diversity

MDPI

Article

Massilia paldalensis sp. nov., Isolated from Stream Bank Soil

Nhi Thi Ai Nguyen and Jaisoo Kim *

Department of Life Science, College of Natural Sciences, Kyonggi University, Suwon 16227, Gyeonggi-do, Republic of Korea; ainhi2389@gmail.com

* Correspondence: jkimtamu@kgu.ac.kr; Tel.: +82-31-249-9648

Abstract: A novel rod-shaped, Gram-negative, motile, aerobic and heavy metal-resistant bacterial strain, designated TN1-12^T, was isolated from stream bank soil in Paldal district, Suwon City, Republic of Korea. Growth occurred at 10–40 °C (opt 30 °C), NaCl concentrations up to 2% (*w*/*v*) and pH 5.0–8.0 (opt pH 7.0). Based on the 16S rRNA gene sequence, the closest relatives of strain, TN1-12^T, are *Massilia putida* 6NM-7^T (98.21% similarity), *Massilia forsythiae* GN2-R2^T (98.00%), *Massilia rhizosphaerae* NEAU-GH312^T (97.79%), *Massilia aurea* AP13^T (97.78%) and *Massilia niabensis* 5420S-26^T (97.71%). The predominant ubiquinone is Q-8. The G+C content of the DNA is 66.7 mol%. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine. The major fatty acids are C16:0, summed feature 3 (C16:1ω7c and/or iso-C15:0 2-OH), 17:0 cyclo and summed feature 8 (C18:1 ω7c and/or C18:1 ω6c). DNA–DNA hybridization and Average Nucleotide Identity data showed values below 26% and 85%, respectively, confirming that TN1-12^T represents a novel species. Based on the genotypic, chemotaxonomic and physiological data presented in this study, we propose that strain TN1-12^T represents a novel species within the genus *Massilia* with the name *Massilia paldalensis* sp. nov. (=KACC 23946^T = CGMCC 1.65296^T).

Keywords: *Massilia paldalensis*; stream bank soil; new species; heavy metal resistance

check for updates

Academic Editors: Michael Wink and Ipek Kurtboke

Received: 24 December 2024
Revised: 12 April 2025
Accepted: 28 April 2025
Published: 1 May 2025

Citation: Nguyen, N.T.A.; Kim, J. *Massilia paldalensis* sp. nov., Isolated from Stream Bank Soil. *Diversity* **2025**, *17*, 327. https://doi.org/10.3390/d17050327

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

The genus *Massilia* was originally described by La Scola et al. with the type species, *Massilia timonae*, isolated from human blood samples of cerebellar lesions [1]. Members of this genus are characterized by Gram-negative, aerobic, motile rods and high G+C contents (62.4–69.4 mol%). The predominant quinone is Q-8. Summed feature 3 and C16:0 are the major fatty acids. Phosphatidylglycerol (PG), phosphatidylethanolamine (PE) and diphosphatidylglycerol (DPG) are the main polar lipids [2]. At the time of writing, 66 species names have been validly reported (https://lpsn.dsmz.de/genus/massilia, accessed on 23 November 2024). The species of the genus *Massilia* can be found in various environmental sources, such as soil, air, humans, drinking water and flowers, etc. [1,3–6]. Genus *Massilia* have members with a variety of functions, such as plant growth-promoting properties, against pathogenic bacteria *Ralstonia solanacearum*, tolerance to heavy metals, and secondary metabolites that have antimicrobial activity, etc. [3,7–9]. Studies have shown that the genus *Massilia* plays an important role in ecosystems, indicating the potential significance of these strains for agricultural applications.

Riverbank soils are host to diverse bacterial communities, often exposed to multiple environmental influences. Bacterial populations in these ecosystems often exhibit adaptive traits that allow them to thrive under fluctuating conditions [10]. Thus, studying the

bacterial diversity in streambank soils in Paldal County may lead to the discovery of new bacterial species with critical functions and unique physiological characteristics.

This study aims to confirm its classification as a new species, contributing to the understanding of soil microbial diversity and the taxonomic expansion of the genus *Massilia*. Furthermore, a comparative analysis of its morphological and physiological traits with closely related strains revealed that TN1-12^T exhibits resistance to certain heavy metals. Given that heavy metals persist in soils indefinitely and pose significant environmental and agricultural concerns, investigating the biosynthetic potential of TN1-12^T may uncover natural solutions for promoting environmental sustainability.

2. Materials and Methods

2.1. Isolation and Cultivation

During the investigation of soil bacteria, strain TN1-12^T was chosen for study due to its undefined taxonomic position. Strain TN1-12^T was isolated from stream bank soil in Paldal district, Suwon city, Republic of Korea (37.28136° N, 127.01818° E). One gram of soil was suspended in sterile 0.85% NaCl (*w*/*v*; saline solution). 100 µL of six-fold serial dilutions were spread on R2A agar (Reasoner's 2A agar, MB Cell) and cultured at 30 °C for 1 week. Single colonies were selected and purified by repeated streaking on R2A agar plates. Strain TN1-12^T was picked up based on colony morphology and then stored in R2A broth supplemented with 25% (*v*/*v*) glycerol at −80 °C for further study.

Strain TN1-12 ^{T} was deposited in KACC 23946 and CGMCC 1.65296. Based on the 16S rRNA gene sequencing result, the closest reference strains *Massilia putida* KACC 21418^T, *Massilia forsythia* KACC 21261^T, *M. rhizosphaerae* DSM 109722^T, *Massilia aurea* KACC 11884^T and *Massilia niabensis* KACC 12632^T were chosen for the comparative study.

