Williamsia soli sp. nov., Isolated from Thermal Power Plant in Yantai

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Research Article

Keywords: Aerobic, Genomic Analysis, Predominant fatty acid, Soil, Thermal power plant, Williamsia soli sp. nov

Posted Date: June 10th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-594776/v1>

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1 ***Williamsia soli* sp. nov., isolated from thermal power plant in Yantai**

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6 **Abstract**

7 Strain C17^T, a novel strain belonging to the phylum *Actinobacteria*, was isolated from
8 thermal power plant in Yantai, Shandong Province, China. Cells of strain C17^T were
9 Gram-stain-positive, aerobic, pink, non-motile and round with neat edges. Strain C17^T
10 was able to grow at 4–42 °C (optimum 28 °C), pH 5.5–9.5 (optimum 7.5) and with
11 0.0–5.0% NaCl (optimum 1.0%, w/v). Phylogenetically, the strain was a member of
12 the family *Gordoniaceae*, order *Mycobacteriales*, class *Actinobacteria*. Phylogenetic
13 analysis based on 16S rRNA gene sequence comparisons revealed that the closest
14 relative was the type strain of *Williamsia faeni* JCM 17784^T with pair-wise sequence
15 similarity of 98.4%. According to the genome, the DNA G + C content obtained from
16 the draft genome sequence was 64.7%, the main resistant antibiotics were rifamycin,
17 aminoglycoside and glycopeptide, the related gene cluster were *clbC*, *clbB*, *clbA*,
18 *vanRI*, *vanRB*, *vanRC* and *vanRD*. The average amino acid identity (AAI), average
19 nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values
20 between genome sequences of strain C17^T and the type strain *Williamsia faeni* of the
21 recognized taxa compared were 77.5, 77.9 and 20.7%, respectively. Predominant fatty
22 acids were C_{16:0} (31.7%), C_{18:1ω9c} (26.8%), 10-methyl-C_{18:0} (16.4%), summed feature

comprising C_{16:1}ω7c and/or C_{16:1}ω6c (12.2%) and sum in feature comprising C_{16:1}ω6c and/or C_{16:1}ω7c (10.9%). The major isoprenoid quinone was MK-9 and the diagnostic phospholipids were phosphatidylethanolamine (PE), diphos-phatidylglycerol (DPG) and phosphatidylinositol (PI). Therefore, the combined phenotypic, chemotaxonomic and phylogenetic data indicated that the strain is considered to represent a novel species of the genus *Williamsia* and the name *Williamsia soli* sp. nov. is proposed for strain C17^T (= KCTC 49567^T= MCCC 1K04355^T).

Keywords: Aerobic; Genomic Analysis; Predominant fatty acid; Soil; Thermal power plant; *Williamsia soli* sp. nov.

Abbreviations:

AAI, Average Amino Acid identity

ANI, Average Nucleotide Identity

cAAI, Core-gene average amino acid identity

CARD, Comprehensive Antibiotic Research Database

COG, Cluster of Orthologous Groups

dDDH, digital DNA-DNA Hybridization

DPG, Diphos-phatidylglycerol

GGD, Genome-to-Genome Distance Calculator

HPLC, High Performance Liquid Chromatography

KCTC, Korean Collection for Type Cultures

KEGG, Kyoto Encyclopedia of Genes and Genomes

LB, Luria-Bertani

45 MCCC, Marine Culture Collection of China
46 MEGA, Molecular Evolutionary Genetics Analysis
47 NCBI, National Center of Biotechnology Information
48 PE, Phosphatidylethanolamine
49 PI, Phosphatidylinositol

