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Pseudoxanthomonas composti sp. nov., isolated from compost

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Abstract A Gram-staining negative bacterium, designated as GSS15^T, was isolated from compost in Guangzhou, China. Cells of strain GSS15^T were rod-shaped and non-motile. The isolate was able to grow at 15–42 °C (optimum 30 °C) and pH 6.0–11.0 (optimum pH 8.0), and tolerate up to 6.0% NaCl (w/v). When the 16S rRNA gene sequence of the isolate was compared with those of other bacteria, the highest similarity was observed with *Pseudoxanthomonas helianthi* roo10^T (96.9%). Furthermore, strain GSS15^T showed low ANI (75.7–79.5%) and DDH (24.2–18.3%) values to the closely related species. Q-8 was the predominant respiratory quinone. The major cellular fatty acids (> 5%) were iso-$C_{15:0}$ (18.7%), $C_{16:1}\omega7c$ (18.6%), anteiso-$C_{15:0}$ (13.2%), $C_{16:0}$ (9.8%), and iso-$C_{16:0}$ (8.8%). The polar lipids consisted of phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol. Based on its phenotypic, chemotaxonomic and genotypic data, strain GSS15^T (= KCTC 52974^T = MCCC 1K03334^T) is designated as the type strain of a novel species of the genus *Pseudoxanthomonas*, for which the name *Pseudoxanthomonas composti* sp. nov. is proposed.

Keywords *Pseudoxanthomonas composti* · Novel species · Polyphasic taxonomy

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Introduction

The genus *Pseudoxanthomonas*, belonging to the family Xanthomonadaceae, was first described by Finkmann et al. (2000) when the type species *Pseudoxanthomonas broegbernensis* was isolated from ammonia-supplied biofilters. Emended descriptions of the genus were later made by Thierry et al. (2004) and Lee et al. (2008). The genus *Pseudoxanthomonas* comprises Gram-staining negative and rod-shaped bacteria that are characterized chemotaxonomically by the presence of ubiquinone 8 (Q-8) as the major isoprenoid quinone and iso-$C_{15:0}$ as the predominant cellular fatty acid (Finkmann et al. 2000; Thierry et al. 2004; Lee et al. 2008). The DNA G + C

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content ranged from 60.1 to 71.1 mol% among different species of this genus (Kittiwongwattana and Thawai 2016). At the time of writing, the genus *Pseudoxanthomonas* comprised 19 species which were isolated from various sources such as roots of Jerusalem artichoke (Kittiwongwattana and Thawai 2016), a hexachlorocyclohexane dumpsite (Kumari et al. 2011), oil-contaminated soil (Young et al. 2007), North Pacific Ocean (Harada et al. 2006), cotton waste composts (Weon et al. 2006), and hot springs (Chen et al. 2002).

In this study, we characterized the taxonomic position of a bacterial strain GSS15^T, and demonstrated, using a polyphasic approach, that it is representative of a novel species of the genus *Pseudoxanthomonas*.

Materials and methods

Bacterial strain and culture conditions

The sample for isolation of strain GSS15^T was obtained from a composting demonstration plant in Guangzhou City, Guangdong Province, China. The compost windrow consisted of sewage sludge and crop straw. The samples were collected after composting for 13 days. Strain GSS15^T was obtained during investigating the cultured aerobic bacteria in the compost samples using LB agar (10.0 g peptone, 5.0 g yeast extract, 10.0 g NaCl and 20.0 g agar; pH 7.2) at 30 °C. The new isolate was cryopreserved at − 80 °C in 15% (v/v) glycerol. The experiments on the new isolate were carried out at 30 °C, pH 8.0 and with 0.5% NaCl (w/v) unless indicated else.

The closely related type strains *Pseudoxanthomonas helianthi* roo10^T, *Stenotrophomonas panacihumi* JCM 16536^T, *Pseudoxanthomonas broegbernensis* B1616/1^T, and *P. spadix* IMMIB AFH-5^T were selected as references. These references were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ) and Japan Collection of Microorganisms (JCM), and were cultured in the media and conditions recommended by these collections.

