Cellulomonas Triticagri Sp. Nov., A Novel Actinomycete Isolated From The Rhizosphere Soil Of Wheat (*Triticum Aestivum* L.)

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Abstract

A Gram-positive, motile, rod-shaped and lignin-degraded novel actinomycete, designated strain NEAU-YY56^T, was isolated from the rhizosphere soil of wheat (*Triticum aestivum* L.) collected from Zhumadian, Henan Province, Central China and characterized using a polyphasic approach. Phylogenetic analysis based on the 16S rRNA gene sequence indicated that strain NEAU-YY56^T belonged to the genus *Cellulomonas* and exhibited 16S rRNA gene sequence similarities of 98.7, 98.2 and 98.1 % to *Cellulomonas pakistanensis* JCM 18755^T, *Cellulomonas denverensis* W6929^T and *Cellulomonas hominis* JCM 12133^T, respectively. The morphological characteristics [Gram-positive and short rod-shaped cells] and chemotaxonomic properties [whole-cell sugars: glucose, rhamnose and ribose; major polar lipids: diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol mannoside and two unknown glycolipids; major menaquinones: MK-9(H_4) and MK-9(H_2); major fatty acids (> 5.0 %): *anteiso*-$C_{15:0}$, $C_{16:0}$, $C_{14:0}$ and *anteiso*-$C_{17:0}$; DNA G+C content: 74.7 %] of strain NEAU-YY56^T have also confirmed the affiliation of the isolate to the genus *Cellulomonas*. However, DNA-DNA relatedness, physiological and biochemical characteristics indicated that strain NEAU-YY56^T can be clearly differentiated from its closest relatives. Therefore, it is concluded that strain NEAU-YY56^T represents a novel species of the genus *Cellulomonas*, for which the name *Cellulomonas triticagri* sp. nov. is proposed. The type strain is NEAU-YY56^T (= DSM 106717^T = JCM 32550^T).

Introduction

The genus *Cellulomonas*, which was first proposed by Bergey et al. (1923) and later amended by Stackebrandt et al. (2006), is a member of the family *Cellulomonadaceae* within the order *Actinomycetales*, and currently comprised 30 validly species (www.bacterio.net/cellulomonas.html). Typical characteristics of this genus are Gram-positive, aerobic or facultatively anaerobic, short rods, with glucose, rhamnose and ribose as the diagnostic whole-cell sugars, MK-9(H_4) as the major menaquinone, straight-chain, saturated or unsaturated, and branched-chain saturated fatty acids of the *iso* and *anteiso* types as the major fatty acids and a high DNA G + C content from 68.5 to 76.0% (Li et al. 2020; Stackebrandt and Schumann 1923). In the course of the continuous effort to discover potential antagonist actinobacteria, which is known to produce a vast diversity of antimicrobials, against *Fusarium graminearum*, a *Cellulomonas*-like strain, NEAU-YY56^T, was isolated. In this article, we report a novel *Cellulomonas* species based on the polyphasic taxonomy analysis, for which the name *Cellulomonas triticagri* sp. nov. is proposed.

Materials And Methods

Isolation and maintenance of the organism

The rhizosphere soil sample of wheat (*Triticum aestivum* L.) collected from Zhumadian, Henan Province, Central China (32°98' N, 114°02' E) was processed as described by Zhao et al. (2018) and grown on

Dulcitol-proline agar (DPA) (Cao et al. 2016) supplemented with cycloheximide (50 mg l^{-1}) and nalidixic acid (20 mg l^{-1}). After 7 days of incubation aerobically at 28 °C, single colonies were transferred and purified on the International *Streptomyces* Project (ISP) medium 3 (Shirling and Gottlieb 1966) and maintained as glycerol suspensions (20%, v/v) at -80 °C. The type strains of *Cellulomonas pakistanensis* JCM 18755^T, *Cellulomonas denverensis* W6929^T and *Cellulomonas hominis* JCM 12133^T were purchased from the Japan Collection of Microorganisms (JCM). All strains were cultured under the same conditions for comparative analysis.

