

Massilia Cellulosilytica Sp. Nov., A Novel Cellulose-degrading Bacterium Isolated from Rhizosphere Soil of Rice (*Oryza Sativa* L.) and whole Genome Analysis

Chuanjiao Du

Northeast Agricultural University

Chenxu Li

Northeast Agricultural University

Peng Cao

Northeast Agricultural University

Tingting Li

Northeast Agricultural University

Dandan Du

Northeast Agricultural University

Xiangjing Wang

Northeast Agricultural University

Junwei Zhao (✉ guyan2080@126.com)

Northeast Agricultural University <https://orcid.org/0000-0003-1250-2756>

Wensheng Xiang

Northeast Agricultural University

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Abstract

A bacterial strain, Gram-stain negative, rod-shaped, aerobic and cellulose-degrading, designated NEAU-DD11^T, was isolated from rhizosphere soil of rice collected from Northeast Agricultural University in Harbin, Heilongjiang province, North-east China. Base on 16S rRNA gene sequence analysis, strain NEAU-DD11^T belongs to the genus *Massilia* and shared high sequence similarities with *Massilia phosphatilytica* 12-OD1^T (98.46 %) and *Massilia putida* 6NM-7^T (98.41 %). Phylogenetic analysis based on the 16S rRNA gene and whole genome sequences indicated that strain NEAU-DD11^T formed a stable cluster with *M. phosphatilytica* 12-OD1^T and *M. putida* 6NM-7^T. The major fatty acids of the strain were C_{16:0}, C_{17:0}-cyclo and C_{16:1} ω7c. The respiratory quinone was Q-8. The polar lipids profile of the strain showed the presence of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, an unidentified polar lipid and an unidentified phospholipid. In addition, the digital DNA-DNA hybridization values between strain NEAU-DD11^T and *M. phosphatilytica* 12-OD1^T and *M. putida* 6NM-7^T were 45.4% and 35.6%, respectively, which are lower than the accepted threshold value of 70%. The DNA G + C content of strain NEAU-DD11^T was 66.2 %. The whole genome analysis showed the strain contained carbohydrate enzymes such as glycoside hydrolase and polysaccharide lyase, which enabled the strain to had the function of degrading cellulose. On the basis of the phenotypic, genotypic and chemotaxonomic characteristics, strain NEAU-DD11^T represents a novel species of the genus *Massilia*, for which the name *Massilia cellulosilytica* sp. nov. is proposed. The type strain is NEAU-DD11^T (= CCTCC AB 2019141^T = DSM 109721^T).

Introduction

The genus *Massilia*, belonging to the family *Oxalobacteraceae*, was first described by La Scola et al. (1998) with the type species *Massilia timonae* isolated from blood of a patient with immunodeficiency. The typical features of the genus *Massilia* are Gram-stain-negative, non-spore-forming, rod-shaped, aerobic and motile (except for the species of *Massilia arvi* THG-RS20^T, '*Massilia humi* THG-S6.8^T', *Massilia glaciei* B448-2^T and *Massilia varians* comb. CCUG35299^T) (Yang et al. 2019). Summed feature 3 (C_{16:1} ω7c and/or iso-C_{15:0} 2-OH), C_{17:0}-cyclo and C_{16:0} are the major fatty acids and Q-8 is the predominant ubiquinone. The major polar lipids are diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG). The DNA G + C contents range from 62.4 to 69.4 %. At present, the genus *Massilia* contains a total of 55 species with validly published names (<https://www.bacterio.net/genus/massilia>), including the recently described species *Massilia arenosa* (Rachel et al. 2020), *Massilia atriviolacea* (Yang et al. 2019) and *Massilia aquatica* (Lu et al. 2020). Members of the genus were isolated from various sources, such as soil (Chaudhary et al. 2017; Singh et al. 2015; Zu et al. 2008; Kim 2014; Zheng et al. 2017), air (Weon et al. 2008, 2009), water (Gallego et al. 2006), ice core (Shen et al. 2013; Guo et al. 2016), glacier permafrost (Wang et al. 2018), rock surface (Sun et al. 2017) and a human clinical specimen (Kämpfer et al. 2008, 2012). Members of the genus *Massilia* are widely distributed and have strong adaptability to the environment, and possess potential

important application values, such as soil remediation, enzyme production and other metabolites. *Massilia putida* (Guang et al. 2016) isolated from ore can produce dimethyl disulfide, which has potential application value for controlling soil borne diseases. *Massilia chloroacetimidivorans* (Lee et al. 2017) was isolated from farmland soil, which can degrade chloracetamide. *Massilia tieshanensis* was isolated from metal ore soil by Du et al. (2012), which was sensitive to As^{3+} , Cu^{2+} , Sb^{3+} , Zn^{2+} , Ni^{2+} and Cd^{2+} . *Massilia phosphatilytica* 12-OD1^T was isolated from a long-term fertilized soil, which was firstly reported that it had the ability of dissolving phosphorus (Zheng et al. 2017). During an investigation of the diversity and community structure and function of microbes in the rhizosphere soil of rice, a bacterial strain NEAU-DD11^T was isolated, which could degrade cellulose. In this study, the taxonomic location of the novel strain was determined by a polyphasic approach, which is closely and phylogenetically related to the genus *Massilia*.