50 **Introduction**

51 The genus *Williamsia* pertained to the family *Gordoniaceae*, was proposed by
52 Kämpfer P (Kämpfer P, 1999) and *Williamsia muralis* was described as the type
53 species. The genus *Williamsia* encompassed 13 species with validly published names
54 (<http://www.bacterio.net/williamsia.html>). The members of the genus were widely
55 distributed in various environments, such as indoor building materials (Kämpfer P,
56 1999), deep-sea sediment (Stach et al., 2004; Yassin et al., 2007; Pathom-Aree et al.,
57 2006), human blood (Yassin and Hupfer, 2006), soil (Yassin et al., 2007), hay
58 meadow (Jones et al., 2010), leaf surface (Kämpfer et al., 2011; Kämpfer et al., 2016;
59 Fang et al., 2013), lake sediment (Sazak and Sahin, 2012), associated with marine
60 organisms (Afonso de Menezes et al., 2017), and marine sponge (De Menezes et al.
61 2019). Generally, they shared common characterisation biochemically. The members
62 of the genus were aerobic, Gram-stain positive, non-motile bacteria. Their fatty acids
63 contained complex mixtures of isobranched and straight chain saturated, and
64 unsaturated components. The major isoprenoid quinone was MK-9. The diagnostic
65 phospholipids were phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG)
66 and phosphatidylinositol (PI). The DNA G + C content ranging between 63.9 mol%

and 71.3 mol%.

In this study, a novel strain C17^T was obtained from thermal power plant in Yantai, Shandong, China, which showed most of features as above. Furthermore, the taxonomic position of *Actinobacterium*, strain C17^T was determined by using a polyphasic taxonomic approach, including morphological, physiological, chemotaxonomic, phylogenetic and genomic characteristics.

Materials and methods

Isolation of the novel strain

Strain C17^T was isolated from soil at thermal power plant in Yantai, Shandong Province, China (sampling location: 120°35'44.51" E, 37°45'28.46" N) on the 1st of April 2019. The isolation of *Williamsia* was performed using Luria-Bertani (LB, Giuseppe Bertani) medium (tryptone 10.0 g, yeast powder leaching 5.0 g, NaCl 10.0 g, pure water 1000 ml, agar 20.0 g, pH 7.3) and incubated at 28 °C. Incubating at 28 °C for 3 days resulted in pink colonies designated as strain C17^T. The strain was later deposited at the Korean Collection for Type Cultures (KCTC) and the China Marine Culture Collection (MCCC). Reference strains *Williamsia faeni* JCM 17784^T and *Williamsia limnetica* KCTC 19981^T were purchased from their respective collection organizations. All strains were cultured under comparable conditions for physiological and chemotaxonomic characterization, unless otherwise indicated. They were preserved at –80 °C in LB liquid medium for longer preservation.

Morphological, physiological and biochemical characterization

Gram staining was performed as described earlier (Smibert and Krieg, 1994). Following incubation on LB at 28 °C for 3 days, cell morphology and flagella were observed using an electron microscope (Jem-1200; JEOL) and gliding motility was observed through oil-immersion phase-contrast microscopy (AX70; Olympus) according to the method by Bowman (Bowman, 2000). In order to consider the growth under anaerobic conditions, the strain was cultured on LB with or without 0.1% (w/v) NaNO₃ at 30 °C for 2 weeks under anaerobic conditions. Bacterial growth was monitored at different temperatures (4, 10, 15, 20, 25, 28, 30, 37, 40, and 42 °C) by growing in the LB medium. The pH range for growth was tested at pH 5.5–9.5 in LB liquid medium and measured using a buffer system as described by Yang (Yang and Cho 2008). The tolerance to salt was examined on the basal medium with different NaCl concentrations (0.0–7.0%, w/v, at intervals of 1.0%) at 28 °C. Activities of catalase and oxidase, cellulose hydrolysis, agar and casein were tested using the Tindall method (Tindall et al., 2014). Other physiological and biochemical properties were performed using the API 20E, API 20NE, and API ZYM kit (bioMérieux) according to the manufacturer's recommendations. The oxidation and fermentation of carbohydrates were determined after growth on LB at 28 °C for 2 days using the Biolog GEN III Micro Plates and API 50CHB Fermentation Kit (bioMérieux). Antibiotic sensitivity was assessed on LB plates with discs (Tianhe) containing various antibiotics for 3 days at 28 °C.

