, strain Y6^T is considered to represent a novel species within the genus *Nocardioides*.

ISOLATION AND CULTIVATION

In October 2019, sludge samples were collected from the sewage pond of Dayang fisheries processing factory, Zhoushan City, Zhejiang Province, PR China. The sample was

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Keywords: *Nocardioides*; *Nocardioides malaquae*; sewage sludge; fisheries processing factory.
Abbreviations: ANI, average nucleotide identity; BHI, brain-heart infusion; DDH, DNA–DNA hybridization; DPG, diphosphatidylglycerol; GGDC, Genome-to-Genome Distance Calculator; KEGG, Kyoto Encyclopedia of Genes and Genomes; MA, marine 2216 agar; MB, marine 2216 broth; NRPS, non-ribosomal peptide synthetase; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PKS, polyketide synthase; PL, phospholipid; RAST, Rapid Annotation using Subsystem Technology.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Y6^T is MW019669. The GenBank accession number for the whole genome sequence of strain Y6^T is JADCSA000000000.
Seven supplementary figures are available with the online version of this article.

diluted by a tenfold dilution series method and then spread onto modified ZoBell 2216E agar plates which were incubated at 28 °C for 10 days. The modified ZoBell 2216E medium contained (per litre distilled water): 0.5 g yeast extract, 0.1 g peptone, 0.1 g ferric citrate, 19.45 g NaCl, 8.8 g $MgCl_2{\cdot}6H_2O$, 1.8 g $CaCl_2{\cdot}2H_2O$, 0.55 g KCl, 0.16 g $NaHCO_3$, 3.24 g Na_2SO_4, 0.08 g KBr, 34 mg $SrCl_2$, 22 mg H_3BO_4, 4 mg $NaSiO_4$, 2.4 mg NaF, 1.6 mg NH_4NO_3, 8 mg Na_2HPO_4, pH 7.4, adjusted with NaOH. A circular, ~1–3 mm and cream-yellow coloured colony named Y6^T was picked out from several distinct colonies and was streaked on marine agar 2216 (MA; BD) plates for purification. The purified strain was preserved at −80 °C in 25% (v/v) glycerol for further study. Meanwhile, strain Y6^T has been deposited in the Marine Culture Collection of China (MCCC) and the Korean Collection for Type Cultures (KCTC).

PHYLOGENETIC ANALYSIS

After extracting and purifying genomic DNA with the Wizard Genomic DNA purification kit (Promega), the 16S rRNA gene of strain Y6^T was amplified with universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACG GYTACCTTGTTACGACTT-3′) [14]. The PCR product was cloned into pMD 19T vector (TaKaRa) for obtaining the almost-complete sequence. The almost-complete sequence (1486 bp) was used for pairwise sequence alignment performed by the BLASTn program (www.ncbi.nlm.nih.gov) and the EzBioCloud server (www. ezbiocloud.net) [15]. The 16S rRNA gene sequences of closely related strains were obtained from the EzBioCloud database and multiple sequence alignment was performed with the MEGA 5.0 program [16] using the neighbour-joining [17], maximum-parsimony [18] and maximum-likelihood [19] methods. *Terrabacter tumescens* DSM 20308^T was used as an outgroup. Bootstrap values of the three phylogenetic trees were based on 1000 replicates and Kimura's two-parameter model was set to calculate the genetic distance in the neighbour-joining method.