2.2. 16S rRNA Gene, Genome and Phylogenetic Analyses

Genomic DNA was extracted and purified by using the InstaGene Matrix Kit (Bio-Rad, Hercules, CA, USA) according to the kit protocol [11]. The 16S rRNA gene was PCR-amplified with the commercial bacterial primer (forward primer 27F and reverse primer 1492R) [12] and sequenced by Macrogen (Seoul, Republic of Korea). The closest phylogenetic neighbors of strain TN1-12^T were identified by performing a search of validly published bacterial species using the EzBioCloud database (www.ezbiocloud.net, accessed on 23 November 2024). The retrieved closest related sequences along with TN1-12^T were aligned by the SILVA alignment program (https://www.arb-silva.de/aligner/, accessed on 23 November 2024) [13]. Phylogenetic trees were reconstructed using the maximum-likelihood (ML), neighbor-joining (NJ) [14] and maximum-parsimony (MP) methods in the software MEGA X version 10.0.5 [15] with 1000 bootstrap replicates [16] and the Kimura two-parameter model [17]. *Parasutterella secuda* JCM 16078^T was used as an outgroup for the phylogenetic tree.

Whole-genome shotgun sequencing of strain TN1-12^T was accomplished by Macrogen (Republic of Korea) using the Illumina HiSeq 2500 platform (San Diego, CA, USA). Raw reads were quality checked using FastQC version 0.11.7 [18], filtered using Trimmomatic version 0.38 [19]. The genome was then assembled using SPAdes de novo version 3.15.0 [20] and the completeness of the genome assembly was assessed using BUSCO version 5.1.3 [21]. The UBCG (updated bacterial core genes) method was used to reconstruct a phylogenetic tree by concatenated alignments of 92 core genes [22]. The DNA G+C content and genome annotation were determined using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) version 6.8 [23] and the Rapid Annotation utilizing Subsystem Technology (RAST) server (https://rast.nmpdr.org/, accessed on 26 November 2024) [24]. To estimate pairwise relatedness between TN1-12^T and close reference strains, Average Nucleotide Identity (ANI) was

calculated utilizing the OrthoANIu (www.ezbiocloud.net/tools/ani, accessed on 23 December 2024) algorithm [25]. DNA–DNA relatedness was clarified using the GGDC web server (http://ggdc.dsmz.de/ggdc.php, accessed on 23 December 2024) [26]. Secondary metabolite biosynthesis gene clusters were analyzed by antiSMASH version 7 [27].

2.3. Physiology and Chemotaxonomy

After 24 h incubation at 30 °C, the cell of TN1-12^T was examined by a zoom stereo microscope (SZ61, Olympus, Tokyo, Japan) and transmission electron microscopy (Bio-TEM H-7650, Hitachi, Tokyo, Japan). Growth of TN1-12^T was observed on different media, including Reasoner's 2A agar (R2A, MB Cell, Seoul, Korea), low salt Luria Bertani agar (LB; MB Cell), MacConkey agar (MB Cell), Nutrient agar (NA; MB Cell), and Tryptone Soya agar (TSA; MB Cell), at 30 °C for a week. Motility was assessed in sulfide indole motility (SIM, CM0435; Oxoid, Basingstoke, UK) medium. Growth under anaerobic conditions was checked in a BBL GasPak anaerobic jar (BD, Franklin Lakes, NJ, USA) at 30 °C for 7 days on R2A agar. The sodium chloride tolerance was evaluated by cultivating the strain on R2A broth at various NaCl concentrations (0–4%, in 0.5% increments) for a week at 30 °C. A growth test at different temperatures (4, 10, 15, 20, 25, 28, 30, 35. 37 and 40 °C) and a pH range of 4.0–10 (at intervals of 0.5 pH unit) was investigated in R2A broth for 7 days. For pH tests, the R2A broth was buffered with phosphate citrate buffer (pH 4.0–6.0), Sorensen's phosphate buffer (pH 6.5–8.0), Tris buffer (pH 8.5–9.0), carbonate buffer (pH 9.5–10.0) and adjusted with NaOH or HCl. A series of phenotypic experiments, such as catalase and oxidase activities, gram staining, hydrolysis of casein, starch, gelatin, DNA, and Tween 80, were evaluated by using the methods of Smibert and Krieg [28]. Additional enzyme activities and carbon source utilization of TN1-12^T and the reference strains were analyzed using API ZYM and API 20NE test kits (bioMérieux, Marcy-l'Étoile, France), following the manufacturers protocol. When comparing the physiological characteristics with related strains, the heavy metal resistance was tested by the agar plate method, strains inoculated on R2A agar supplemented with $CdCl_2.5H_2O$, $CuSO_4.5H_2O$ and $ZnSO_4.7H_2O$ at different concentrations (0, 0.2, 0.5, 1.0, 1.5, 2.0 mM) [7]. Growth was observed after 7 days at 30 °C.

For the determination of cellular fatty acids, strain TN1-12^T and reference strains were harvested under the same conditions (at 30 °C for 2 days). The fatty acid compositions were analyzed with a GC system (Agilent 6890N, Santa Clara, CA, USA), according to the Sherlock Microbial Identification System (MIDI) protocol, with the TSBA6 database used for standard identification and quantification [29]. The lyophilized biomass of TN1-12^T was used for the analysis of respiratory quinones and polar lipids. Quinones were extracted and analyzed according to the methods described by Minnikin et al. [30] and Hiraishi et al. [31] using a reversed-phase HPLC system. Polar lipids were extracted as previously described by Minnikin et al. [30]. The lipids were separated on thin-layer chromatography (TLC) and identified by spraying with detective reagents (ethanolic molybdatophosphoric acid (Sigma-Aldrich, St. Louis, MO, USA) for total lipids, molybdenum blue spray reagent (Sigma-Aldrich) for phospholipids, and ninhydrin (Sigma Life Science, Burlington, MA, USA) for aminolipids).