Phenotypic characteristics

Gram staining was carried out by using the standard Gram stain and morphological characteristics were observed by light microscopy (Nikon ECLIPSE E200) and scanning electron microscopy (Hitachi SU8010) using cultures grown on trypticase soy agar (TSA) medium at 28 °C for 2 weeks. Cultural characteristics were determined on yeast extract-malt extract agar (ISP 2), oatmeal agar (ISP 3), inorganic salts-starch agar (ISP 4), glycerol-asparagine agar (ISP 5), peptone-yeast extract iron agar (ISP 6), tyrosine agar (ISP 7) (Shirling and Gottlieb 1966) and TSA media after 2 weeks at 28 °C. Color determination was done with ISCC-NBS color charts Standard Samples No 2106 (Kelly 1964). Growth at different temperatures (4, 10, 15, 18, 20, 25, 28, 30, 35, 37, 40, 45 and 50 °C) was determined on TSA after incubation for 2 weeks. The pH range for growth (pH 3.0–11.0, at intervals of 1 pH unit) was tested in trypticase soy broth (TSB). The buffer systems were described by Cao et al. (2020) and Zhao et al. (2019). NaCl tolerance was determined in TSB medium supplemented with various concentrations of NaCl (0–15.0%, w/v, in 0.5% increments) incubation in shake flasks (250 rpm) at 28 °C for 2 weeks. Hydrolysis of Tweens (20, 40 and 80) and production of urease were tested as described by Smibert and Krieg (1994). The utilization of sole carbon and nitrogen sources, decomposition of cellulose, hydrolysis of starch and aesculin, reduction of nitrate, coagulation of milk, liquefaction of gelatin and production of H_2S were examined as described previously (Gordon et al. 1974; Yokota et al. 1993). The ability of lignin degradation was determined by the azure B plate decolorization method (Xu et al. 2015).

Chemotaxonomic characteristics

Biomass for chemotaxonomic studies was obtained by growing the organisms in TSB medium in shake flasks at 28 ºC for 7 days. Biomass was harvested by centrifugation at 8000 rpm, washed with distilled water twice and then freeze-dried. The whole-cell sugars were tested according to a procedure developed by Lechevalier and Lechevalier (1980). Polar lipids were extracted and examined by two-dimensional TLC and identified using the method of Minnikin et al. (1984). Menaquinones were extracted from freeze-dried biomass and purified according to Collins (1985) and analyzed by a HPLC-UV method (Wu et al. 1989) using an Agilent Extend-C18 Column (150 × 4.6 mm, i.d. 5 μm), monitored at 270 nm. The mobile phase was acetonitrile/propyl alcohol (60:40, v/v). Cellular fatty acids were extracted from the biomass as described by Gao et al. (2014) and analyzed by GC-MS using the method of Xiang et al. (2011).

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

Extraction of chromosomal DNA and PCR amplification of the 16S rRNA gene sequence were performed using procedures described by Kim et al. (2000). 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-YY56^T (1,513 bp) was multiply aligned in Molecular Evolutionary Genetics Analysis (MEGA) using the Clustal W algorithm and trimmed manually where necessary. Phylogenetic trees were generated with the neighbour-joining (Saitou and Nei 1987) and maximum-likelihood (Felsenstein 1981) algorithms using the software packages MEGA version X (Kumar et al. 2018). The stability of the topology of the phylogenetic tree was evaluated by using the bootstrap method of Felsenstein (1985) with 1000 replicates. The distance matrix was generated using Kimura's two-parameter model (Kimura 1980). All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). Pairwise alignment analysis of 16S rRNA gene sequence similarities between strains was calculated on the EzBioCloud server (https://www.ezbiocloud.net) (Yoon et al. 2017a).