The almost-complete sequence (1486 bp) and the complete sequence (1510 bp) obtained from the whole genome had identical alignment results. The EzBioCloud results showed that strain Y6^T had the highest 16S rRNA sequence similarities to *Nocardioides jishulii* dk3136^T (97.84%) and *Nocardioides solisilvae* Ka25^T (97.60%). In phylogenetic trees using different methods, strain Y6^T formed a distinct branch and located in a cluster with *Nocardioides daphniae* D287^T and *Nocardioides yefusunii* HY056^T (Figs 1, S1 and S2, available in the online version of this article). According to these results, *N. jishulii* CGMCC 4.7570^T, *N. daphniae* DSM 18664^T, *N. solisilvae* KCTC 39528^T and *N. yefusunii* CGMCC 4.7563^T were selected as reference strains in this study and obtained from the KCTC, the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) and the China General Microbiological Culture Collection Center (CGMCC), respectively. Unless described otherwise, *N. jishulii* CGMCC 4.7570^T and *N. yefusunii* CGMCC 4.7563^T were incubated in brain-heart infusion (BHI) medium at 28 °C, the other strains were incubated on MA or in marine 2216 broth (MB) at 28 °C. The BHI medium contained (per litre distilled water): 10.0 g tryptone, 5.0 g NaCl, 2.5 g Na_2HPO_4, 2.0 g dextrose, 9.8 g heart extract powder, 7.7 g brain extract powder, pH 7.4.

MORPHOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERISTICS

The cell morphology of strain Y6^T was observed by transmission electron microscope (JEM-1230, JEOL) described by Ren *et al.* [20] and optical microscope (BX40, Olympus) after Gram staining performed with a commercial Gram-stain kit (BD). The motility of strain Y6^T was tested using the semi-solid culture method with a pipette tip and the gliding motility was tested by observing the motility of cells in the drop on the cover glass [21].

To test the temperature range for growth, 1% (v/v) culture at the end of logarithmic growth was inoculated in MB medium and incubated at different temperatures (4, 10, 15, 20, 25, 28, 30, 35, 37, 40, 42 and 45 °C). The tolerance of NaCl was tested from 0 to 12% (w/v) in 0.5% intervals and cultivating at the optimum temperature. The growth range of pH was tested at pH 5.0–10.0 at intervals of 0.5 pH units with 40 mM different buffers including MES (for pH 5.5–6.0), MOPS (for pH 6.0–7.5), Tricine (for pH 8.0–8.5) and Bis–Tris propane (for pH 9.0–10.0). Optimal growth was observed after 3 days of incubation, and the growth limits were determined after 14 days. All the growth results monitored by measuring OD_{590} values using the UV/visible spectrophotometer (Ultrospec 6300 pro, Amersham Biosciences). Anaerobic growth was tested by using the anaerobic system (AnaeroPack-MicroAero, 2.5l, MGC). *N. jishulii* CGMCC 4.7570^T and *N. yefusunii* CGMCC 4.7563^T were incubated on BHI medium and other strains were cultured on MA, to which 20 mM sodium thiosulfate, 5 mM sodium sulfite, 20 mM sodium sulphate, 5 mM sodium nitrite or 20 mM sodium nitrate were added as potential electron acceptors [22].

The activity of catalase and oxidase was observed by dropping 3% hydrogen peroxide solution and 1% *p*-aminodimethylaniline oxalate solution, respectively [23]. Nitrate reduction, indole and H_2S production, methyl red and Voges–Proskauer reactions were tested as described by Sun *et al.* [24]. Hydrolysis of starch, tyrosine, CM-Cellulose, Tweens (20, 40, 60, 80), gelatin, casein and filter paper were evaluated as described by Chen *et al.* [25]. As for the single carbon source test, the profiles of MB without peptone were used as the basic medium, 0.01% (w/v) of the yeast extract and 0.2% (w/v) of different carbon sources were added as growth factors and substrates. Other physiological and biochemical activities were detected by using API 20NE, API ZYM and API 50CH systems (bioMérieux), according to the manufacturer's instructions. Reference strains and strain Y6^T were tested under the same conditions simultaneously.