3. Results

3.1. 16S rRNA Gene, Genome and Phylogenetic Analyses

The 16S rRNA gene sequence had 1477 bp and has been deposited at GenBank database under the accession PQ538617. Strain TN1-12^T was closest to *M. putida* 6NM-7^T (98.21% sequence similarity), followed by *M. forsythiae* GN2-R2^T (98.00%), *M. rhizosphaerae* NEAU-GH312^T (97.79%), *M. aurea* AP13^T (97.78%) and *M. niabensis* 5420S-26^T (97.71%). All three phylogenetic trees clearly showed that strain TN1-12 is a member of the genus *Massilia*

(Figure 1, Figures S1 and S2). The NB phylogenetic tree based on 16S rRNA gene sequences showed that strain TN1-12 is closely related to *M. forsythiae* GN2-R2^T, *M. phosphatilytica* 12-OD1^T, and *M. putida* 6NM-7^T (Figure 1). This phylogenetic relationship was confirmed by the UBCG phylogenetic tree based on the whole genome. Strain TN1-12^T was clustered with species in the genus *Massilia* and forms a clear clade (Figure 2).

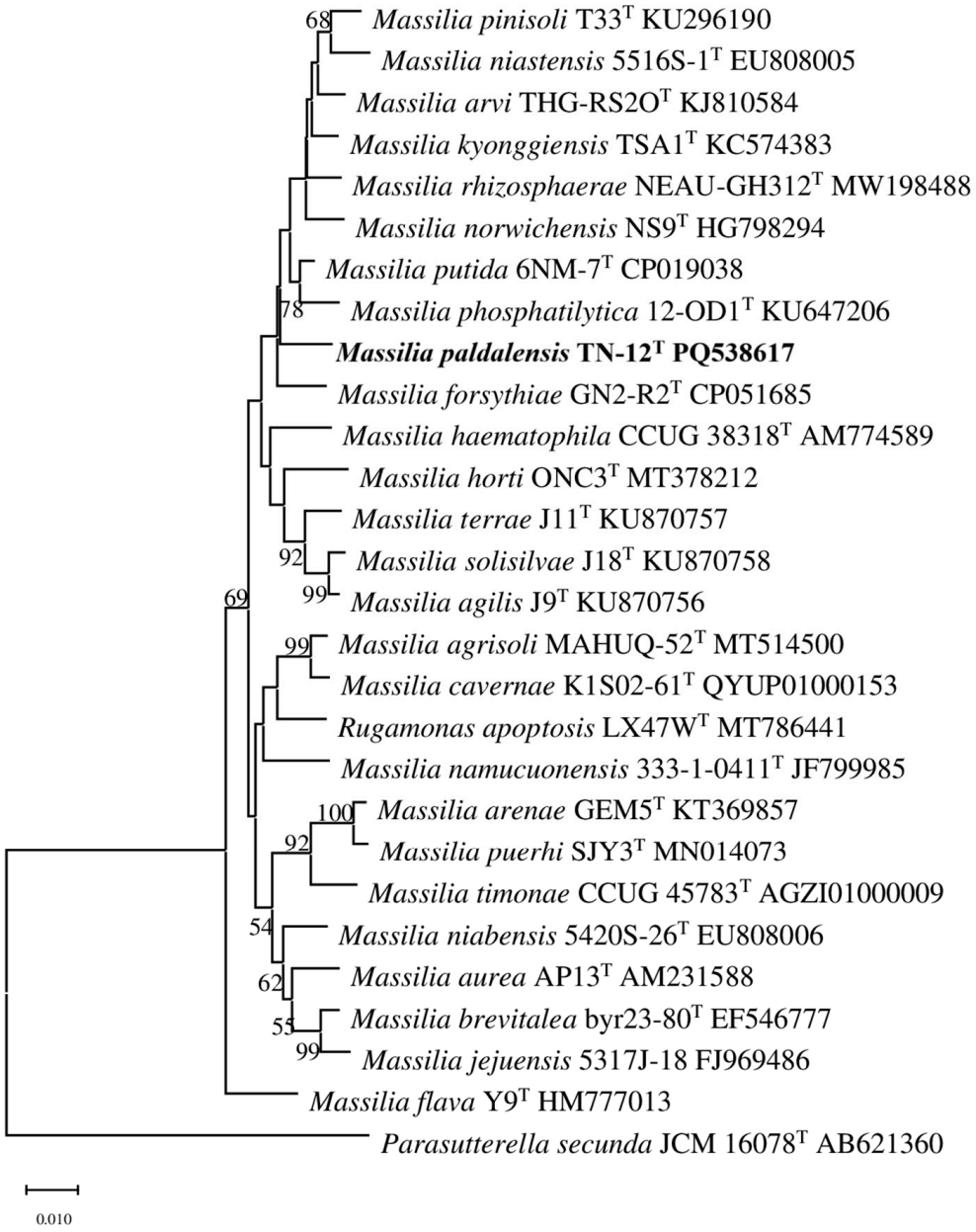


Figure 1. Neighbor-joining tree created using mega X based on 16S rRNA gene sequences showing relationships between strain TN1-12^T (bold; a novel type strain as a new species) and other related species. Only bootstrap values above 50% (expressed as percentages of 1000 replications) are shown. Bar, 0.01 nucleotide exchanges per site.