Genomic analysis, determination of housekeeping genes sequences, DNA-DNA hybridization and DNA G + C content

For draft genome sequencing and assembly, the genomic DNA of strain NEAU-YY56^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 version 2.04 (Li et al. 2008, 2010) (http://soap.genomics.org.cn/soapdenovo.html) into several scaffolds. The draft genome of strain NEAU-YY56^T was deposited in GenBank under the accession number RFFI00000000. Phylogenetic relationship of strain NEAU-YY56^T was confirmed using partial sequences of housekeeping genes including *gyr*B, *rpo*B, *rec*A, *rel*A and *atp*D obtained from the draft genome sequences. The sequences of each locus were aligned using MEGA software version X and trimmed manually at the same position before being used for further analysis. Phylogenetic analysis was performed as described above.

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 ANI values and dDDH levels were determined between the genomes of strain NEAU-YY56^T and *C. pakistanensis* JCM 18755^T (BONO00000000), *C. denverensis* W6929^T (BONL00000000) and *C. hominis* JCM 12133^T (JAHLPH00000000) online at www.ezbiocloud.net/tools/ani using the ChunLab's online ANI Calculator (Yoon et al. 2017b) and at

http://ggdc.dsmz.de using the Genome-to-Genome Distance Calculation (GGDC 3.0) (Meier-Kolthoff et al. 2022), respectively.

Results And Discussion

Phenotypic characteristics

The morphological characteristics of strain NEAU-YY56^T showed that the strain had the typical characteristics of the genus *Cellulomonas*. The cells of NEAU-YY56^T were found to be Gram-positive, motile and rod-shaped about 1.6-1.7 μm in length and 0.3-0.4 μm in width (Fig. 1). Colonies are smooth, circular and pale yellow on TSA medium. Strain NEAU-YY56^T was observed to grow moderately on TSA, ISP 2 and ISP 3 agar media and poorly on ISP 4, ISP 5, ISP 6 and ISP 7 agar media. No diffusible or melanoid pigments were observed on the tested media. The colonies were in yellow-color-series, cultural characteristics of the isolate are shown in Table S1. The strain was found to grow at a temperature range of 18-40 ^oC (optimum temperature 28 ^oC), pH 6.0-8.0 (optimum pH 7.0) and tolerated 0-3.0 % NaCl (optimum NaCl of 0 %). A decolorizing circle was observed around the colony of strain NEAU-YY56^T on azure B plate, indicating that the strain had lignin degradation ability with a degradation diameter of 29.2 mm (Fig. S1). Certain data of physiological and biochemical characteristics could clearly distinguish strain NEAU-YY56^T from its closely related strains, *C. pakistanensis* JCM 18755^T, *C. denverensis* W6929^T and *C. hominis* JCM 12133^T (Table 1). For instance, strain NEAU-YY56^T could not grow at pH 9.0, while the closely related strains could. The tolerance to NaCl of strain NEAU-YY56^T was up to 3.0 %, which was lower than the closely related strains. Other phenotypic differences include the decomposition of aesculin, hydrolysis of starch, reduction of nitrate and patterns of carbon and nitrogen utilization. Detailed physiological and biochemical characteristics are presented in the species description.

Chemotaxonomic characterization

Strain NEAU-YY56^T was found to exhibit a range of chemotaxonomic properties that are consistent with the description of the genus *Cellulomonas.* Whole-cell sugars contained glucose, rhamnose and ribose. The polar lipids consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol mannoside and two unknown glycolipids (Fig. S2). The predominant menaquinones were MK-9(H_4) (56.1 %) and MK-9(H_2) (43.9 %). The cellular fatty acids of strain NEAU-YY56^T were *anteiso*-$C_{15:0}$ (37.1 %), $C_{16:0}$ (28.3 %), $C_{14:0}$ (15.4 %), *anteiso*-$C_{17:0}$ (9.4 %), *iso*-$C_{16:0}$ (4.0 %), $C_{18:0}$ (3.2 %), $C_{17:0}$ (1.2 %), $C_{18:1}$ω9c (0.9 %), $C_{16:1}$ω9c (0.4 %) and $C_{19:0}$ (0.1 %). The DNA G+C content of strain NEAU-YY56^T was 74.7 %. All these chemotaxonomic data showed that strain NEAU-YY56^T should be assigned to the genus *Cellulomonas*.