Cells of strain Y6^T were Gram-stain-positive, strictly aerobic, non-motile and rod-shaped (0.6–0.8×0.8–1.3 μm) without

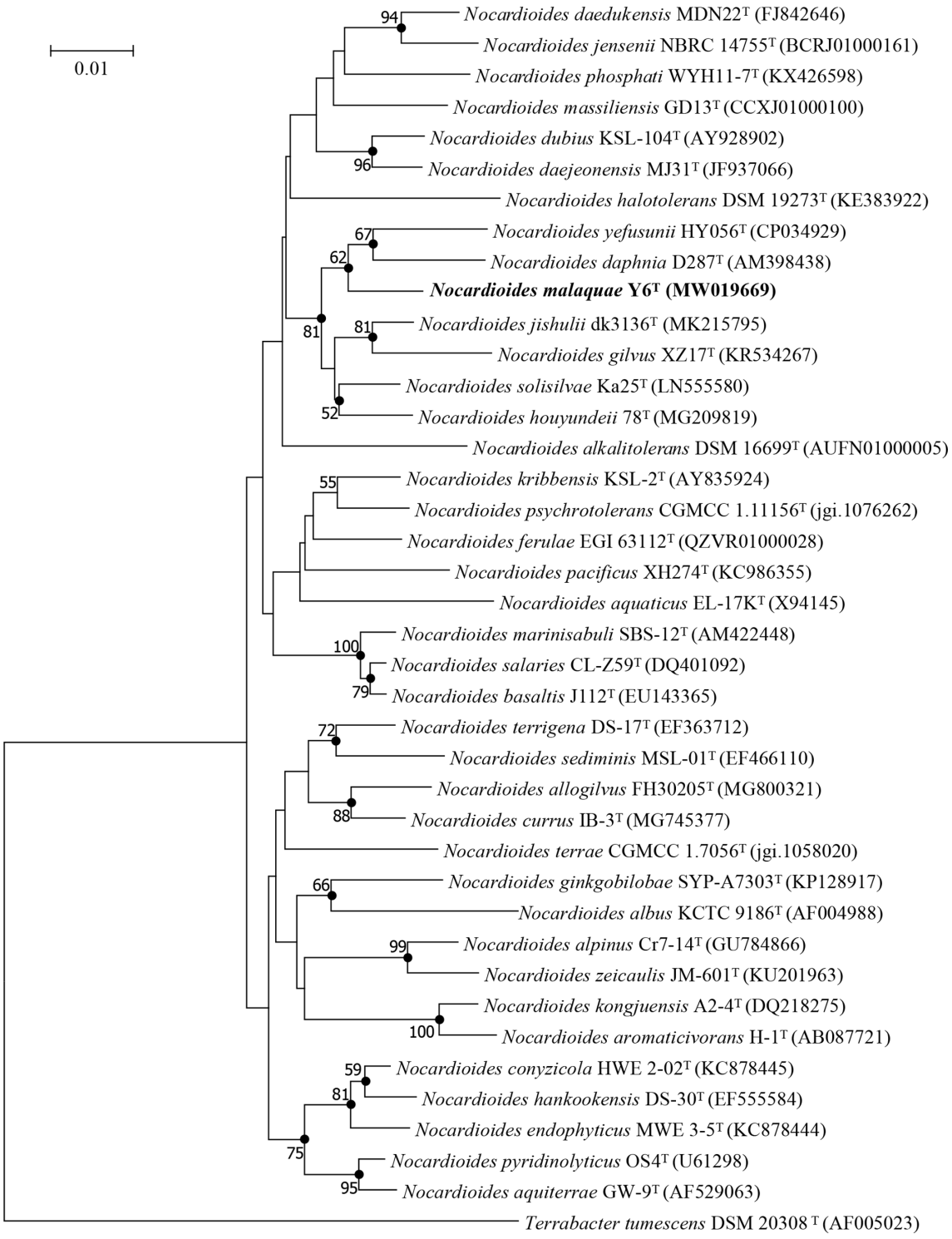

Fig. 1. Neighbour-joining phylogenetic tree based on the 16S rRNA gene sequences of strain Y6^T and representatives of related taxa. Bootstrap values were expressed as a percentage of 1000 replicates and only those higher than 50% are given at the branch points. Bar, 0.01 substitutions per nucleotide position. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with maximum-parsimony and maximum-likelihood algorithms. *Terrabacter tumescens* DSM 20308^T (AF005023) was used as an outgroup.