Materials And Methods

Isolation, maintenance and cultural conditions of strain

Strain NEAU-DD11^T was isolated from rhizosphere soil of rice (*Oryza sativa* L.) collected from Northeast Agriculture University, Harbin, Heilongjiang province, North-east China (45°44'N, 126°43'E). In order to obtain the bacteria, two grams of rice rhizosphere soil were added to a 100 ml conical flask containing 18 ml of sterile distilled water and stirred in a rotating shaker at 200 rpm for 30 min and continuously diluted to a final dilution of 10^{-3} , 10^{-4} and 10^{-5} . 200 μ l of each soil suspension was plated on Reasoner's 2A (R2A) medium (0.5g yeast extract, 0.5g peptone, 0.5g casamino acid, 0.5g glucose, 0.5g soluble starch, 0.3g K_2HPO_4 , 0.05g $MgSO_4 \cdot 7H_2O$, 0.3g sodium pyruvate, 20g agar, 1l sterile distilled water, pH 7.2) supplemented with cycloheximide (50 mg l^{-1}) and chloramphenicol (0.1 g l^{-1}). The strain NEAU-DD11^T was isolated and purified on R2A agar medium. After 3 days of aerobic incubation at 28 °C, the cultures were preserved as a suspension in R2A broth with glycerol (40 %, w/v) and stored at -80 °C. The reference strains *Massilia phosphatilytica* 12-OD1^T was obtained from the China Center for type Culture Collection (CCTCC) and *Massilia putida* 6NM-7^T was obtained from Guangdong Microbial Culture Center (GDMCC). These strains were cultured under the same conditions for comparative analysis.

Morphological, cultural and physiological Characteristics

Cell morphology and the presence of flagella were determined by light microscopy (ECLIPSE E200, Nikon) and transmission electron microscopy (Hitachi H-7650) using cells grown on R2A agar medium at 28 °C for 72 h. The cell motility was established by observing the growth and diffusion of cells in the medium of semi-solid R2A containing 0.3 % agar at 20 °C incubation for 48 h (Yang et al. 2019). Growth of strain NEAU-DD11^T was tested on R2A agar, Luria-Bertani agar (5 g yeast extract, 10 g tryptone, 10 g NaCl, 20 g agar and 1 l distilled water) and nutrient agar (5 g peptone, 3 g meat extracts, 15 g agar, 1 l distilled water, pH 7.2–7.4) at 28 °C for 3 days. Color determination was done with color chips from the ISCC-NBS color charts (Kelly 1964). Bacterial growth at 4, 10, 15, 20, 25, 28, 37, 40 and 45 °C was assessed after 3 days

of incubation on R2A agar. The pH range for growth was determined after 3 days of incubation at 28 °C in R2A broth adjusted to pH 1.0–12.0 at intervals of 1.0 pH units that was buffered with 0.1 M citric acid/0.1 M sodium citrate; 0.1 M KH₂PO₄/0.1 M NaOH; 0.1 M NaHCO₃/0.1 M Na₂CO₃; 0.2 M KH₂PO₄/0.1 M NaOH (Cao et al. 2020; Zhao et al. 2019). Tolerance to NaCl was tested in R2A broth supplemented with 0–4.0 % NaCl (w/v) with an interval of 0.5 % (w/v) after 3 days of incubation. The Gram reaction was determined by using the Hucker staining method (Lányi 1987). Degradation of cellulose and starch was determined using the methods of Smibert and Krieg (Smibert et al. 1994) after 3 days of incubation. Oxidase activity was tested using an oxidase reagent (bioMérieux) according to the manufacturer's instructions. Hydrolysis of Tweens (20, 40 and 80) was tested by using the methods of Lányi (1987). Other physiological and biochemical characteristics were examined with the API ZYM, API 20NE and API 50CH systems (bioMérieux). The API 20NE and API 50CH tests were read after 72 h incubation at 28 °C. The API ZYM tests were read after 4 h incubation at 37 °C. Cells of strain NEAU-DD11^T grown on R2A agar at 28 °C for 2 days were used for API tests. Congo red dyeing plate (Teather et al. 1982) was used for cellulose degradation test.

Chemotaxonomic characterisation

For analysis of fatty acids, strain NEAU-DD11^T and its reference strains were grown in R2A broth at 28 °C for 3 days. Fatty acid methyl esters were extracted from the biomass according the modified method (cells were harvested by centrifugation and freeze-dried) as described by Gao et al. (2014) and analyzed by GC-MS using the method of Xiang et al. (2011) and identified with the NIST 14 database. The respiratory quinone was extracted from freeze-dried biomass and purified according to Collins (1985) and analyzed by using reversed-phase HPLC according to the method described by Zhao et al. (2020). The polar lipids were examined by two-dimensional TLC and identified using the method of Minnikin et al. (1984). Polar lipids were analyzed by using chloroform/methanol/water (65:25:4) in the first dimension, followed by chloroform/acetic acid/methanol/water (80:18:12:5) in the second dimension.