Molecular characteristics

Identification using the EzBioCloud server revealed that strain NEAU-YY56^T belongs to the genus *Cellulomonas* and shared high sequence similarities with *C. pakistanensis* JCM 18755^T (98.7 %), *C. denverensis* W6929^T (98.2 %) and *C. hominis* JCM 12133^T (98.1 %). In addition, the phylogenetic trees based on 16S rRNA gene sequences (1,446 bp) reconstructed using the neighbour-joining (Fig. 2) and maximum-likelihood (Fig. S3) algorithms showed that strain NEAU-YY56^T formed a cluster with *C. pakistanensis* JCM 18755^T, *C. denverensis* W6929^T and *C. hominis* JCM 12133^T, which was further confirmed by the phylogenetic tree based on the concatenated sequences (3,594 bp) of the partial sequences of housekeeping genes including *gyr*B, *rpo*B, *rec*A, *rel*A and *atp*D reconstructed by the neighbour-joining (Fig. 3) and maximum-likelihood (Fig. S4) algorithms. Thus, based on these phylogenetic relationships and 16S rRNA sequences similarities, the isolate was grouped with members of the genus *Cellulomonas*.

The genome sequence of the assembled strain NEAU-YY56^T was 4,391,169 bp and has a DNA G+C content of 74.7 %. Also, it was made up of 280 scaffolds with an N50 of 30,351 bp. The genome sequence was deposited in the GenBank/EMBL/DDBJ with the accession number RFFI00000000. And NCBI Prokaryotic Genome Annotation Pipeline (PGAP) showed that there were three copies of 5S rRNA genes, four copies of 16S rRNA genes, one copy of 23S rRNA gene, 48 tRNA genes, three copies of noncoding RNA genes. More specific genomic information and obvious features are presented in Table 2. AntiSMASH 6.0 was used genome mining analysis led to the identification of 9 putative gene clusters responsible for the production of diverse secondary metabolites. Of which 8 putative gene clusters displayed low similarity to the known gene clusters of tylactone, terpene and so on. And one putative gene cluster was 100% similar to alkylresorcinol. Of the genome of 4,174 genes, 3,294 genes were annotated and classified into known functions according to the KEGG database. By analyzing the genome of the strain NEAU-YY56^T, there were 855 genes involved in metabolism-associated pathways. Genome analysis of the COG database revealed that strain NEAU-YY56^T contained 272 amino acid transport and metabolism genes, 413 carbohydrate transport and metabolism genes, 335 transcription genes, 222 inorganic ion transport and metabolism genes and 299 general function prediction only genes. Moreover, the genomic analysis of the COG database showed that strain NEAU-YY56^T contained a gene encoding laccase (GM002387) and a gene encoding catalase (GM003718), both of which are key enzymes in lignin degradation (Blánquez et al. 2017; Zhang 2006). Therefore, the genomic analysis also confirmed that strain NEAU-YY56^T could decompose lignin.

ANI value and dDDH level were employed to determine whether the strain could be considered to represent a new species. The ANI values between strain NEAU-YY56^T and its reference strains, *C. pakistanensis* JCM 18755^T, *C. denverensis* W6929^T and *C. hominis* JCM 12133^T, were 83.5 %, 79.0 % and 83.1 %, respectively, which were lower than the 95 %-96 % threshold defined by prokaryotic species (Richter and Rossello-Mora 2009; Chun and Rainey 2014). Similarly, the low dDDH levels between strain NEAU-YY56^T and *C. pakistanensis* JCM 18755^T (26.8 ± 2.4 %), *C. denverensis* W6929^T (21.8 ± 2.3 %) and *C. hominis* JCM 12133^T (26.2 ± 2.4 %) were also well below the threshold (70 %) recommended by

Wayne et al. (1987) for assigning strains to the same genomic species. These results supported the conclusion that strain NEAU-YY56^T represents a novel species of the genus *Cellulomonas*.