Table 1. Differential phenotypic characteristics of strain Y6^T and its most closely related species

Strains: 1, Y6^T; 2, *Nocardioides jishulii* CGMCC 4.7570^T; 3, *Nocardioides daphniae* DSM 18664^T; 4, *Nocardioides solisilvae* KCTC 39528^T; 5, *Nocardioides yefusunii* CGMCC 4.7563^T. Unless stated otherwise, all data were obtained from this study under identical growth conditions. +, Positive; –, negative; w, weakly positive.

Characteristic	1	2	3	4	5
Colony colour	Cream yellow	Yellowish	Yellowish	Cream	Pale yellow
Temperature range for growth (°C)	10–42	8–37*	4–38†	20–45‡	16–37§
Optimal growth temperature (°C)	37	28*	30†	30‡	28–30§
NaCl (%, w/v)	0.0–6.0	0.0–4.0*	0.0–5.0†	0.0–14.0‡	0.0–2.0§
Optimal NaCl for growth (%, w/v)	3.0	1.0*	0–0.5†	4.0‡	0.5§
pH range for growth	7.0–10.0	6.0–9.5*	5.5–10.5†	6.0–11.0‡	0.0–0.5§
Optimal pH for growth	9.0	7.5*	8.0†	7.5‡	7.0§
Degradation of:					
Tyrosine	–	w	+	+	–
Casein	+	+	w	+	–
Tween 20	+	w	+	+	–
API ZYM tests:					
Alkaline phosphatase	+	+	+	+	–
Lipase(C14)	–	+	+	+	–
Cystine arylamidase	–	+	+	+	–
α-Chymotrypsin	–	+	–	–	–
α-Glucosidase	–	–	–	+	–
β-Glucosidase	–	+	–	–	+
N-Acetyl-glucosaminidase	–	–	w	–	w
API 20NE tests:					
Glucose fermentation	–	–	+	+	+
Arginine dihydrolase	–	–	–	+	–
Aesculin hydrolysis	–	+	–	–	+
Gelatin hydrolysis (protease)	+	+	+	–	–
β-Galactosidase	–	–	+	–	+
D-Mannose assimilation	w	–	–	–	–
Potassium gluconate assimilation	w	–	–	–	w
Acid production from:					
Sucrose	+	–	+	+	–
Salincin	–	–	+	–	+
Cellobiose	–	+	–	+	+
Utilization of single carbon source:					
D-Galactose	w	w	+	–	+
D-Xylose	–	w	–	–	+
Sucrose	+	–	–	–	+

Continued

Table 1. Continued

Characteristic	1	2	3	4	5
Raffinose	–	–	+	+	+
L-Arginine	–	–	+	w	w

*Data from Dong *et al.* [13].
†Data from Toth *et al.* [9].
‡Data from Sultanpuram *et al.* [5].
§Data from Huang *et al.* [12].