DNA preparation, amplification and determination of 16S rRNA gene sequences and phylogenetic relationships

Extraction of chromosomal DNA and PCR amplification of the 16S rRNA gene sequence were carried out using the methods of Kim *et al.* (2014) PCR amplification was carried out using the universal primers 27F and 1492R (Embley 1991). The PCR product was purified and cloned into the vector pMD19-T (Takara) and sequenced using an Applied Biosystems DNA sequencer (model 3730XL). Almost full-length 16S rRNA gene sequence of strain NEAU-DD11^T was obtained and compared with type strains available in the EzBioCloud server (<https://www.ezbiocloud.net/identify>) (Yoon et al. 2017a) and retrieved using NCBI BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), and then submitted to the GenBank database.

Phylogenetic trees were constructed based on the 16S rRNA gene sequences of strain NEAU-DD11^T and related reference species. Sequences were multiply aligned in Molecular Evolutionary Genetics Analysis (MEGA) using the Clustal W algorithm and trimmed manually where necessary. Phylogenetic trees were constructed with neighbour-joining (NJ) (Saitou et al. 1987) and maximum-likelihood (ML) (Felsenstein

1981) algorithms using MEGA software version X (Kumar et al. 2018). The stability of the clades of the phylogenetic trees was assessed using the bootstrap method with 1000 replications (Felsenstein 1985). A distance matrix was generated using Kimura's two-parameter model (Kimura 1980). 16S rRNA gene sequence similarities between strains were calculated on the basis of pairwise alignment using the EzBioCloud server (Yoon et al. 2017a). Phylogenetic analysis was performed as described above. Whole-genome phylogeny was generated using TYGS server (<http://tygs.dsmz.de>) (Meier-Kolthoff et al. 2019). The strain NEAU-DD11^T and all the species with validly published names of the genus *Massilia* were included in the phylogenetic trees. *Burkholderia metallica* LMG 24068^T was taken as an outgroup.

For draft genome sequencing and assembly, the genomic DNA of strain NEAU-DD11^T was extracted with the SDS method (Nikodinovic et al. 2003). The harvested DNA was detected by the agarose gel electrophoresis and quantified by Qubit. Whole-genome sequencing was performed on the Illumina NovaSeq PE150 platform. A-tailed, ligated to paired-end adaptors and PCR amplified with a 350 bp insert was used for the library construction at the Beijing Novogene Bioinformatics Technology Co., Ltd. Illumina PCR adapter reads and low quality reads from the paired-end were filtered using the readfq (Version 10) remove reads with less than a certain percentage of low-quality bases (mass value B 38 or default is 40 bp), a certain percentage of reads with N bases (default is 10 bp), overlap exceeds a certain threshold (default is 15 bp) and the possibility reads originating from the host. All good quality paired reads were assembled using the SOAP denovo (Li et al. 2008, 2010).

(<http://soap.genomics.org.cn/soapdenovo.html>) into a number of scaffolds. Two genomic metrics are now available to distinguish between orthologous genes of closely related prokaryotes, including the calculation of average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH). The dDDH and ANI values were determined between the genomes of strain NEAU-DD11^T and *M. phosphatilytica* 12-OD1^T and *M. putida* 6NM-7^T online at <http://ggdc.dsmz.de> using the Genome-to-Genome Distance Calculation (GGDC 2.0) (Meier-Kolthoff et al. 2013) and the ChunLab's online ANI Calculator (www.ezbiocloud.net/tools/ani) (Yoon et al. 2017b), respectively.

Results And Discussion

Phenotypic characteristics

Morphological observation of strain NEAU-DD11^T grown on R2A medium at 28 °C for 3 days were conducted by light microscopy and transmission electron microscopy. It revealed that it has the typical characteristics of the members of the genus *Massilia*. Cells of strain NEAU-DD11^T were observed to be rod-shaped (1.1-1.2 µm long and 0.3-0.4 µm in diameter) and motile by peritrichous flagella (Fig. 1). The strain NEAU-DD11^T grew well on R2A agar, nutrient agar and Luria-Bertani agar. Colonies were circular, convex, smooth and ivory-white on R2A agar plates. The strain NEAU-DD11^T was found to grow at 10-40 °C, pH 4.0-10.0 and 0-2 % (w/v) NaCl. Optimal growth occurred at 28 °C and pH 7.0 without NaCl. The cells are Gram-stain negative. The strain was oxidase positive, but Tweens (20, 40 and 80) were negative. The results of morphological, physiological and biochemical characteristics that differentiated strain