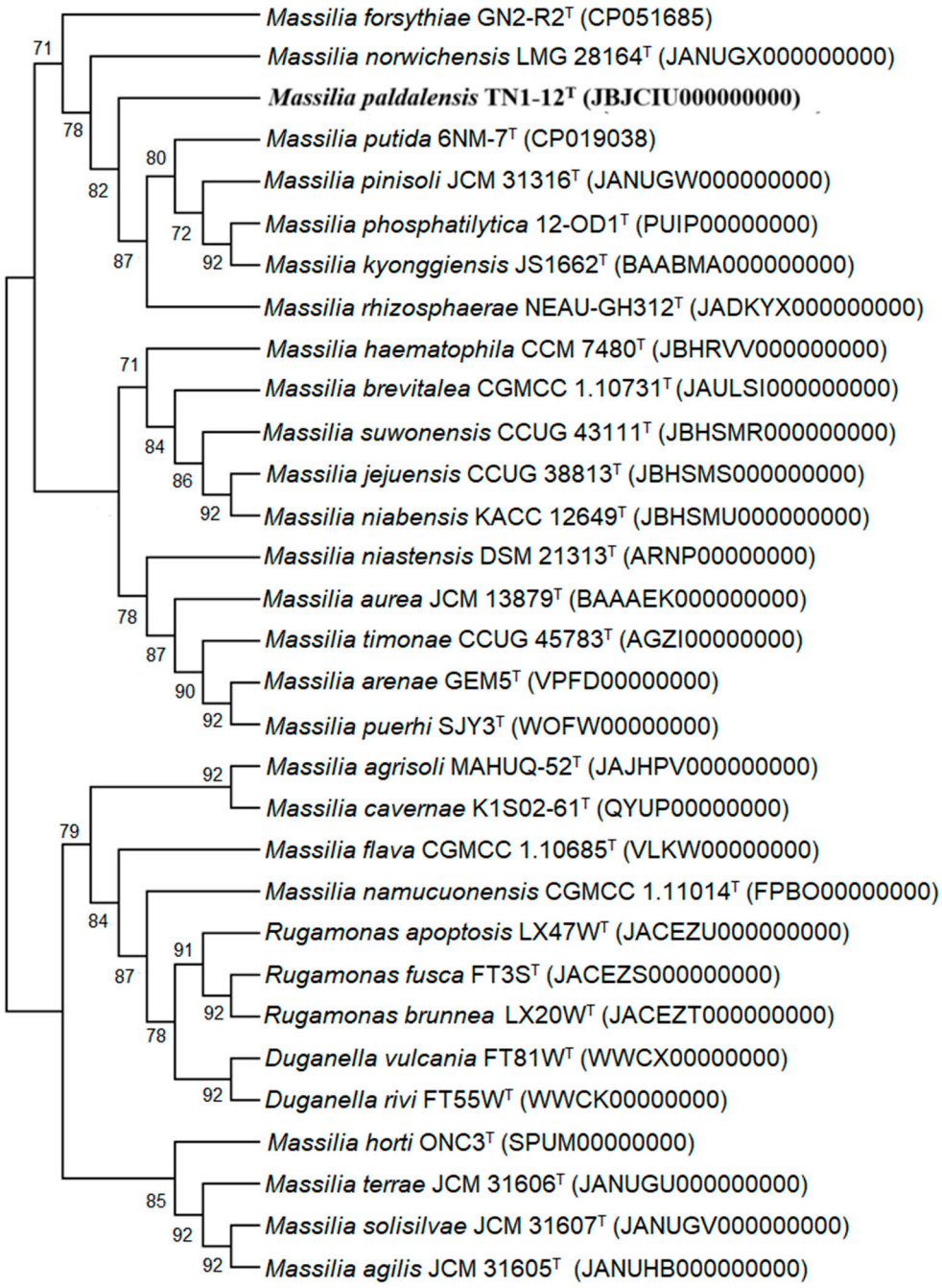


Figure 2. Genome-based phylogenetic tree is reconstructed using the UBCG showing the phylogenetic placement of the strain TN1-12^T (bold; a novel type strain as a new species) with closely related species of the genus *Massilia*. GenBank accession numbers are given in parentheses. Bar, 0.1 substitutions per site.

The de novo assembled genome sequence of strain TN1-12^T was found to be 6,225,423 bp long and composed of 37 contigs with an N50 of 1,047,983 bp, a G+C content of 66.7 mol%. High sequencing depth of 130.77 ensures genome assembly accuracy and reliability. Additionally, BUSCO analysis identified 98.39% of the expected single-copy orthologs, further confirming good assembly with minimal contamination. According to the PGAP annotation, the complete genome of strain TN1-12^T was annotated to have 5578 protein-coding sequences (CDSs), 88 RNA genes (9 rRNA, 75 tRNA and 4 ncRNA genes) and 30 pseudo genes. Detailed genomic features are presented in the Table S1. The RAST analysis results showed that 23% (1307) of the genes were involved in essential

biological processes, as detailed in Figure S3. The analysis revealed 55 genes indicating virulence, disease and defense, of which 41 genes were involved in antibiotic and toxic compound resistance (explaining the heavy metal resistance capacity of strain TN 1-12^T). In addition, 5 genes related to secondary metabolisms consisting of one alkaloid biosynthesis from L-lysine, four auxin biosynthesis, and several genes responsible for flagella motility were identified. Based on the antiSMARH software, five BGCs were identified as follows: terpene, NAPAA, RiPP-like, acyl amino-acids and redox-cofactor (Figure S4). DNA–DNA hybridization results between strain TN1-12^T and the close relatives *M. putida* KACC 21418^T, *M. forsythiae* KACC 21261^T, *M. rhizosphaerae* DSM 109722^T, *M. aurea* KACC 11884^T and *M. niabensis* KACC 12632^T were 25.4, 25.5, 25.5, 21.4, and 21.9%, respectively. Strain TN1-12^T revealed OrthoANI values of 77.21–82.22% compared with 15 related strains (Table S2). Both DNA–DNA relatedness and ANI values were all below the criteria for species delineation (threshold value of 70% for dDDH and 95–96% for ANI) [32]. These results supported strain TN1-12^T being considered to be a new species of the genus *Massilia*.