flagella (Fig. S3). Colonies of strain Y6^T were 2 mm in diameter, circular, convex, smooth and cream yellow after growing on MA at 28 °C for 5 days. The isolate grew at 10–42 °C (optimum, 37 °C), pH 7.0–10.0 (optimum, pH 9.0) and with 0–6.0% (w/v) NaCl (optimum, 3.0%). Strain Y6^T and the reference strains were positive for catalase, reduction of nitrates to nitrites, Voges–Proskauer reaction, esterase (C4), esterase lipase (C8), leucine arylamidase, hydrolysis of trypsin and Tweens (40, 60, 80) and negative for oxidase, reduction of nitrites to nitrates, indole production, H_2S production, methyl red reaction, *α*-chymotrypsin, *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, *α*-mannosidase, *α*-fucosidase, urease, tyrosinase, hydrolysis of starch, CM-cellulose, filter paper and gelatin. The main difference of physiological and biochemical characteristics between strain Y6^T and the most closely related species are shown in Table 1. For example, the optimum growth temperature of strain Y6^T was 37 °C, which was much higher than all reference strains. Unlike the reference strains, strain Y6^T could not hydrolyse aesculin. The results for lipase (C14) and cystine arylamidase were negative in strain Y6^T, but positive in *N. jishulii* CGMCC 4.7570^T, *N. daphniae* DSM 18664^T and *N. solisilvae* KCTC 39528^T. In contrast to the closest phylogenetic neighbour *N. jishulii* CGMCC 4.7570^T, strain Y6^T could not produce *α*-chymotrypsin and *β*-glucosidase. Casein and Tween 20 could be degraded by strain Y6^T, which was different from *N. yefusunii* CGMCC 4.7563^T. Strain Y6^T could produce acid from sucrose, but *N. jishulii* CGMCC 4.7570^T and *N. yefusunii* CGMCC 4.7563^T could not. Unlike *N. solisilvae* KCTC 39528^T, strain Y6^T could utilize sucrose but not raffinose.

CHEMOTAXONOMY

The fatty acid analyses of strain Y6^T and the reference strains were based on the method described by Sasser [26]. All strains were cultured on MA at 28 °C and harvested until the logarithmic phase except *N. jishulii* CGMCC 4.7570^T and *N. yefusunii* CGMCC 4.7563^T which were cultured on BHI plate, then cells were analysed using the Microbial Identification System (MIDI) with the standard MIS Library Generation Software version 4.5 [27]. Respiratory quinones were determined according to the method of Minnikin *et al.* [28], and identified using HPLC–MS (Agilent 1200 and Thermo Finnigan LCQ DECA XP MAX mass spectrometer). The polar lipids were extracted and separated by two-dimensional TLC on silicon gel 60 F254 (Merck) plates (10×10 cm) according to Tindall [29]. The aminolipids, phospholipids and glycolipids were tested after spraying with molybdophosphoric acid, ninhydrin reagent, molybdenum blue and *α*-naphthol/H_2SO_4 reagent, respectively. The amino acid composition of the cell-wall peptidoglycan was determined according to the procedure described by Schleifer and Kandler [30].

The fatty acid profiles of strain Y6^T and the reference strains are listed in Table 2. The major cellular fatty acids (≥10%) of the novel isolate were iso-$C_{16:0}$ (46.3%), 10-methyl-$C_{17:0}$ (13.1%) and $C_{18:1}$ *ω9c* (11.0%). In brief, all reference strains contained the typical major fatty acid of the genus (iso-$C_{16:0}$ and $C_{18:1}$ *ω9c*), but the presence of 10-methyl-$C_{17:0}$ as a major one can be a distinguishing feature of the novel isolate. The respiratory quinone was MK-8(H_4) in all strains, which is also a common feature of members in the genus *Nocardioides*.

The polar lipids of the novel isolate included diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylcholine (PC), four unidentified phospholipid, three unidentified aminolipid, five unidentified lipids (Fig. S4). The major known polar lipids were similar to those of reference strains. However, compared with the closest phylogenetic neighbour *N. jishulii* CGMCC 4.7570^T, strain Y6^T contained PE and PC, but no glycolipid was detected in *N. jishulii* CGMCC 4.7570^T. Meanwhile, PI was not found in strain Y6^T but detected in *N. solisilvae* KCTC 39528^T and *N. yefusunii* CGMCC 4.7563^T. However, similar to the result of respiratory quinone, the diagnostic amino acid of the cell-wall peptidoglycan detected in strain Y6^T was LL-2,6-diaminopimelic acid as well as in all reference strains.

GENOME SEQUENCING AND ANALYSIS

The draft genome of strain Y6^T was sequenced by the Illumina HiSeq 2000 platform at Beijing Genomics Institute with Solexa PE150 sequencing technology to generate a sub-read set. The *de novo* assembly of the reads was performed using ABySS 1.5.2 [31]. The assembly k-value was tested from 32 to 64 to find the optimal *k*-value using abyss-pe script. Contigs shorter than 2000 bp were removed. The quality of microbial genomes was assessed using the bioinformatic tool CheckM version 1.0.7 [32].