NEAU-DD11^T from closely related species, *M. phosphatilytica* 12-OD1^T and *M. putida* 6NM-7^T, are listed in Table 1. In API ZYM test, it was positive for lipase, leucine arylamidase, valine arylamidase, quatic arylamidase, trypsin, acid phosphatase, α -galactosidase and β -galactosidase, weakly positive for α -chymotrypsin, and negative for N-acetyl- β -glucosaminidase and α -mannosidase, which were different from *M. phosphatilytica* 12-OD1^T. In API 20NE test, adipic acid, citrate and phenylacetic acid are assimilated except for malic acid, these also showed a distinct difference from the reference strain *M. phosphatilytica* 12-OD1^T. The strain could utilize N-acetyl-glucosamine, arbutin, salicin and D-fibrodiose, while its reference strains *M. phosphatilytica* 12-OD1^T and *M. putida* 6NM-7^T could not. These phenotypic characteristics could clearly distinguish strain NEAU-DD11^T from its closely related phylogenetic neighbours of the genus *Massilia*. Cellulose degradation test result showed that the strain NEAU-DD11^T had degradation ability and the degradation diameter reached 32.1 mm (Fig. S1).

Chemotaxonomic characteristics

Cellular fatty acid profiles of strains NEAU-DD11^T, *M. phosphatilytica* 12-OD1^T and *M. putida* 6NM-7^T are shown in Table S1. The fatty acid profile of strain NEAU-DD11^T was similar to those of members of genus *Massilia*, with minor differences from its reference strains of some fatty acids. Strain NEAU-DD11^T contained C_{16:0} (41.6 %), C_{17:0}-cyclo (40.3 %) and C_{16:1} ω 7c (10.8 %) as the major components, which were the same with those of reference strains; but the strain did not contain C_{12:0} and C_{15:0}, while the reference strain *M. phosphatilytica* 12-OD1^T contained C_{15:0}, and the reference strain *M. putida* 6NM-7^T contained C_{12:0}. The respiratory quinone was Q-8. The polar lipids included phosphatidylglycerol (PG), phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), an unidentified polar lipid (UL) and an unidentified phospholipid (PL) (Fig. S2), which was distinct differences from its reference strains (Table 1). All these chemotaxonomic data showed that strain NEAU-DD11^T should be assigned to the genus *Massilia*.

Table 1

Differential characteristics of strain NEAU-DD11^T, *M. phosphatilytica* 12-OD1^T and *M. putida* 6NM-7^T.

Strains: 1, NEAU-DD11^T; 2, *M. phosphatilytica* 12-OD1^T; 3, *M. putida* 6NM-7^T. All data were determined in the present study except where marked. +, positive; -, negative; w, weakly positive.

Characteristic	1	2	3
Colonies on R2A agar			
Color	Ivory-white	Light-yellow	Grey-white
Smooth	+	-	-
NaCl (% w/v)	0-2	0-0.5	0-0.5
Temperature (°C)	10-40	4-37	25-37
pH	4-10	5-8	6-8
API ZYM:			
Lipase C14	+	-	+
Leucine arylamidase	+	-	+
Valine arylamidase	+	-	+
Cystine arylamidase	+	-	+
Trypsin	+	-	-
α-chymotrypsin	w	+	+
Acid phosphatase	+	-	+
α-galactosidase	+	-	+
β-galactosidase	+	-	+
N-acetyl-β-glucosaminidase	-	+	+
α-mannosidase	-	w	-
API 20NE:			
Nitrate reduction	+	+	-
Aesculin hydrolysis	w	-	-
β-galactosidase	-	+	-
Adipic acid	-	+	-
Malic acid	+	+	-

Citrate	-	+	-
Phenylacetic acid	-	+	-
API 50CH:			
D-Arabinose	-	+	-
L-Arabinose	-	+	+
D-ribose	W	-	-
D-xylose	-	+	-
D-galactose	-	+	+
D-glucose	+	-	+
D-fructose	+	+	W
N-Acetylglucosamine	+	-	-
Amygdalin	-	+	-
Arbutin	+	-	-
Salicin	+	-	-
Cellobiose	+	+	-
Raffinose	-	+	-
Glycogen	W	+	-
Gentiobiose	-	-	W
D-Tagatose	-	-	W
D-Fucose	-	+	-
L-Fucose	-	+	-
Potassium gluconate	-	+	+
Potassium 2-ketogluconate	-	+	-
Polar lipids	DPG, PE, PG, UL, PL	DPG, PE, PG, UL	DPG, PE, PG, UL, PL, APL
Major fatty acids	C _{16:0} , C _{16:1} ω7c, C _{17:0} -cyclo	C _{16:0} , C _{16:1} ω7c, C _{17:0} -cyclo	C _{16:0} , C _{16:1} ω7c, C _{17:0} -cyclo
DNA G+C content (mol %)	66.2	67.7 ^a	66.8±0.6 ^b
*Data taken from: a, Zheng <i>et al.</i> (2017); b, Guang <i>et al.</i> (2016)			

Molecular characteristics

An almost-complete 16S rRNA gene sequence of strain NEAU-DD11^T (1521 bp) was subjected to comparative analysis. Sequence analysis of the 16S rRNA gene showed that strain NEAU-DD11^T is closely related to *M. phosphatilytica* 12-OD1^T (98.46 %) and *M. putida* 6NM-7^T (98.41 %). Furthermore, the neighbour-joining (NJ) phylogenetic tree based on 16S rRNA gene sequences showed that strain NEAU-DD11^T formed a stable cluster with *M. phosphatilytica* 12-OD1^T and *M. putida* 6NM-7^T with a bootstrap value of 56 % (Fig. 2). This similar phylogenetic relationship was also observed in the maximum-likelihood (ML) tree with a bootstrap value of 54 % (Fig. S3). The phylogenetic trees generated with the NJ and ML methods showed that strain NEAU-DD11^T was grouped with members of the genus *Massilia*.