Therefore, based on the distinct morphological, physiological and biochemical, chemotaxonomic and phylogenetic and DNA-DNA relatedness data mentioned above, strain NEAU-YY56^T represents a novel species of the genus *Cellulomonas*, for which the name *Cellulomonas triticagri* sp. nov. is proposed.

Description of *Cellulomonas triticagri* sp. nov.

Cellulomonas triticagri (tri.tic.a'gri. L. neut. n. *triticum* wheat; L. masc. n. *ager* field; N.L. gen. n. *triticagri* of a wheat field)

Short rods, about 1.6-1.7 μm in length and 0.3-0.4 μm in width. Gram-stain positive, aerobic and motile actinomycete that produces pale yellow and smooth mycelium after 2 weeks at 28 °C on TSA medium. Moderate growth is observed on TSA, ISP 2 and ISP 3 agar media; poor growth on ISP 4, ISP 5, ISP 6 and ISP 7 agar media. Colonies are in yellow-color-series. No diffusible or melanoid pigments are observed on the tested media. Grows at 18-40 ℃ (optimum 28 ℃), at pH 6.0-8.0 (optimum, pH 7.0) and with 0-3.0 % (w/v, optimum 0 %) NaCl. Hydrolysis starch and Tweens (20, 40 and 80), but not aesculin. Catalase is produced, but H_2S and urease are not. Besides that, negative for decomposition of cellulose, peptonization and coagulation of milk, reduction of nitrate and liquefaction of gelatin. D-Fructose, *meso*-inositol, lactose, D-maltose, D-mannose, L-rhamnose and D-sorbitol are utilized as sole carbon sources, but not L-arabinose, dulcitol, D-galactose, D-glucose, D-mannitol, D-raffinose, D-ribose, D-sucrose or D-xylose. L-Arginine, L-glutamic acid, L-glutamine and L-tyrosine are utilized as sole nitrogen sources, but not L-alanine, L-asparagine, L-aspartic acid, creatine, glycine, L-proline, L-serine or L-threonine. Whole-cell sugars contain glucose, rhamnose and ribose. The polar lipid profile consists of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol mannoside and two unknown glycolipids. The predominant menaquinones are MK-9(H_4) and MK-9(H_2). The predominant cellular fatty acids (> 5.0 %) are *anteiso*-$C_{15:0}$, $C_{16:0}$, $C_{14:0}$ and *anteiso*-$C_{17:0}$. The G+C content of the DNA of the type strain is 74.7 %.

The type strain is NEAU-YY56^T (= DSM 106717^T = JCM 32550^T), was isolated from the rhizosphere soil of wheat (*Triticum aestivum* L.) collected from Zhumadian, Henan Province, Central China. The GenBank accession numbers for the 16S rRNA gene sequence and the draft genome sequence of the type strain are MH144588 and RFFI00000000, respectively.

Abbreviations

ANI, average nucleotide identity; *atp*D, ATP synthase F1, beta subunit; dDDH, digital DNA:DNA hybridization; DPA, dulcitol-proline agar; DPG, diphosphatidylglycerol; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen; GC-MS, Gas Chromatography-Mass Spectrometer; GL, unknown glycolipid; GO, Gene Ontology; *gyr*B, DNA gyrase subunit B; ISCC-NBS, Inter-society color council-national