3.2. Physiology and Chemotaxonomy

Strain TN1-12^T was rod-shaped (0.4–0.8 × 2.0–3.8 μm) and motile by means of a single polar flagellum (Figure S5). Colonies were yellowish white, round, smooth with distinct margins and 0.2–1.0 mm in diameter when cultured for 1 day on R2A agar and turned yellow, irregular and rough after 2–3 days at 30 °C. This difference was also observed in the species *M. tieshanensis* KACC 14940^T; these colonies were difficult to scrape after 2–3 days incubation [3]. Strain TN1-12^T is characterized by Gram-negative staining, aerobic, catalase, motile, capable of hydrolyzing urea and arginine, similar to the closely related species. Besides some common features with its closest relatives, strain TN1-12^T revealed some morphological, physiological and biochemical differences from related type strains. Growth of strain TN1-12^T occurs at pH between 5.0 and 8.0 (optimum pH 7.0), temperature between 10 and 40 °C (optimum 30 °C) with 0–2% (*w*/*v*) NaCl (optimum 0%). Detailed differential characteristics from the closest type strain in the genus *Massilia* related to physiological, biochemical and morphological traits are provided in Table 1. The main distinctions are oxidase positive and hydrolytic activity of the different substrates (starch, casein, gelatin, esculin and Tween 80), as well as assimilation and enzyme activity properties. In addition, among the strains tested, strain TN1-12^T had the highest resistance to 1.5 mM Cd^{2+}, 1.5 mM Cu^{2+} and 2.0 mM Zn^{2+}. The related strain *M. putida* KACC 21418^T was resistant to 1.0 mM Cd^{2+}, 0.5 mM Cu^{2+} and 0.5 mM Zn^{2+}. *M. forsythia* KACC 21261^T and *M. niabensis* KACC 12632^T were hardly found to be resistant to heavy metals (Table S3). The metal resistance level of strain TN1-12^T was found to be quite high when compared with other strains in previous reports. The strain had the highest Cd^{2+} tolerance (1.5 mM) in the genus *Massilia*, followed by *M. timonae* AY512824 (1 mM) [33]. Cu^{2+} tolerance was only lower than *M. tieshanensis* KACC 14940^T [3] and Zn^{2+} tolerance was lower than *M. timonae* AY512824 [33]. These differences support the strain TN1-12^T as a potential novel species within the genus *Massilia*.

The fatty acid result of strain TN1-12^T and two reference strains are shown in Table 2. The major fatty acids (>10%) were partially similar to the characteristics of genus *Massilia* as follows: summed feature 3 (C15:0 iso 2-OH and/or C16:1 ω7c, 54.2%), C16:0 (31.5%), 17:0 cyclo (18.82%) and summed feature 8 (C18:1 ω7c and/or C18:1 ω6c, 10.41%). The proportions of anteiso-branched chain in *M. forsythia* KACC 21261^T and some iso-branched chain in *M. putida* KACC 21418^T, *M. rhizosphaerae* DSM 109722^T, *M. aurea* KACC 11884^T, and *M. niabensis* KACC 12632^T are less than 0.5%. The difference in fatty acid proportions (Table 2) and the absence of anteiso- and iso-branched chain fatty acids distinguished strain TN1-12^T from its reference strains. The major respiratory quinone of strain TN1-12^T was

ubiquinone 8 (Q-8), the same as a common feature among members of the genus. The main polar lipids consisted of diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), followed by phospholipid (PL), two aminophospho lipid (APL) and three lipids (L) (Figure S6).

Table 1. Differential characteristics of strain TN1-12^T and closely related species: 1, strain TN1-12^T; 2, *M. putida* KACC 21418^T; 3, *M. forsythiae* KACC 21261^T; 4, *M. rhizosphaerae* DSM 109722^T; 5, *M. aurea* KACC 11884^T; 6, *M. niabensis* KACC 12632^T. Data in columns 1–3, 5–6 are from this study except where marked. Data for 4, *M. rhizosphaerae* DSM 109722^T are from Chenxu Li et al. (2021) [8]. +, Positive; −, negative; ND, not determined.

Characterictics	1	2	3	4	5	6
Isolation source	Soil	Wolfram mine tailing [7]	Flowers [6]	Rhizosphere soil of rice	Drinking water [5]	Air [4]
Media	R2A, TSA, NA, LB	R2A, PYE [7]	R2A [6]	R2A, NA, ISP2	PCA, R2A, TSB, NA [5]	R2A, NA [4]
Oxidase	+	−	−	+	+	+
Catalase	+	+	+	ND	+	+
Urea	−	−	−	−	−	−
NaCl tolerance (%, *w*/*v*)	0–2	0–1	0–1	0–5	0–2	0–1
Temperature for growth	10–40	20–37	10–30	10–40	10–30	10–35
pH (optimum)	5–8 (7)	6–8 (7)	4.5–8.5 (7)	4–8 (7)	4.5–9 (7.5)	6.5–9 (7)
Hydrolysis of:						
Casein	+	−	−	ND	+	−
Starch	+	+	+	−	+	+
Gelatin	+	−	+	+	−	−
Esculin	+	−	+	−	+	−
Tween 80	+	−	−	−	−	+
Assimilation of:						
d-Glucose	+	+	+	+	+	−
l-Arabinose	+	+	+	−	−	−
d-Mannose	+	+	−	+	+	−
d-Mannitol	−	−	−	−	−	−
N-acetyl-glucosamine	−	+	−	+	−	−
Maltose	+	+	+	+	+	−
Potassium gluconate	+	+	−	+	−	−
Capric acid	+	−	−	+	−	−
Adipic acid	−	−	−	+	+	−
Malic acid	+	−	+	−	+	+
Trisodium citrate	+	−	−	−	+	−
Phenylacetic acid	−	−	−	+	−	−
Enzyme activities:						
Lipase (C14)	−	−	−	+	−	−
Cystine arylamidase	−	−	−	+	−	−
Trypsin	−	−	−	+	+	−
α-chymotrypsin	+	+	−	+	−	−
α-galactosidase	−	+	+	−	−	−
β-galactosidase	−	+	+	−	−	+
β-glucuronidase	−	−	−	−	−	+
β-glucosidase	+	+	+	+	+	−
N-acetyl- β-glucosaminidase	−	+	−	+	+	−
α-mannosidase	−	−	−	−	+	−
DNA G+C content (mol%)	66.7	66.8 [7]	66.5 [6]	66.3	66.0 [5]	67.8 [4]