Phylogenies and average nucleotide identity analyzes

Genomic DNA of strain GSS15^T was extracted using a DNA Extraction Kit (Aidlab; China). The 16S rRNA gene was amplified by PCR using the universal primers 27F and 1492R (Baker et al. 2003), and then double-checked by sequencing both strands. The pairwise sequence similarity was calculated using the EzBiocloud server (https://www.ezbiocloud.net/; Yoon et al. 2017a). Phylogenetic analysis was carried out using MEGA version 7.0 with the neighbor-joining (NJ), maximum-likelihood (ML) and maximum-parimony (MP) models after multiple alignments of the sequence data with Clustal_W (Kumar et al. 2016). Statistical support for the branches of the phylogenetic trees was determined using bootstrap analysis (based on 1000 re-samplings) (Felsenstein 1985).

The draft genome of strain GSS15^T was sequenced using paired-end sequencing method with the Illumina PE150 platform by Novogene Bioinformatics Technology Co., Ltd., China. Reads of each data set were filtered and high quanlity paired-end reads were assembled using SOAP denovo (version 2.04). Contigs with length greater than 500 bp were kept for gene prediction by GeneMarkS (version 4.17). In silico DNA-DNA hybridization (DDH) values were calculated by genome-to-genome distance calculator (GGDC) (Meier-Kolthoff et al. 2013). Values of average nucleotide identity (ANI) were calculated by using ANI Calculator (https://www.ezbiocloud.net/tools/ani) (Yoon et al. 2017b). Further multilocus sequence analysis (MLSA) based on sequences of housekeeping genes (*recA*, recombinase A; *gyrA*, DNA gyrase A subunit; *rpoD*, RNA polymerase, sigma 70) was performed with NJ, ML and ME methods supported with bootstrap values based on 1000 replications. The genome sequences for DDH and ANI calculation and the sequences of the housekeeping genes were downloaded from the NCBI database.

Morphological, physiological and biochemical characterization

Cell morphology was investigated with a transmission electron microscope (JEOL, Japan) after cultivation on LB plate at 30 °C for 24 h (Yang et al. 2013). Cell motility was tested by observing the growth spread in a test tube containing semi-solid LB medium. The Gram reaction was determined by a Gram staining kit HB8278 (Qingdao Hope-Bio Technology Co., Ltd; China). Oxidase activity was determined using an oxidase reagent (BioMérieux), and catalase activity

was determined by observing bubble production in 3% (v/v) hydrogen peroxide solution. The temperature range for growth was determined at 10, 15, 25, 30, 37, 42, 45, and 50 °C. The pH range for growth was determined at pH 4.0–11.0 (at intervals of 0.5 pH unit) using the following buffer systems: pH 4.0–5.0, 0.1 M citric acid/0.1 M sodium citrate; pH 6.0–8.0, 0.1 M KH_2PO_4/0.1 M NaOH; pH 9.0–10.0, 0.1 M $NaHCO_3$/0.1 M Na_2CO_3; pH 11.0, 0.05 M Na_2HPO_4/0.1 M NaOH (Zhang et al. 2009). The tolerance for NaCl concentrations was determined with 0–8.0% (w/v) NaCl (with increments of 0.5%). Growth at different temperature, pH and NaCl concentration was investigated for up to 1 week. Hydrolysis of gelatin and starch was tested as described by Dong and Cai (2001). Other enzyme activities and acid production characteristics were characterized with the API 20NE and API 50CH systems (BioMérieux) according to the manufacturer's instructions.