The draft genome of strain NEAU-DD11^T consisted of 7 341 311 bp and 30 contigs with an N50 contig length of 705160 bp, a DNA G+C content of 66.2 % and a coverage of 130×. It was deposited in GenBank under the accession number WSES00000000. The NCBI Prokaryotic Genome Annotation Pipeline (PGAP) revealed that the genome contained four copies of the 5S rRNA genes, five copies of the 16S rRNA genes, three copies of the 23S rRNA genes, 76 tRNA genes and four copies of noncoding RNA genes. In the draft genome of strain NEAU-DD11^T, the 16S rRNA gene sequence (MN784464) determined by PCR method was found and confirmed. Detailed genomic information is presented in Table S2. The digital DNA-DNA hybridization values between strain NEAU-DD11^T and *M. phosphatilytica* 12-OD1^T and *M. 6NM-7^T* were 45.4 % and 35.6 %, respectively, which were all below the threshold value of 70% proposed for species discrimination by Wayne et al. (1987). Similarly, the low ANI values of genome sequences between strain NEAU-DD11^T and its reference strains were 91.7 % and 88.4 %, respectively, results well below the threshold of 95-96 % used to delineate prokaryote species (Richter et al. 2009; Chun et al 2014). In whole genome phylogeny (Fig. 3), the strain NEAU-DD11^T formed a cluster with *M. phosphatilytica* 12-OD1^T and *M. putida* 6NM-7^T, the closest relative based on 16S rRNA gene identity. It was also supported by the highest pairwise averagenucleotide identity (ANI) value, indicating that they are closely related in many ways. These results support the conclusion that strain NEAU-DD11^T represents a novel species of the genus *Massilia*.

Gene function annotation can be found in the GO function classification diagram (Fig. S4). The genome has been identified as free of contamination. The genome of strain NEAU-DD11^T contained a total of 5935 genes and 5412 genes were annotated and assigned to putative functions based on the KEGG database. The result showed that more than 2489 genes of strain NEAU-DD11^T were annotated into metabolism associated pathways. The detailed distribution of genes in the KEGG functions of metabolism is shown in Fig. S5. Moreover, the genome of strain NEAU-DD11^T contained 47 carbohydrate-binding modules, 26 carbohydrate esterases, 207 glycoside hydrolases, 94 glycosyl transferases, 20 polysaccharide lyases, 3 auxiliary activities were annotated based on Carbohydrate-Active EnZymes database (CAZy) (Cantarel et al. 2009). These carbohydrate enzymes catalyze carbohydrate degradation,

modification, and biosynthesis, and have important application value. There were many types of glycoside hydrolases, which can hydrolyze polysaccharides such as cellulose, starch, xylan and mannan (Suleiman et al. 2020). Furthermore, the strain comprised gene encoding of polysaccharide lyases that degrades cellulose into small molecules, thus playing a key role in agricultural waste utilization (Kikuchi et al. 2020). AntiSMASH version 2.0.2 (Medema et al. 2011) was used to investigate the genome contained secondary metabolites gene clusters including Terpene (3 clusters), Bacteriocin (2 clusters), Siderophore (1 cluster). For pathogenicity and drug resistance analyses, the genome of strain NEAU-DD11^T was compared with the Virulence Factors of Pathogenic Bacteria Database (VFDB) (Chen et al. 2012) and Antibiotic Resistance Genes Database (Liu et al. 2009) (ARDB). The results showed that 486 virulence factors and 27 antibiotic resistance genes were annotated.

Based on the distinct phenotypic, biochemical, chemotaxonomic and phylogenetic data mentioned above, the strain NEAU-DD11^T represents a novel species of the genus *Massilia*, for which the name *Massiliacellulosilytica* sp. nov. is proposed.

Description of *Massilia cellulosilytica* sp. nov.