Phylogenetic analysis and genome assembly and annotation

The 16S rRNA sequence of strain C17^T was determined by PCR using bacterial

universal primers, and the PCR product was purified using a PCR purification kit (Sangon Biotech, China) (Kim et al., 2014). The resulting array was assembled using Bioedit (Hall, 1999). This step was carried out to ensure that the isolated strain selected for further characterisation represent members of the phylum *Actinobacteria*, compared with matching sequences of closely related strains extracted from the EzBioCloud 16S database and National Center of Biotechnology Information (NCBI). (Yoon et al., 2017a). Based on all the genus-type strains with 16S rRNA sequence similarity of 95.0% above, multiple alignments of their sequences were performed using Clustal_X version 1.83 with default settings (Thompson et al., 1997).

Phylogenetic trees were inferred using three tree-making algorithms, maximum-likelihood (Felsenstein, 1981), maximum-parsimony (FITCH, 1971) and neighbor-joining (Saitou and Nei, 1987). The topologies of the evolutionary trees were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 replicates. All phylogenetic and molecular evolutionary studies cited above were conducted using MEGA version 7 (Kumar et al., 2016).

The draft genome sequence of strain C17^T was determined using paired-end sequencing method coupled to the Illumina HiSeq-PE150 platform. Assembly of raw reads was performed using the SOAP, Spades, and Abyss software. Annotation for the genomic sequence was processed using GeneMarkS, rRNAmmer and Rfam software. These operations were all implemented by Beijing Novogene Bioinformatics Technology Co, Ltd. (Beijing, China). The functional annotation of the sequences was done using Blast compared to the non-redundant GenBank

database. The genes involved in metabolic pathways were analysed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) databases (Kanehisa et al., 2016). In order to assess the antibiotic gene cluster of the strain C17^T, comprehensive Antibiotic Research Database (CARD) database was used to provide the names of genes associated with drug resistance, the type of antibiotic tolerated and integrate sequences, antibiotic resistance and mechanisms of action.

The assembly was also uploaded to RAST (Rapid Annotation using Subsystem Technology) server (<https://rast.nmpdr.org/>) (Aziz et al., 2008) and antiSMASH server (<https://antismash.secondarymetabolites.org/>) (Medema et al., 2011; Blin et al., 2019) for metabolic reconstruction analysis and prediction of secondary metabolite gene clusters, respectively.

The average amino acid identity (AAI) (Konstantinidis and Tiedje, 2005; Rodriguez-R and Konstantinidis, 2014b) calculator was used to determine the genomic similarities between strain C17^T and neighbor genera (<http://en-veomics.ce.gatech.edu/aai/>). The average nucleotide identity (ANI) values and Digital DNA–DNA hybridization (dDDH) values were used to determine the genome-based similarities. These were calculated by using the EzBioCloud integrated database (Yoon et al., 2017a), OrthoANIu algorithm of the EzGenome web service (Yoon et al., 2017b) and the Genome-to-Genome Distance Calculator (GGDC) version (Meier-Kolthoff et al., 2013).

Chemotaxonomic characterization

Standard procedures were used to determine the menaquinone and polar lipids profiles (Minnikin et al., 1984) of strain C17^T. For the fatty acid analysis, cells were harvested from LB which was incubated at 28 °C for 3 days. Fatty acids extracted from the isolate strain C17^T were methylated, separated by gas chromatography (Agilent 6850 GC) and analysed using the standard Sherlock microbial identification (MIDI) system, ACTINO version 5 (Sasser, 1990).