Chemotaxonomic characterization

For respiratory quinone and polar lipids analysis, strain GSS15^T was cultured in LB medium at 30 °C to the exponential growth phase. Cells were collected by centrifugation at 12,000 rpm at 4 °C, and free-dried using the vacuum freeze drying apparatus (FD-1A-50, Beijing Biocool, China). The quinones were extracted with methanol according to Collins et al. (1977) and analyzed by HPLC as described by Groth et al. (1997). Polar lipids were extracted from the freeze-dried cells, separated by two-dimensional thin layer chromatography (TLC) and identified according to Minnikin et al. (1984) by spraying the plates with detection reagent molybdophosphate and ninhydrin. For cellular fatty acid analysis, cells of strain GSS15^T and the reference strains grown on LB at 30 °C for 36 h to exponential phase were saponified, methylated and extracted using the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.0B). The fatty acids were analyzed with GC (Agilent 6850) and identified by using the TSBA6.0 database of the Microbial Identification (MIDI) System (Sasser 1990). The groups of two or three fatty acids that cannot be identified using the MIDI system were further analyzed using GC–MS (Agilent) as previously described (Olivier and Loots 2012). The G + C content of the genomic DNA was determined by HPLC according to the method of Mesbah et al. (1989).

Results and discussion

Phylogenetic analysis and ANI values

A nearly complete 16S rRNA gene sequence (1443nt) was determined for strain GSS15^T. Comparison between the 16S rRNA gene sequence of strain GSS15^T and those of the validly named species showed that, strain GSS15^T shares high sequence similarity with *P. helianthi* roo10^T (96.9%), *S. panacihumi* JCM 16536^T (96.7%) and *Xanthomonas maliensis* M97^T (96.4%). The NJ, ML and MP trees depicting the phylogenetic relationships between strain GSS15^T and its near phylogenetic neighbours are shown in Fig. 1 and supplementary Fig. S1 and S2, respectively. In addition, strain GSS15^T formed a subcluster with *P. spadix* in the neighbor-joining tree. The phylogenetic analysis indicated that the new isolate is closely related to the genus *Pseudoxanthomonas* and is relatively distant from the genera *Stenotrophomona* and *Xanthomonas*.

The draft genome of strain GSS15^T was deposited at DDBJ/EMBL/GenBank under the accession SAWZ00000000. The assembled genome sequence consists of 4,340,084 bp with a DNA G + C content of 68.2%, which falls within the range observed for the other members of the genus *Pseudoxanthomonas* (60.1–71.1 mol%) (Kim et al. 2015). The genome was predicted to contain a total of 3695 genes, including 3640 protein-coding genes, 50 tRNA genes and 5 rRNA genes. The MLSA showed that strain GSS15^T formed a subcluster with *P. spadix* within the genus *Pseudoxanthomonas* (Fig. S3), which supported the affiliation of the new isolate to the genus *Pseudoxanthomonas*. The calculated digital DDH values between strain GSS15^T and the known species of the genus *Pseudoxanthomonas* (24.2–18.3%) was well below the proposed threshold of 70% for prokaryotic species delineation. In addition to low DDH values, ANI values calculated on the EzBioCloud web server ranged between 75.7 and 79.5% (values below the threshold of 95–96%; Richter and Rosselló-Móra 2009), which confirmed the