Massilia cellulosilytica (cel.lu.lo.si.ly'ti.ca. N.L. neut. n. cellulorum cellulose; N.L. fem. adj. lytica able to loose, able to dissolve; from Gr. fem. adj. lytikê able to loose, able to dissolve; N.L. fem. adj. cellulosilytica dissolving cellulose)

Cells are Gram-stain negative, aerobic, non-spore-forming, rod-shaped, and 0.3-0.4 × 1.1-1.2 µm in size after cultivation for 2 days at 28°C on R2A agar. Colonies on R2A agar are circular, convex, smooth, and ivory-white. Good growth occurs on R2A, nutrient agar and Luria-Bertani agar. Growth occurs aerobically at 10-40 °C (optimum, 28 °C) and at pH 4.0-10.0 (optimum, 7.0). Cells grow in the presence of 0-2 % (w/v) NaCl (optimum, 0 %). Gelatin, cellulose and esculin are hydrolyzed, but starch and urea are not hydrolyzed. Arginine dihydrolase is not produced. Nitrate is reduced to nitrite. H₂S is produced, but indole is not produced. In API ZYM, results are positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, quatic arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase and β-glucosidase, and weakly positive for α-chymotrypsin, but negative for β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase. In API 20NE test, D-glucose, L-arabinose, D-mannose, N-acetylglucosamine, D-maltose, potassium gluconate and malic acid are assimilated. The following substrates are not assimilated: D-mannitol, capric acid, adipic acid, citrate, phenylacetic acid. In API 50CH tests, acid is produced from D-glucose, D-fructose, D-mannose, N-acetylglucosamine, arbutin, aesculin ferric citrate, salicin, D-cellobiose, D-maltose, D-sucrose, D-trehalose and starch, and weakly produced from D-ribose and glycogen. Acid is not produced from glycerol, erythritol, D-arabinose, L-arabinose, D-xylose, L-xylose, D-adonitol, methyl β-D-xylopyranoside, D-galactose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, D-lactose, D-melibiose, inulin, D-melezitose, D-raffinose, xylitol, D-gentiobiose, D-turanose, D-lyxose, D-

tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate. The major cellular fatty acids are C_{16:0}, C_{17:0}-cyclo and C_{16:1}ω7c. The respiratory quinone is ubiquinone Q-8. The polar lipids include phosphatidylglycerol (PG), phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), an unidentified polar lipid (UL) and an unidentified phospholipid (PL). The DNA G+C content of the strain NEAU-DD11^T is 66.2 %.

The type strain is NEAU-DD11^T (=CCTCC AB 2019141^T = DSM 109721^T), isolated from the rhizosphere soil of rice collected from Northeast Agricultural University in Harbin, Heilongjiang Province, China. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NEAU-DD11^T is MN784464. This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession WSES00000000. The version described in this paper is version WSES00000000.1.

Declarations

Consent to participate and/or Consent to publish

This research doesn't involve in human subjects, so the informed consent to participate and consent to publish are not obtained.

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Conflicts of interest

The authors declare that they have no conflict of interest.

Ethics approval

This article does not contain any studies with human participants and/or animals performed by any of the authors. The formal consent is not required in this study.

Consent

All authors have seen a copy of the manuscript and have approved its submission.

Data availability

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NEAU-DD11^T is MN784464. The Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession WSES00000000. The version described in this paper is version WSES00000000.1.

Author's Contributions

Chuanjiao Du performed the laboratory experiments, analyzed the data, and drafted the manuscript. Chenxu Li contributed to the biochemical characterization. Peng Cao contributed to the morphological analyzes. Tingting Li contributed to the fatty acids determination. Dandan Du contributed to the polyphasic taxonomy. Xiangjing Wang participated to the discussions of each section of experiments. Junwei Zhao and Wensheng Xiang designed the experiments and revised the manuscript.