Table 2. Fatty acid compositions of strain TN1-12^T and two closely related species. Strain: 1, TN1-12^T; 2, *M. putida* KACC 21418^T; 3, *M. forsythiae* KACC 21261^T; 4, *M. rhizosphaerae* DSM 109722^T; 5, *M. aurea* KACC 11884^T; 6, *M. niabensis* KACC 12632^T. Data in columns 1–3, 5–6 are from this study. Data for 4, *M. rhizosphaerae* DSM 109722^T are from Chenxu Li et al. (2021) [8]. –, <0.5% or not detected.

Fatty Acid	1	2	3	4	5	6
10:0	–	–	–	–	0.62	0.56
10:0 3OH	6.09	3.69	4.83	–	–	5.73
12:0	3.73	1.45	2.27	–	–	8.32
12:0 3OH	–	0.88	–	–	0.5	–
14:0	1.79	3.52	2.68	–	1.33	–
14:0 2OH	3.27	2.31	2.93	–	–	–
14:0 iso	–	–	–	0.7	–	–
15:0 iso	–	–	–	–	0.89	–
15:0 anteiso	–	–	–	–	14.83	–
16:0	31.50	26.21	30.82	39.7	7.26	22.55
16:0 iso	–	–	–	1.4	16.46	–
17:0 anteiso	–	–	–	–	17.22	–
17:0 iso	–	–	–	–	2.49	–
17:0 cyclo	18.82	6.69	–	–	–	–
17:1 w8c	–	–	–	–	0.59	–
18:0	–	–	–	–	0.56	–
18:1 w9c	–	–	–	–	7.86	–
18:1 w7c 11-methyl	–	1.31	–	–	–	–
Summed Feature 3 *	23.85	26.37	46.50	47.9	3.16	51.10
Summed Feature 8 **	10.41	26.06	9.37	9.8	25.08	10.83

* Summed feature 3 consists of C16:1 w7c/C16:1 w6c; ** Summed feature 8 consists of C18:1 w7c/C18:1 w6c.

4. Discussion

Strain TN1-12^T exhibited major fatty acids, polar lipids, G+C content, quinones, and several characteristics (such as being Gram-negative, aerobic, and motile) similar to its closest relatives in the genus *Massilia*. However, its morphological, physiological, and biochemical features clearly differentiate it from reference strains. Notable differences include phenotypic changes after 2–3 days of cultivation and the absence of anteiso- and iso-branched chain fatty acids. Moreover, average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values, based on species delineation thresholds, strongly support that strain TN1-12^T represents a novel species within the genus *Massilia*, for which the name *Massilia paldalensis* sp. nov. is proposed. The discovery of this new soil species and its detailed taxonomic and genetic characterization contribute to a deeper understanding of microbial diversity in soil ecosystems.

Additionally, strain TN1-12^T demonstrated remarkably high resistance to heavy metals compared to previously reported *Massilia* species (Table S3). Studying this resistance may offer insights into the strain's ecological role in soil environments and its potential in bioremediation applications. The presence of genes related to antibiotic and toxic compound resistance indicates the strain's adaptability to polluted environments and its possible capability to metabolize pollutants. Further research is required to experimentally confirm

the mechanisms underlying its metal resistance and to investigate microbial interactions with soil and plants.

Moreover, the detection of secondary metabolism genes, including those involved in alkaloid biosynthesis from L-lysine and auxin biosynthesis, suggests that *M. paldalensis* may influence plant growth and provide various beneficial biological functions to host organisms. These characteristics indicate that *Massilia paldalensis* may play an essential role in maintaining ecosystem balance through pollutant detoxification and nutrient cycling. The identification of this novel bacterial species is a significant step toward expanding our knowledge of microbial ecology and discovering organisms with valuable applications in agriculture and environmental biotechnology.

Description of Massilia paldalensis sp. nov.

Massilia paldalensis (pal.dal.en'sis. N.L. fem. adj. paldalensis of or belonging to Paldal, referring to the Paldal district where the soil sample was collected).

Its characteristics include being an aerobic, rod-shaped (0.4–0.8 × 2.0–3.8 μm), Gram-stain-negative, oxidase-positive, catalase-positive, motile with a single polar flagellum, non-spore-forming bacterium. The colonies are yellowish white, circular, and smooth with entire edges on nutrient agar plates, and 0.2–1.0 mm in diameter after 24 h of incubation at 30 °C. Some differences are observed after 2–3 days of incubation, on which colonies are yellow, irregular and rough. Growth is found to occur at 10–40 °C (opt 30 °C), and at pH 5.0–8.0 (opt pH 7.0) in the presence of 0–2% (*w*/*v*) NaCl. Cells can grow on R2A agar, TSA agar, NA agar, LB agar but not on MacConkey agar. Urease reaction is negative. Hydrolyses of substrates, such as starch, casein, Tween 80, esculin and gelatin, can be conducted. β-galactosidase and nitrate reduction is positive but indole production, glucose fermentation, and arginine dihydrolase are negative. In API 20NE strips, the assimilation of d-glucose, l-arabinose, d-mannose, maltose, potassium gluconate, capric acid, malic acid and trisodium citrate is positive. The assimilation is negative for d-mannitol, N-acetyl-glucosamine, adipic acid, and phenylacetic acid. In API ZYM test strip, α-glucosidase, β-glucosidase, alkaline phosphatase, esterase (C4), lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase are positive, but α-galactosidase, β-galactosidase, lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are negative. The predominant ubiquinone is Q-8. The G+C content of the DNA is 66.7 mol%. The main polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phospholipid, phosphatidylethanolamine, two aminophospho lipid and three lipids. The major fatty acids are C16:0, summed feature 3 (C16:1ω7c and/or iso-C15:0 2-OH), 17:0 cyclo and summed feature 8 (C18:1 ω7c and/or C18:1 ω6c).