Results and discussion

Phylogenetic inference

Based on an almost complete 16S rRNA genes sequence (1360 bp) of strain C17^T, phylogenetic analysis shown the isolate belongs to genus *Williamsia* (Fig. 1). Strain C17^T shared its highest 16S rRNA similarity with the type strain of *W. faeni* namely (98.4%), followed by *W. limnetica* (98.4%), *W. muralis* (97.7%), *W. marianensis* (97.6%) among species with validly published names. The isolate strain C17^T formed a new phyletic line closely associated with the type strain of *W. limnetica* L1505^T and the result was supported by all tree-making algorithm and by a high bootstrap value (100%). In the neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, strain C17^T formed a separate branch from *Absiella argi* (Fig.1). The phylogenetic analysis was also recovered with the maximum-likelihood tree-making algorithms and displayed the same tree topologies. (Fig. S1).

Morphological and physiological analyses

Cells were Gram-stain-positive, aerobic, non-motile, non-gliding, lacking flagella,

and rod-shaped. A round pink colony with a diameter of 2 µm (Fig. S2) was obtained after incubating on LB for 2–3 days at 28 °C. The strain was catalase-positive and oxidase-negative. Growth was observed at 4–42 °C (optimum 28 °C), pH 5.0–9.5 (optimum 7.5). It could grow at 4 °C, which was different from related strains. The NaCl concentrations for growth were 0.0–5.0% (optimum 1.0%). NaCl was essential for growth, which also distinguished strain C17^T from the most closely related strains, *W. faeni* JCM 17784^T and *W. limnetica* KCTC 19981^T. It was sensitive to ampicillin, penicillin and chloramphenicol. Other phenotypic characteristics of the strain C17^T and related strains were shown in Table 1.

Chemotaxonomic characterization

The main fatty acids of strain C17^T were iso-C_{16:0} (31.7%), C_{18:1}ω₉c (26.8%), 10-methyl-C_{18:0} (16.4%), summed feature 3 (C_{16:1}ω₇c and/or C_{16:1}ω₆c 12.2%) and sum in feature 3 (C_{16:1}ω₆c and/or C_{16:1}ω₇c 10.9%), which were consistent with the reference strains, and other fatty acids that made up less than 10% could distinguish strain C17^T from reference strains. Detailed fatty acid profiles of the strain C17^T and related strains were shown in Table 2. The menaquinone of strain C17^T was MK-9, which was consistent with other members of the genus *Williamsia*. Polar lipid analysis showed that the phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylinositol as the characteristic phospholipids (Fig. S3).

Genomic characteristics

General features of strain C17^T genome were summarised in Table S1. The genome of strain C17^T was comprised of 5701 genes and 25 contigs with a total length of

6072852 bp. The mean coverage was 260 ×. The N50 value was 506168 bp and N90 value was 151305 bp. There were 5S rRNAs of 4, 16S rRNA of 1, 23S rRNAs of 1, tRNAs of 46 and sRNAs of 2. According to the genome sequence, the DNA G+C content was 64.7%, which belonged to the range of family *Gordoniaceae* (above 50 mol%) (Bernardet J-F et al. 2012) and the membership to the genus *Williamsia*.

In the gene annotation results, the predicted gene length distribution map showed the gene length distribution. The abscissa was the interval of different gene length distribution, and the ordinate was the number of genes whose length fell in this region (Fig. S4). There were 2468 genes annotated by KEGG pathway annotation and 256 genes related to amino acid metabolism predicted. A large number of genes were found to be involved in global and overview maps (717). Based on the result of the gene cluster prediction derived from the ‘antiSMASH’ database, strain C17^T contained genes which shared similarity with four known pathways at 40% or higher; non-ribosomal peptide synthetase cluster (NRPS), T3pks-terpene, ectoine, bacteriocin and terpene (Table S2). As shown in table S3, the main classes of antibiotics tolerated were tetracycline, rifamycin, aminoglycoside and glycopeptide. Based on the genome sequence annotation, the genome contained several genes coding for Glycoside Hydrolases (GHs), Glycosyl transferases (GTs) and Carbohydrate esterases (CEs), such as cellulase (CE1, GT2 and GH23). Based on the sequence annotation, the genome contained several genes coding for amino acid transport and metabolism, translation, ribosomal structure and biogenesis, inorganic ion transport and metabolism and several functions unknown, which participated in several aspects of