Rapid Annotation using Subsystem Technology (RAST) was used for the DNA G+C content and the open reading frames using the draft genome sequence [33]. For the

Table 2. Cellular fatty acid profiles (%) of strain Y6^T and the reference strains

Strains: 1, Y6^T; 2, *Nocardioides jishulii* CGMCC 4.7570^T; 3, *Nocardioides daphniae* DSM 18664^T; 4, *Nocardioides solisilvae* KCTC 39528^T; 5, *Nocardioides yefusunii* CGMCC 4.7563^T. The major fatty acids (>10%) are in bold text. –, Not detected; TR trace amount (<1%).

Fatty acid	1	2	3	4	5
Saturated:					
$C_{16:0}$	TR	TR	1.6	1.5	5.9
$C_{17:0}$	–	TR	TR	TR	2.5
$C_{18:0}$	TR	TR	TR	TR	4.2
$C_{17:0}$ 10 methyl	**13.1**	8.8	4.0	2.1	2.4
$C_{18:0}$ 10 methyl	TR	TR	TR	4.3	**18.5**
Unsaturated:					
iso-$C_{16:1}$ h	1.2	2.4	TR	3.8	–
$C_{17:1}$ ω8*c*	3.4	7.2	3.4	4.6	2.3
$C_{18:1}$ ω9*c*	**11.0**	**11.9**	**13.3**	**12.0**	**21.4**
Hydroxy branched:					
iso-$C_{14:0}$	4.0	4.3	1.7	1.8	TR
iso-$C_{15:0}$	TR	1.8	2.6	3.7	–
iso-$C_{16:0}$	**46.3**	**43.4**	**29.4**	**33.3**	**24.8**
iso-$C_{17:0}$	1.4	3.0	8.2	7.2	2.8
iso-$C_{18:0}$	1.4	2.0	TR	TR	1.0
anteiso-$C_{17:0}$	2.4	1.6	4.4	3.3	–
Summed feature 3*	5.6	1.9	8.2	6.7	2.3
Summed feature 8*	TR	TR	1.6	2.8	TR
Summed feature 9*	3.3	3.7	**13.5**	6.8	3.7

*Summed features are fatty acids that cannot be resolved reliably from another fatty acid using the chromatographic conditions chosen.The MIDI system groups these fatty acids together as one feature with a single percentage of the total. Summed feature 3 includes $C_{16:1}$ ω7*c* and $C_{16:1}$ ω6*c*, summed feature 8 includes $C_{18:1}$ ω6*c* and $C_{18:1}$ ω7*c*, and summed feature 9 includes iso-$C_{17:1}$ ω9*c* and $C_{16:0}$ 10 methyl.

annotation of the function, the Kyoto Encyclopedia of Genes and Genomes (KEGG) [34] and the Clusters of Orthologous Groups [35] databases were used via the KEGG Automatic Annotation Server and the WebMGA server (http://weizhong-lab.ucsd.edu/webMGA/server/cog/). The antiSMASH 5.0 program was used as a tool for the identification of the secondary metabolism gene clusters [36]. The genome sequences of *N. jishulii* CGMCC 4.7570^T (CP040748), *N. daphniae* DSM 18664^T (CP038462), *N. solisilvae* KCTC 39528^T (QHKX00000000) and *N. yefusunii* CGMCC 4.7563^T (CP034929) were obtained from the NCBI GenBank database (www.ncbi.nlm.nih.gov). The OrthoANIu algorithm of Chun lab's online average nucleotide identity (ANI) calculator in the EzBioCloud was used for the ANI calculation [37]. The *in silico* DNA–DNA hybridization (*in silico* DDH) values were calculated using the Genome-to-Genome Distance Calculator (GGDC) [38]. The phylogenomic tree based on the whole genome was reconstructed using the TYGS (https://tygs.dsmz.de/).