bureau of standards; ISP, International *Streptomyces* Project; ISP 2, yeast extract-malt extract agar; ISP 3, oatmeal agar; ISP 4, inorganic salts-starch agar; ISP 5, glycerol-asparagine agar; ISP 6, peptone-yeast extract iron agar; ISP 7, tyrosine agar; JCM, Japan Collection of Microorganisms; KEGG, Kyoto Encyclopedia of Genes and Genomes; MEGA, Molecular Evolutionary Genetics Analysis; MLSA, multilocus sequence analysis; PE, phosphatidylethanolamine; PGAP, Prokaryotic Genome Annotation Pipeline; PIM, phosphatidylinositol mannoside; *rec*A, recombinase A; *rel*A, ppGpp synthetase; *rpo*B, RNA polymerase, beta subunit; TSA, trypticase soy agar; TSB, trypticase soy both.

Declarations

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Author Contributions

Chuanyu Han and Yuting Zhang performed the laboratory experiments, analyzed the data, and drafted the manuscript. Bing Yu contributed to the biochemical characterization. Qiqi Shan and Yuanyuan Tian contributed to the morphological analyzes. Haoran Shi contributed to the fatty acids determination. Yan Zhang contributed to the Genomic analysis. Junwei Zhao participated to the discussions of each section of experiments. Wensheng Xiang and Chunyu Zhu designed the experiments and revised the manuscript.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical standards

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.

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Tables

Table1. Differential characteristics between strain NEAU-YY56^T and the closest related *Cellulomonas* species.

Characteristics	1	2	3	4
Growth temperature range (°C)	18-40	10-37	35-45	15-40
Growth pH	6.0-8.0	6.0-9.0	5.0-10.0	5.0-9.0
NaCl Tolerance (%, w/v)	3.0	12.0	11.0	9.0
Aesculin decomposition	–	+	+	+
Nitrate reduction	–	+	+	+
Starch hydrolysis	+	–	–	–
Utilize as sole carbon source:				
L-Arabinose	–	–	–	+
D-Fructose	+	+	+	–
D-Galactose	–	+	+	–
D-Glucose	–	–	+	–
D-Mannitol	–	+	–	–
D-Sucrose	–	+	+	+
Utilize as sole nitrogen source:				
L-Aspartic acid	–	+	+	+
L-Glutamic acid	+	–	+	–
Glycine	–	+	+	–

Strains: 1, NEAU-YY56^T; 2, *C. pakistanensis* JCM 18755^T; 3, *C. denverensis* W6929^T; 4, *C. hominis* JCM 12133^T. Abbreviation: +, positive; –, negative.

Table 2. Genome sequence features of strain NEAU-YY56^T and the closest related *Cellulomonas* species.

Features	1	2	3	4
Accession No.	RFFI00000000	BONO00000000	BONL00000000	JAHLPH00000000
BioProject	PRJNA498149	PRJDB9993	PRJDB9957	PRJNA733006
Sequencing Technology	Illumina HiSeq	Illumina HiSeq 1000	Illumina HiSeq 1000	Illumina
Assembly method	SOAP denovo v. 2.04	newbler v. 3.0	newbler v. 3.0	SPAdes v. 3.9.0
Genome coverage	306x	141x	143x	99x
Contigs	282	57	54	14
Genome size (bp)	4,391,169	4,003,496	3,997,997	4,117,008
DNA G+C content (%)	74.7	75.8	72.9	75.4
Genes (coding)	4,020	3,679	3,677	3,731
tRNAs	48	47	45	46
rRNA	8	3	3	3
ncRNA	3	3	3	3

Strains: 1, NEAU-YY56^T; 2, *C. pakistanensis* JCM 18755^T; 3, *C. denverensis* W6929^T; 4, *C. hominis* JCM 12133^T.

Figures

Figure 1

Scanning electron micrograph of strain NEAU-YY56^T grown on TSA medium at 28 ^oC for 2 weeks.