cell life activities. The results of the COG annotation of strain C17^T were showed in Fig. S5. Compared with reference strains, genes code of general function prediction only and extracellular structures were characteristic of the strain C17^T. ANI, dDDH and AAI values between strain C17^T and neighbor type strains were shown in Table S4. The mean levels of dDDH between isolate of strain C17^T and the type strains of *W. faeni* and *W. limnetica* were 63.9% and 64.5%, respectively, which were below 70% cutoff point recommended for the assignment of prokaryotic strains to the same genomic species (Wayne et al. 1987). The AAI values between strain C17^T and the related type strains *W. faeni* and *W. limnetica* were 77.9% and 88.9%. Commonly, it was suggested that AAI values of 90% and 60% were probably the threshold value for species and genus, respectively (Rodriguez-R and Konstantinidis, 2014a). The ANI valued between strain C17^T and the related type strains *W. faeni* and *W. limnetica* were 77.5% and 86.1%, respectively, which were below the standard ANI criteria identity (95.0-96.0%) (Kim et al., 2014). Strain C17^T shared dDDH of 20.7% and 30.7% with *W. faeni* and *W. limnetica*, respectively, which were below the 70% thresholds for species delineation (Meier-Kolthoff et al., 2013). A complete 16S rRNA gene sequence (1522 bp) obtained from the genome sequence was 99.9% similar with the clone sequence (1360 bp). Based on the genome sequence analysis, strain C17^T should be located within the genus *Williamsia* as a novel species.

DESCRIPTION OF *WILLIAMSLIA SOLI* SP. NOV

Williamsia soli (so'li. L. neut. gen. n. soli of soil, referring to the isolation of the type strain from a soil sample).

In addition to the features assigned by the genus, the following features are display. Cells are 2 μ m on LB at 28 °C for 2 days. Growth is observed at 4–42 °C (optimum 28 °C) at pH 5.0–9.5 (optimum 7.5), with 0.0–5.0% NaCl (optimum 1.0%). Major fatty acids are iso-C_{16:0} (31.7%), C_{18:1} ω 9*c* (26.8%), 10-methyl-C_{18:0} (16.4%), summed feature 3 (C_{16:1} ω 7*c* and/or C_{16:1} ω 6*c* 12.2%) and sum in feature 3 (C_{16:1} ω 6*c* and/or C_{16:1} ω 7*c* 10.9%). The major menaquinone is MK-9. Phosphatidylethanolamine, diphos-phatidylglycerol and phosphatidylinositol are the major polar lipids. The type strain, C17^T = (KCTC 49567^T=MCCC 1K04355^T) was isolated from thermal power plant at Yantai, Shandong Province, China. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain C17^T is MW762712 and the number for the whole genome sequence is JAFRDR000000000.

Declarations

Funding information

This work was supported by the National Natural Science Foundation of China (31700116), the Natural Science Foundation of Shandong Province (ZR2017MC019), the China Postdoctoral Science Foundation (2183), and the Key Science and Technology Program of Weihai (1070413421511).

Conflict of interest

The authors declare that there is no conflict of interest.

Authors' contribution

XHL and ZCL contributed to the sample collection and biochemical characterization.

MJZ analyzed the data, and drafted the manuscript. STY and LYP contributed to genome analyses. YXZ designed the experiments and revised the manuscript. All authors read and approved the final manuscript.

Acknowledgement

Thanks to the expert Aharon Oren for helping to view and modify the name of the new species.

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Table 1 Differential characteristics of strain C17^T and other closely related members of the genus *Williamsia*.