The draft genome sequence of strain Y6^T generated 1388 Mb of clean data. The genome completeness of strain Y6^T was 99.48% with a contamination percentage of 0.95%. The genome sequence was considered an excellent reference genome for the future analysis (≥95% completeness, ≤5% contamination) [39]. The DNA G+C content of strain Y6^T calculated from the draft genome sequence was 67.8%, similar to *N. jishulii* CGMCC 4.7570^T (70.4%), *N. daphniae* DSM 18664^T (73.7%), *N. solisilvae* KCTC 39528^T (71.0%) and *N. yefusunii* CGMCC 4.7563^T (68.9%). The reconstructed phylogenomic tree showed that strain Y6^T formed a cluster with *N. jishulii* CGMCC 4.7570^T and *N. daphniae* DSM 18664^T, which was located in the clade of genus *Nocardioides* (Fig. S5). The *in silico* DDH values between strain Y6^T and *N. jishulii* CGMCC 4.7570^T, *N. daphniae* DSM 18664^T, *N. solisilvae* KCTC 39528^T and *N. yefusunii* CGMCC 4.7563^T were 23.2, 23.6, 20.6 and 21.2%, respectively, which were below the 70% threshold value of the GGDC [40]. Strain Y6^T showed ANI values of 80.85, 81.28, 77.72, 76.95% with *N. jishulii* CGMCC 4.7570^T, *N. daphniae* DSM 18664^T, *N. solisilvae* KCTC 39528^T and *N. yefusunii* CGMCC 4.7563^T, respectively, which were lower than the 95% threshold value of the delineation of bacterial species [41].

The different genomic features of the novel isolate and reference strains based on the RAST result are listed in Table 3. On basis of the genome annotation and the KEGG analysis, a considerable number of genes associated with environmental adaptation were found in strain Y6^T, of which, 23 genes were related to the phosphorus metabolism, 22 genes for nitrogen metabolism and 13 genes for sulphur metabolism. Compared with the reference strains, strain Y6^T contained 13 genes responsible for sulphur metabolism, which was higher than the reference strains. In addition, 18 genes responsible for stress response were also found in the genome of strain Y6^T, including nine genes associated with oxidative stress, five genes associated with detoxification and one gene associated with osmotic pressure. Additionally, strain Y6^T contained 88 proteins genes for synthesis, transport and catabolism of secondary metabolites. The result of antiSMASH 5.0 indicated that the novel isolate contained two gene clusters for non-ribosomal peptide synthetase (NRPS), which is a multifunctional key enzyme in the process of biosynthesis of secondary metabolites, one gene cluster for NRPS-like fragment which shown only 3% similarity to polyketide and two gene clusters belong to terpenoid type which shown 50% similarity to carotenoids (Fig. S6). In addition, three gene clusters belonging to Type I PKS (polyketide synthase) including heterocyst glycolipid synthase-like PKS and beta-lactone containing protease inhibitor were found. The terpenoid backbone showed the main synthetic directions of the strain included zeatin, monoterpenoids, diterpenoids, carotenoids, indole iron alkaloids, sesquiterpenoids, triterpenoid and so on (Fig. S7).

Table 3. Genomic features of strain Y6^T and the reference strains

Strains: 1, Y6^T; 2, *Nocardioides jishulii* CGMCC 4.7570^T; 3, *Nocardioides daphniae* DSM 18664^T; 4, *Nocardioides solisilvae* KCTC 39528^T; 5, *Nocardioides yefusunii* CGMCC 4.7563^T.