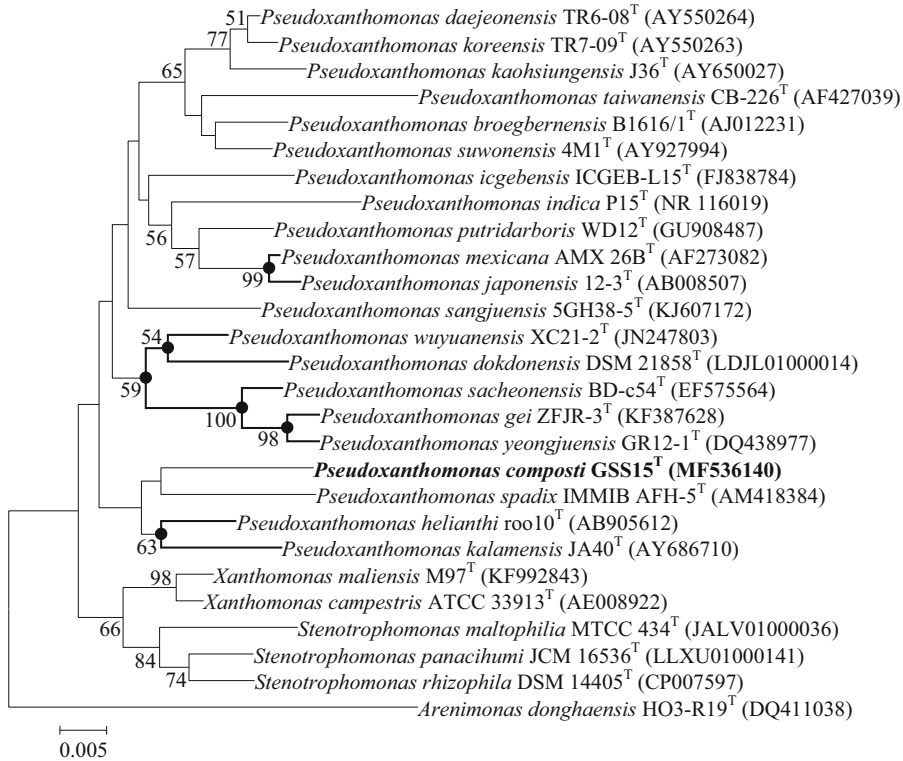

Fig. 1 Neighbour-joining phylogenetic tree of strain GSS15^T and its closely related taxa, based on 16S rRNA gene sequences. Numbers at branching points represent bootstrap value from 1000 replicates. Bootstrap values $\geq$ 50% are shown at each nod. Bar, 0.005 substitutions per nucleotide position. *Arenimonas donghaensis* HO3-R19^T is selected as the outgroup. Filled circles indicated branches that were also found in phylogenetic trees reconstructed with the maximum-likelihood and maximum-parimony methods

differentiation of strain GSS15^T from its phylogenetic neighbors.

Morphological, physiological and biochemical characterization

Cells of strain GSS15^T were Gram-staining negative, aerobic, motile, and rod-shaped (2.4–2.5 μm long and 0.6–0.8 μm wide) (Supplementary Fig. S4). Colonies of this strain were circular, convex and yellow-pigmented with a diameter of 1.6–2.3 mm after incubation at 30 °C for 48 h on LB agar. Strain GSS15^T was positive for catalase but negative for oxidase reaction, indole production, arginine dihydrolase, and urease. Strain GSS15^T can hydrolyze gelatin, esculin and starch. Growth occurred at 10–42°C (optimum 30 °C) and at pH 6.0–11.0 (optimum 8.0). Growth occurred in the presence of 0–6.0% (w/v) NaCl with the optimal growth at 0.5% (w/v) NaCl. Acid was produced from D-galactose, D-glucose, D-fructose, D-mannose, *N*-acetylglucosamine, amygdalin, arbutin, salicin, D-cellobiose, D-maltose, D-lactose, D-saccharose, D-trehalose, starch, gentiobiose, D-turanose, and D-fucose, but not from glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, methyl-*β*-D-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-α-D-mannopyranoside, methyl-α-D-glucopyranoside, D-melibiose, inulin, D-melezitose, D-raffinose, glycogen, xylitol, D-lyxose, D-tagatose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate, or potassium 5-ketogluconate. D-glucose, D-mannose, *N*-acetyl-glucosamine, D-maltose, malic acid and trisodium citrate are utilized as substrates, and the following substrates are not utilized: L-arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid and phenylacetic acid. A comparison of selective characteristics with its closely related type strains is given in Table 1.