Strains: 1, C17^T; 2, *W. faeni* JCM 17784^T; 3, *W. limnetica* KCTC 19981^T. +, Positive; –, negative; w, weak. All data from this study except DNA G+C contents of the related strains, which were from the original species description:

Characteristic	1	2	3
Growth at/with:			
4 °C	+	–	–
Assimilation of:			
Gluconate	+	–	–
Adipic acid	+	–	–
Malic acid	+	–	–
Maltose	–	+	+
Nitrate reduction	–	–	+
Hydrolysis of:			
Leucine arylamidase	+	–	+
Cystine arylamidase	w	–	+
β -galactosidase	w	–	w
β -glucosidase	w	+	–
Urease	–	+	+
Gelatin	+	–	–
Arginine dihydrolase	+	–	+

Arginine dihydrolase	—	—	+
Acids from:			
D-arabinose	—	+	—
L-arabinose	—	+	—
D-galactose	—	+	—
D-glucose	—	—	+
D-fructose	+	—	+
L-rhamnose	+	—	—
D-sorbito	+	—	+
Saccharose	+	—	+
Trehalose	+	+	—
Xylitol	+	—	—
Salicin	+	—	—
D-maltose	—	+	+
Carbon source oxidation:			
D-cellobiose	—	—	+
D-mannose	—	+	+
D-fructose	+	—	+
D-galactose	—	+	+
D-mannitol	+	—	+
D-arabitol	+	+	—
D-sorbitol	+	—	—

L-alanine	—	+	+
L-arginine	—	+	+
Acetoacetic acid	+	—	—
Propionic acid	+	—	—
DNA G+C content (mol%)	64.7	63.9 ^a	64.5 ^b

416 ^aData from (Jones et al., 2010)

417 ^bData from (Sazak and Sahin, 2012)

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Table 2 Cellular fatty acid composition of strain C17^T and the closest relatives.

Strains: 1, C17^T; 2, *W. faeni* JCM 17784^T; 3, *W. limnetica* KCTC 19981^T. All data were taken from this study. TR, Traces (<1.0%); -, not detected. Fatty acids amounting to <1.0% of the total fatty acids in both strains are not shown.

Fatty acid	1	2	3
Saturated fatty acids			
C _{10:0}	TR	1.3	TR
C _{14:0}	1.9	3.8	2.4
C _{16:0}	31.7	34.8	30.1
C _{18:0}	2.2	4.9	2.2
Branched fatty acids			
iso-C _{10:0}	1.0	2.0	1.5
anteiso-C _{11:0}	1.01	-	-
10-methyl-C _{18:0}	16.4	13.9	5.4
Unsaturated fatty acids			
C _{18:1} ω ⁹ <i>c</i>	26.8	23.9	33.5
iso-C _{17:1} ω ⁵ <i>c</i>	TR	1.4	TR
Summed Feature 3*	12.2	11.5	17.7
Sum In Feature 3[#]	10.9	9.6	17.7
Summed Feature 7^{&}	-	-	1.5

*Summed feature 3, C_{16:1}ω⁷*c* and/or C_{16:1}ω⁶*c*.

[#]Sum In Feature 3, C_{16:1}ω⁶*c* and/or C_{16:1}ω⁷*c*.

439 $\&$ Summed Feature 7, un 18.846 and/or $C_{19:1}\omega 6c$.

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Figures

Figure 1

Neighbor-joining phylogenetic tree based on 16s rRNA gene sequences of strain C17T and high similarity of genus. Bootstrap support values (1000 replications) above 50% are shown at nodes. The filled circles indicate that the corresponding node is also restored in the maximum-likelihood tree. *Absiella argi* N6H1-5T was used as an outgroup. Bar 0.05 substitutions per nucleotide position.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [KCTC49567.pdf](#)
- [MCCC1K04355.pdf](#)
- [Supplementmaterials.pdf](#)