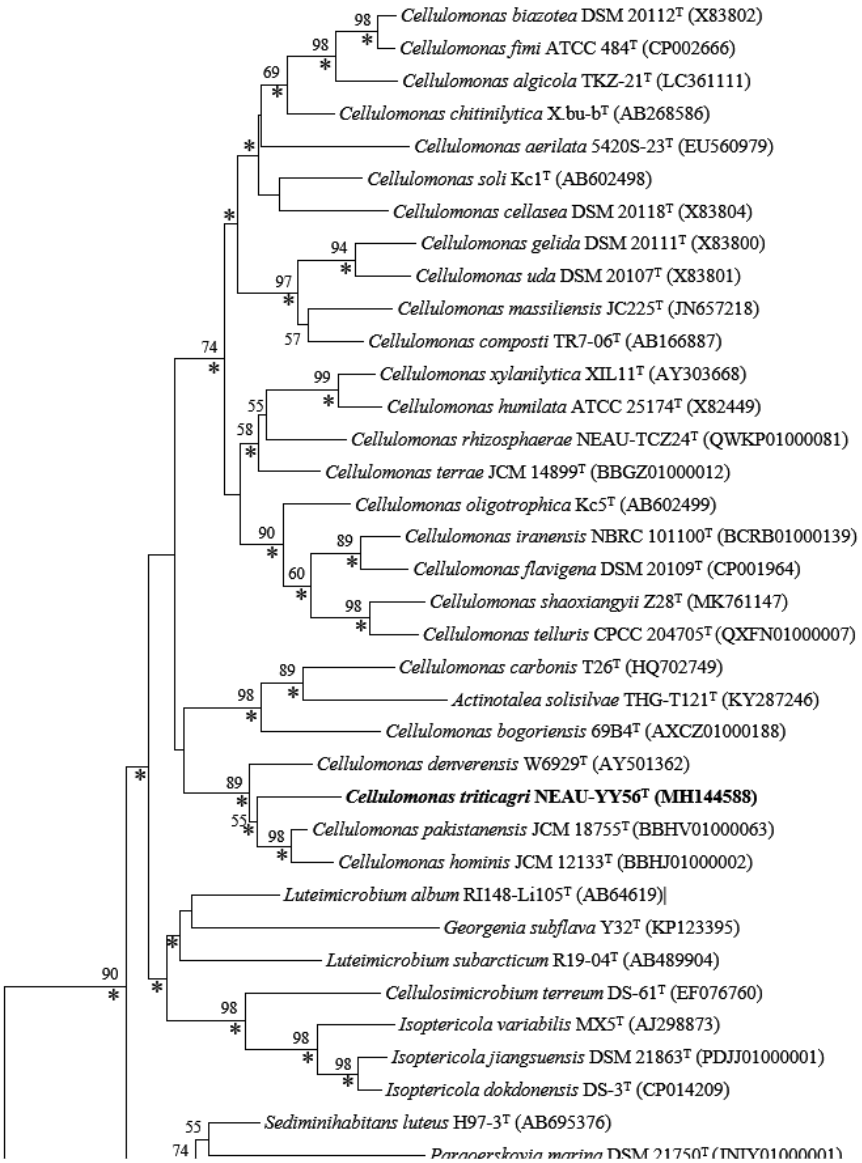

Figure 2

Neighbour-joining tree based on 16S rRNA gene sequence (1446) showing relationship between strain NEAU-YY56^T (in bold) and its near neighbours based on 16S rRNA gene sequence. The out-group used was *Kitasatospora setae* DSM 43861^T. 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.