The type strain TN1-12^T (=KACC 23946^T = CGMCC 1.65296^T) was isolated from stream bank soil in Suwon City, Korea. The GenBank accession numbers are PQ538617 (16S rRNA) and JBJCIU000000000 (genome).

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d17050327/s1, Figure S1: Maximum-likelihood phylogenetic tree based on the 16S rRNA gene sequence showing the positions of strain TN1-12^T and other reference strains. *Parasutterella secuda* JCM 16078^T was used as an outgroup for the phylogenetic tree. Bar, 0.02 substitutions per nucleotide position; Figure S2: Maximum-parsimony tree showing the phylogenetic position of strain TN1-12^T and its related taxa. Only bootstrap values over 50% (percentages of 1000 replications) are shown at branch points; Figure S3: Functional classes of protein-coding genes of *Massilia paldalensis* TN1-12^T; Figure S4: The Prediction Informatics for Secondary Metabolomes of *Massilia paldalensis* TN1-12^T; Figure S5: Transmission electron microscopy of *Massilia paldalensis* TN1-12^T grown on R2A broth for 24 h at 28 °C; Figure S6: Polar lipids of strain TM1-12^T after

spraying 5% ethanolic phosphomolybdic acid (1); ninhydrin (2); molybdenum blue spray reagent (3); Table S1: General genome features of *Massilia paldalensis* TN1-12^T with closely related strains; Table S2: The average nucleotide identity and digital DNA–DNA hybridization values between the genome sequences of *Massilia paldalensis* TN1-12^T and closely related strains; Table S3: Minimum inhibitory concentrations (MICs) of three metal ions tested on bacterial strains TN1-12^T and reference strain.

Author Contributions: N.T.A.N. conceived, designed, and conducted all of the experiments. N.T.A.N. and J.K. interpreted all of the data and read, discussed, edited, and approved the final draft of the manuscript. J.K. coordinated and supervised the study. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by Kyonggi University's Graduate Research Assistantship 2023.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The whole-genome sequence of *Massilia paldalensis* TN1-12^T has been deposited in GenBank under the accession number JBJCIU000000000. The 16S rRNA gene sequence of strain TN1-12^T is available in GenBank under the accession number PQ538617. All other relevant data supporting the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. La Scola, B.; Birtles, R.J.; Mallet, M.N.; Raoult, D. *Massilia timonae* gen. nov., sp. nov., isolated from blood of an immunocompromised patient with cerebellar lesions. *J. Clin. Microbiol.* **1998**, *36*, 2847–2852. [CrossRef] [PubMed]
2. Kämpfer, P.; Lodders, N.; Martin, K.; Falsen, E. Revision of the genus *Massilia* La Scola et al. 2000, with an emended description of the genus and inclusion of all species of the genus *Naxibacter* as new combinations, and proposal of *Massilia consociata* sp. nov. *Int. J. Syst. Evol. Microbiol.* **2011**, *61*, 1528–1533. [CrossRef] [PubMed]
3. Du, Y.; Yu, X.; Wang, G. *Massilia tieshanensis* sp. nov., isolated from mining soil. *Int. J. Syst. Evol. Microbiol.* **2012**, *62 Pt 10*, 2356–2362. [CrossRef]
4. Weon, H.-Y.; Kim, B.-Y.; Hong, S.-B.; Jeon, Y.-A.; Koo, B.-S.; Kwon, S.-W.; Stackebrandt, E. *Massilia niabensis* sp. nov. and *Massilia niastensis* sp. nov., isolated from air samples. *Int. J. Syst. Evol. Microbiol.* **2009**, *59 Pt 7*, 1656–1660. [CrossRef]
5. Gallego, V.; Sánchez-Porro, C.; García, M.T.; Ventosa, A. *Massilia aurea* sp. nov., isolated from drinking water. *Int. J. Syst. Evol. Microbiol.* **2006**, *56 Pt 10*, 2449–2453. [CrossRef]
6. Heo, J.; Won, M.; Lee, D.; Han, B.-H.; Hong, S.-B.; Kwon, S.-W. *Duganella dendranthematis* sp. nov. and *Massilia forsythiae* sp. nov., isolated from flowers. *Int. J. Syst. Evol. Microbiol.* **2022**, *72*, 005487. [CrossRef]
7. Feng, G.-D.; Yang, S.-Z.; Li, H.-P.; Zhu, H.-H. *Massilia putida* sp. nov., a dimethyl disulfide-producing bacterium isolated from wolfram mine tailing. *Int. J. Syst. Evol. Microbiol.* **2016**, *66*, 50–55. [CrossRef] [PubMed]
8. Li, C.; Cao, P.; Du, C.; Zhang, X.; Bing, H.; Li, L.; Sun, P.; Xiang, W.; Zhao, J.; Wang, X. *Massilia rhizosphaerae* sp. nov., a rice-associated rhizobacterium with antibacterial activity against *Ralstonia solanacearum*. *Int. J. Syst. Evol. Microbiol.* **2021**, *71*, 005009. [CrossRef]
9. Dahal, R.H.; Chaudhary, D.K.; Kim, J. Genome insight and description of antibiotic producing *Massilia antibiotica* sp. nov., isolated from oil-contaminated soil. *Sci. Rep.* **2021**, *11*, 12864.
10. Kour, D.; Kaur, T.; Devi, R.; Yadav, A.; Singh, M.; Joshi, D. Beneficial microbiomes for bioremediation of diverse contaminated environments for environmental sustainability: Present status and future challenges. *Environ. Sci. Pollut. Res.* **2021**, *28*, 24917–24939. [CrossRef]
11. Weisburg, W.G.; Barns, S.M.; Pelletier, D.A.; Lane, D.J. 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* **1991**, *173*, 697–703. [CrossRef] [PubMed]
12. Frank, J.A.; Reich, C.I.; Sharma, S.; Weisbaum, J.S.; Wilson, B.A.; Olsen, G.J. Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Appl. Environ. Microbiol.* **2008**, *74*, 2461–2470. [CrossRef]
13. Pruesse, E.; Peplies, J.; Glöckner, F.O. SINA: Accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* **2012**, *28*, 1823–1829. [CrossRef]
14. Saitou, N.; Nei, M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **1987**, *4*, 406–425. [PubMed]

15. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547–1549. [CrossRef] [PubMed]
16. Felsenstein, J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **1985**, *39*, 783–791. [CrossRef]
17. Kimura, M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **1980**, *16*, 111–120. [CrossRef]
18. Andrews, S. FastQC: A quality control tool for high throughput sequence data. In *Babraham Bioinformatics*; Babraham Institute: Babraham, UK, 2010.
19. Bolger, A.M.; Lohse, M.; Usadel, B. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* **2014**, *30*, 2114–2120. [CrossRef]
20. Bankevich, A.; Nurk, S.; Antipov, D.; Gurevich, A.A.; Dvorkin, M.; Kulikov, A.S.; Lesin, V.M.; Nikolenko, S.I.; Pham, S.; Prjibelski, A.D.; et al. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* **2012**, *19*, 455–477. [CrossRef]
21. Simão, F.A.; Waterhouse, R.M.; Ioannidis, P.; Kriventseva, E.V.; Zdobnov, E.M. BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* **2015**, *31*, 3210–3212. [CrossRef]
22. Na, S.I.; Kim, Y.O.; Yoon, S.H.; Ha, S.M.; Baek, I.; Chun, J. UBCG: Up-to-date bacterial core gene set and pipeline for phylogenomic tree reconstruction. *J. Microbiol.* **2018**, *56*, 280–285. [CrossRef]
23. Tatusova, T.; DiCuccio, M.; Badretdin, A.; Chetvernin, V.; Nawrocki, E.P.; Zaslavsky, L.; Lomsadze, A.; Pruitt, K.D.; Borodovsky, M.; Ostell, J. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res.* **2016**, *44*, 6614–6624. [CrossRef] [PubMed]
24. Aziz, R.K.; Bartels, D.; Best, A.A.; DeJongh, M.; Disz, T.; Edwards, R.A.; Formsma, K.; Gerdes, S.; Glass, E.M.; Kubal, M.; et al. The RAST Server: Rapid annotations using subsystems technology. *BMC Genom.* **2008**, *9*, 75. [CrossRef]
25. Yoon, S.-H.; Ha, S.-M.; Lim, J.; Kwon, S.; Chun, J. A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie Van Leeuwenhoek* **2017**, *110*, 1281–1286. [CrossRef]
26. Meier-Kolthoff, J.P.; Auch, A.F.; Klenk, H.-P.; Göker, M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinform.* **2013**, *14*, 60. [CrossRef] [PubMed]
27. Blin, K.; Shaw, S.; Augustijn, H.E.; Reitz, Z.L.; Biermann, F.; Alanjary, M.; Fetter, A.; Terlouw, B.R.; Metcalf, W.W.; Helfrich, E.J.; et al. antiSMASH 7.0: New and improved predictions for detection, regulation, chemical structures and visualisation. *Nucleic Acids Res.* **2023**, *51*, W46–W50. [CrossRef] [PubMed]
28. Smibert, R.M.; Krieg, N.R. Phenotypic characterization. In *Methods for General and Molecular Bacteriology*; Gerhardt, P., Murray, R.G.E., Wood, W.A., Krieg, N.R., Eds.; American Society for Microbiology: Washington, DC, USA, 1994; pp. 607–654.
29. Sasser, M. Identification of bacteria by gas chromatography of cellular fatty acids. *Tech. Note* **1990**, *101*, 1–6.
30. Minnikin, D.E.; O'donnell, A.G.; Goodfellow, M.; Alderson, G.; Athalye, M.; Schaal, A.; Parlett, J.H. An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J. Microbiol. Methods* **1984**, *2*, 233–241. [CrossRef]
31. Hiraishi, A.; Ueda, Y.; Ishihara, J.; Mori, T. Comparative lipoquinone analysis of influent sewage and activated sludge by high-performance liquid chromatography and photodiode array detection. *J. Gen. Appl. Microbiol.* **1996**, *42*, 457–469. [CrossRef]
32. Wayne, L.G. International Committee on Systematic Bacteriology: Announcement of the report of the ad hoc Committee on Reconciliation of Approaches to Bacterial Systematics. *Zentralblatt Bakteriol. Mikrobiol. Hygiene A* **1988**, *268*, 433–434.
33. Abou-Shanab, R.A.; van Berkum, P.; Angle, J.S. Heavy metal resistance and genotypic analysis of metal resistance genes in gram-positive and gram-negative bacteria present in Ni-rich serpentine soil and in the rhizosphere of *Alyssum murale*. *Chemosphere* **2007**, *68*, 360–367. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.