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Figures

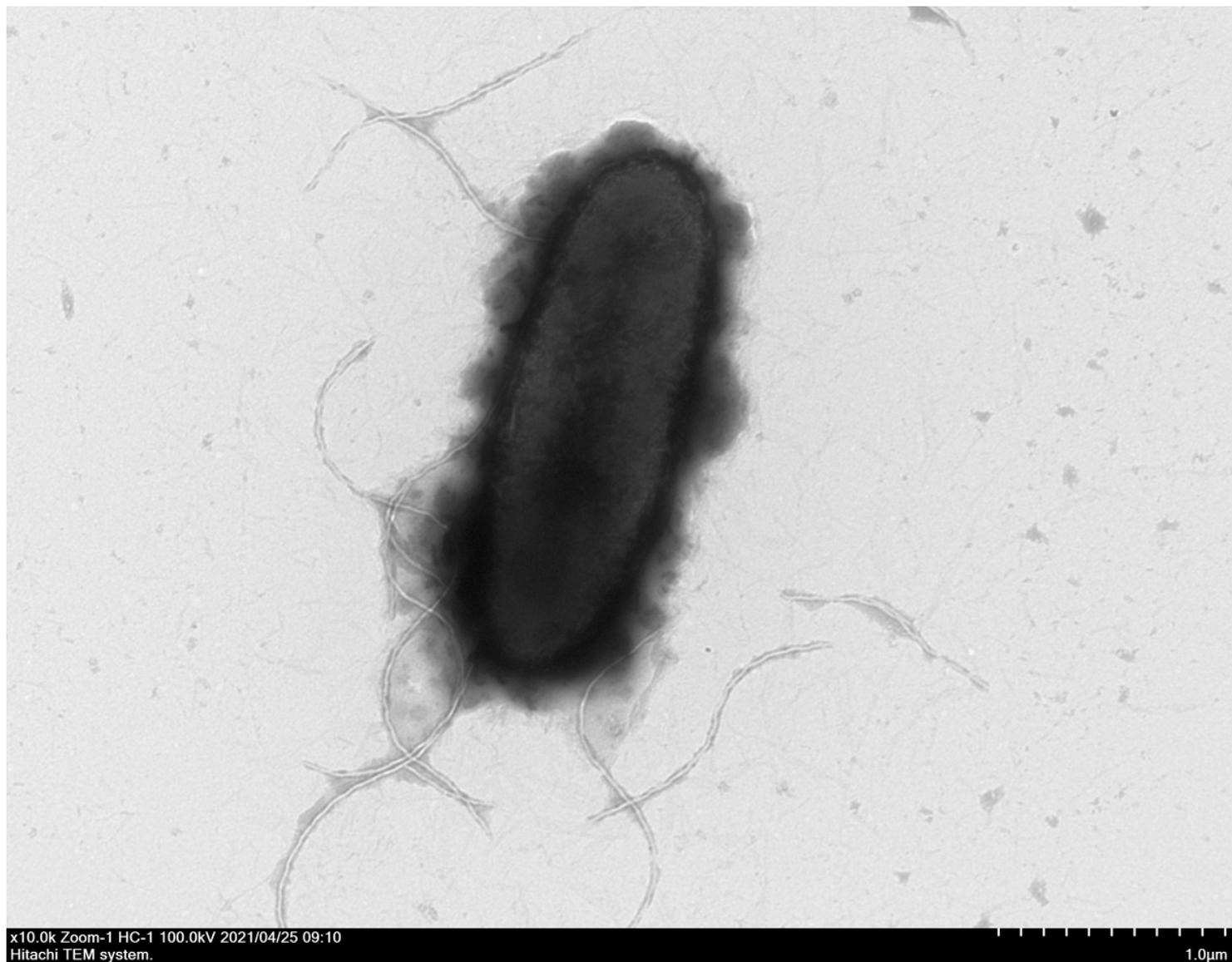


Figure 1

Transmission electron micrograph of strain NEAU-DD11T grown on R2A agar for 3 days at 28 oC. Bar, 1 μm .

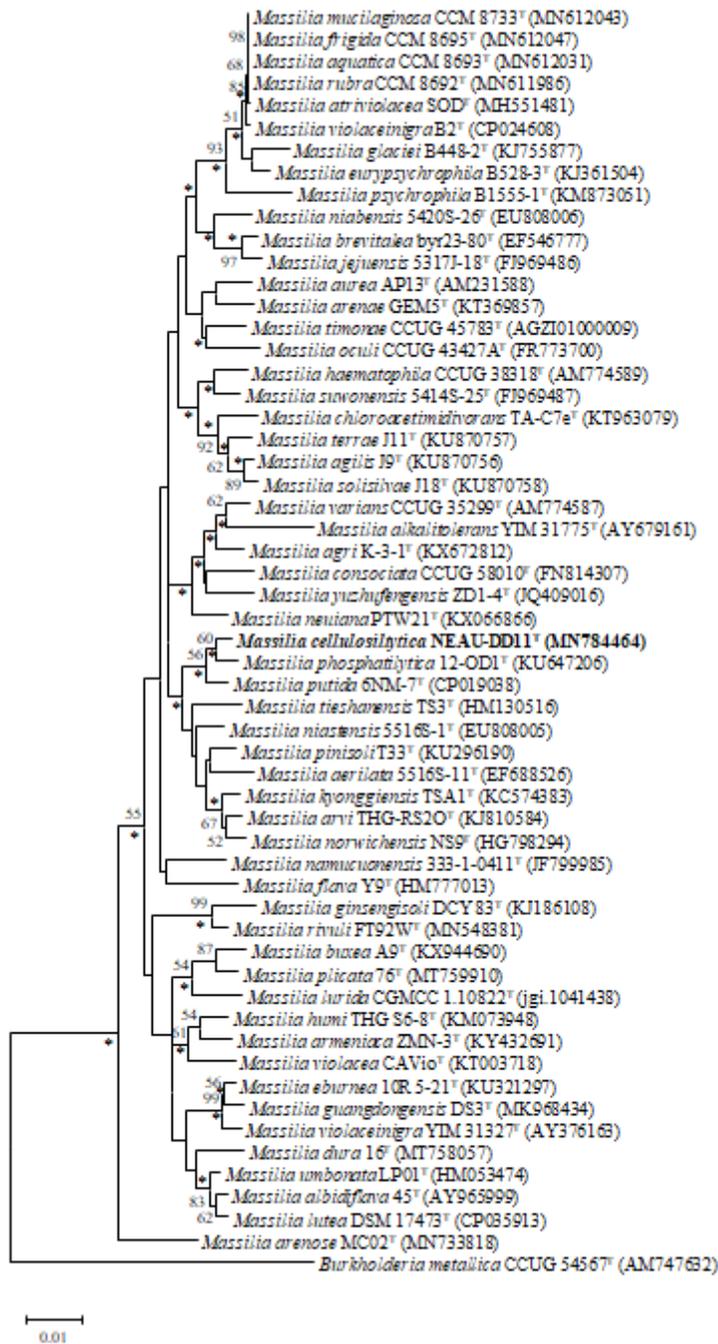


Figure 2

Neighbour-joining tree showing the phylogenetic position of strain NEAU-DD11T (1381 bp) and species of 55 16S rRNA gene sequence of the genus *Massilia*. The out-group used was *Burkholderia metallica* LMG 24068T (AM747632). Only bootstrap values above 50 % (percentages of 1000 replications) are indicated. Asterisks (*) indicate branches also recovered in the maximum-likelihood tree. Bar, 0.01 nucleotide substitutions per site.