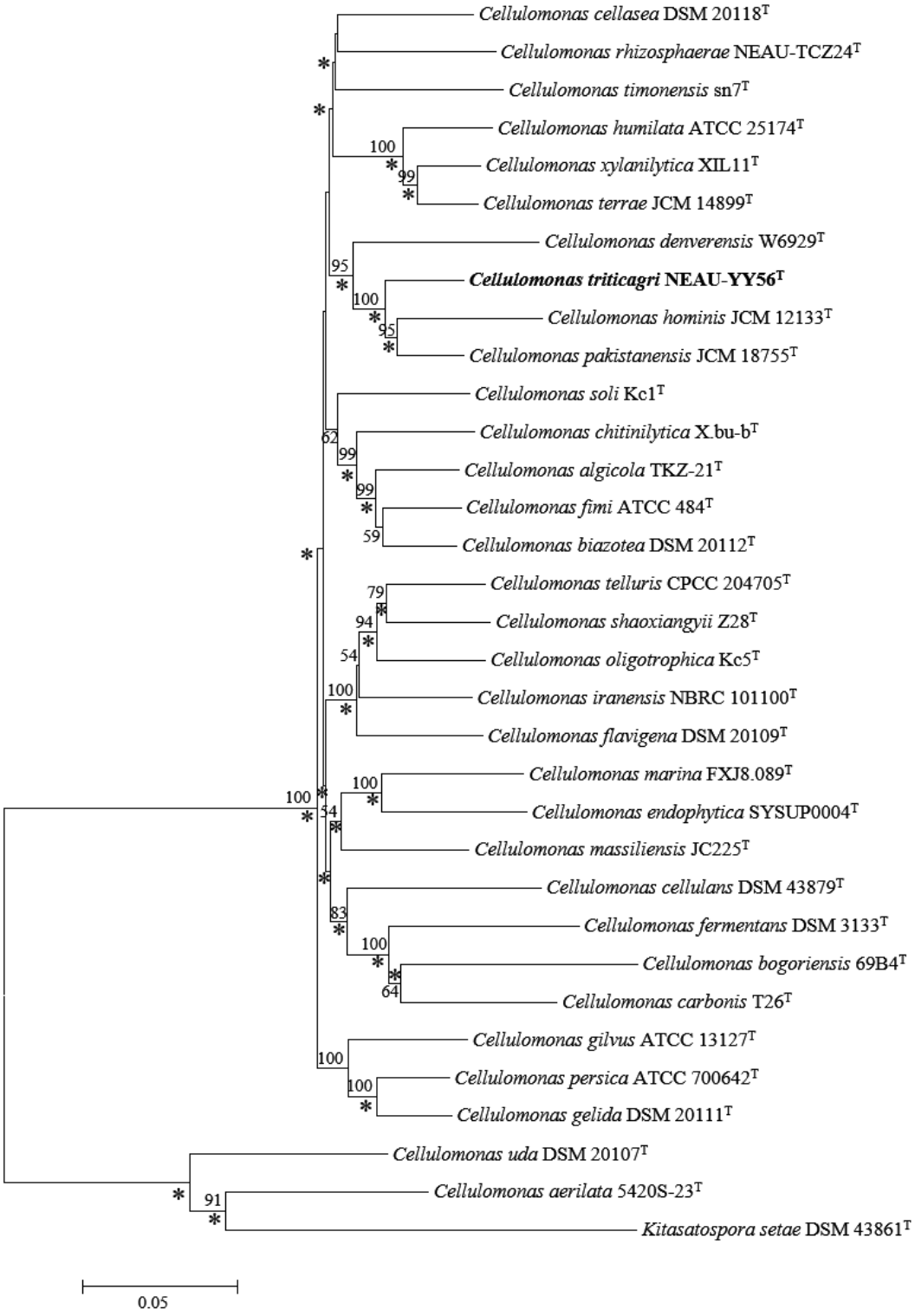

Figure 3

Neighbour-joining tree based on MLSA analysis of the concatenated partial sequences from five housekeeping genes (*gyr*B, *rpo*B, *rec*A, *rel*A and *atp*D) of strain NEAU-YY56^T (in bold) and related taxa. Only bootstrap values above 50 % (percentages of 1000 replications) are indicated. *Kitasatospora setae* DSM 43861^T was used as an out-group. Asterisks indicate branches also recovered in the maximum-likelihood tree; Bar, 0.05 nucleotide substitutions per site.

Supplementary Files

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- SupplementaryMaterial.doc