Genomic characteristics	1	2	3	4	5
Size (bp)	3636387	3536142	3558816	3583817	3321975
DNA G+C contents (mol%)	67.8	70.3	71.0	73.7	68.9
N50	174980	3536126	3560159	1103360	3321975
L50	7	1	1	2	1
Number of coding sequences	3298	3395	3889	3447	3072
Number of RNAs	62	60	60	45	82
Subsystem feature counts					
Cofactors, vitamins, prosthetic groups, pigments	152	141	159	135	136
Virulence, disease and defence	29	24	37	26	18
Membrane transport	31	36	46	19	28
Iron acquisition and metabolism	6	14	8	3	8
RNA metabolism	31	35	39	34	30
DNA metabolism	101	80	94	85	62
Protein metabolism	174	179	187	173	178
Regulation and cell signalling	12	12	12	10	10
Secondary metabolism	5	4	4	0	4
Fatty acids, lipids and isoprenoids	94	101	117	112	100
Nitrogen metabolism	22	21	27	18	8
Respiration	68	61	85	69	60
Stress response	18	18	25	15	14
Metabolism of aromatic compounds	9	13	14	21	21
Amino acids and derivatives	238	259	303	300	211
Sulphur metabolism	13	8	8	6	6
Phosphorus metabolism	23	25	23	25	22
Carbohydrates	114	139	127	138	97

In conclusion, based on the phenotypic, genotypic, phylogenetic and chemotaxonomic properties presented in this study, strain Y6^T represents a novel species in the genus *Nocardioides*, for which the name *Nocardioides malaquae* sp. nov. is proposed.

DESCRIPTION OF *NOCARDIOIDES MALAQUAE* SP. NOV.

Nocardioides malaquae (mal.a'quae. L. masc. adj. *malus* bad; L. fem. n. *aqua* water; N.L. gen. n. *malaquae* of bad water, effluent).

Cells are Gram-stain-positive, strictly aerobic, rod-shaped, non-motile and approximately 0.6–0.8×0.8–1.3 µm in diameter. Colonies on MA are 2 mm in diameter, circular, smooth and cream yellow-pigmented. Growth occurs at 10–42 °C (optimum, 37 °C), pH 7.0–10.0 (optimum, pH 9.0) and with 0–6.0% (w/v) NaCl (optimum, 3.0%). Positive for catalase activity, reduction of nitrates to nitrites, Voges–Proskauer reaction, degradation of casein, and Tweens 20, 40, 60 and 80. Negative for oxidase, reduction of nitrites to nitrates, indole production, H_2S production, methyl red reaction, hydrolysis of starch, tyrosinase, CM-cellulose, filter paper and gelatin. The activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, trypsin and acid phosphatase are present; those of valine arylamidase, D-glucose, potassium gluconate, naphthol-AS-BI-phosphohydrolase are weakly positive; and they negative for lipase (C14), cystine arylamidase, α-chymotrypsin, α-galactosidase, β-galactosidase,

β-glucuronidase, *α*-glucosidase, *β*-glucosidase, *N*-acetyl-glucosaminidase, *α*-mannosidase and *α*-fucosidase. Acid can be produced from aesculin ferric citrate, sucrose and potassium 2-ketogluconate. D-Glucose, maltose, lactose, D-galactose, L-arabinose, inulin, sorbitol, glycine, D-mannitol, inositol, erythritol, cellobiose, sucrose and tartaric acid can be utilized as carbon sources while D-ribose, raffinose, D-xylose and L-arginine are not. The major respiratory quinone is MK-8(H_4). The major cellular fatty acids are iso-$C_{16:0}$, 10-methyl-$C_{17:0}$ and $C_{18:1}$ ω9*c*. The polar lipid profile is composed of DPG, PG, PE, PC, four PLs, three aminolipids and five unidentified lipids. LL-2,6-Diaminopimelic acid is the diagnostic diamino acid in the cell-wall peptidoglycan.

The type strain, Y6^T (=KCTC 49504^T=MCCC 1K04765^T), was isolated from the sewage pond at Dayang fisheries processing factory, Zhoushan City, Zhejiang Province, PR China. The DNA G+C content of the type strain is 67.8mol% based on the genome sequence.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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