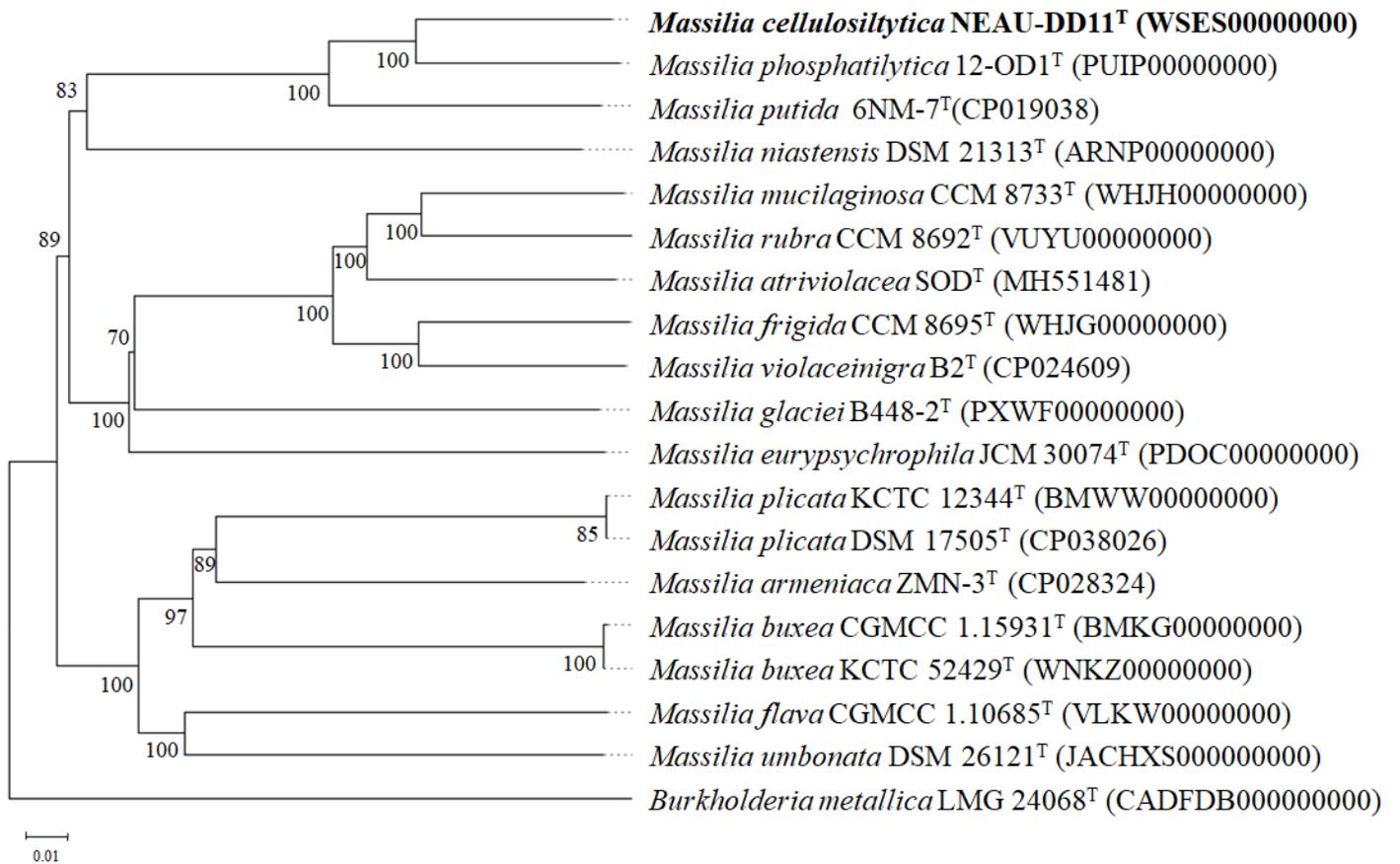


Figure 3

Whole-genome sequence tree generated with TYGS for NEAU-DD11T and closely related species of the genus *Massilia*. The out-group used was *Burkholderia metallica* LMG 24068T. Tree inferred with FastME from GBDP distances calculated from genome sequences. Branch lengths are scaled in terms of GBDP distance formula d5; numbers above branches are GBDP pseudo-bootstrap support values from 100 replications; Bar, 0.01 nucleotide substitutions per site.