Table 1 Differential characteristics of strain GSS15^T and strains of related taxa

Characteristics	1	2	3	4	5
Habitat	Compost	Root	Soil	Biofilters	Soil
Motility	+	−	−	+	+
Flagellum	+	−	−	+	+
Temperature range (°C)	15–42	20–37	10–37	10–37	20–37
Optimum temperature (°C)	30	30	25	25	30
pH range	6–11	7–9	6–9	6.5–10	6–10
Optimum pH	8	8	7	8	7
NaCl tolerance (%, w/v)	0–6	0–5	0–3	0–4	0.5–3
Optimum NaCl concentration (%, w/v)	0.5	0	2	0	2
Oxidase	−	+	−	+	+
Gelatin hydrolysis	+	−	−	−	+
Utilization of					
Trisodiumcitrate	+	−	+	−	−
D-maltose	+	+	−	+	−
N-acetyl-glucosamine	+	+	+	−	−
D-mannose	+	+	+	+	−
D-glucose	+	+	+	+	−
DNA G + C content (mol%)*	69.7	65.7[a]	65.7[b]	66.5[c]	68.5[d]

Taxa: 1, strain GSS15^T; 2, *P. helianthi* roo10^T; 3, *S. panacihumi* JCM 16536^T; 4, *P. broegbernensis* B1616/1^T; 5, *P. spadix* IMMIB AFH-5^T. Data for all strains were obtained from this study unless indicated elsewhere. +, positive; −, negative

*Data were obtained from: a, Kittiwongwattana and Thawai (2016); b, Yi et al. (2010); c, Finkmann et al. (2000); d, Young et al. (2007)

Chemotaxonomic characteristics

Strain GSS15^T contained a quinone system with ubiquinone Q-8 as the predominant component, which is consistent with the description of the genus *Pseudoxanthomonas* (Finkmann et al. 2000). Cellular fatty acid analysis revealed that iso-$C_{15:0}$ (18.7%), $C_{16:1}\omega7c$ (18.6%) and anteiso-$C_{15:0}$ (13.2%) were the major fatty acids in strain GSS15^T (Table 2). According to previous reports, the *Pseudoxanthomonas* species can be easily differentiated from the *Xanthomonas* and *Stenotrophomonas* species by the characteristic lack or traces of iso-C13:0 3-OH fatty acid (Thierry et al. 2004; Li et al. 2014). Therefore, the fatty acid profile (absence of iso-C13:0 3-OH) of strain GSS15^T clearly assigned this strain to the genus *Pseudoxanthomonas*. However, strain GSS15^T could be distinguished from its close phylogenetic neighbours, both qualitatively and quantitatively; for example, relatively higher proportion of $C_{16:1}\omega7c$ (18.6%) was present in strain GSS15^T than in its phylogenetic neighbours (0–5.4%). The polar lipid pattern of strain GSS15^T consisted of diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine (Fig. S5). The major polar lipids of strain GSS15^T were consistent with that of the reference strain *P. helianthi* (Kittiwongwattana and Thawai 2016).

The phylogenetic tree based on 16S rRNA gene sequences and the chemotaxonomic characteristics clearly suggested that strain GSS15^T is a member of the genus *Pseudoxanthomonas*. However, besides the low 16S rRNA gene similarities, comparison in physiological and biochemical characteristics between strain GSS15^T and its neighbouring strains demonstrated significant differences, suggesting that strain GSS15^T can be distinguished from all recognized *Pseudoxanthomonas* species. On the basis of these data, strain GSS15^T represents a novel species of the genus *Pseudoxanthomonas*, and for this novel species the name *Pseudoxanthomonas composti* sp. nov. is proposed.

Description of *Pseudoxanthomonas composti* sp. nov.

Pseudoxanthomonas composti (com.pos'ti. N.L. gen. n. *composti* referring to compost, where the strain was isolated).

Cells are Gram-staining negative, aerobic, rod-shaped, and motile with polar flagellum. Cells are approximately 2.4–2.5 μm long and 0.6–0.8 μm wide. Colonies are circular, convex and yellow-pigmented. Growth occurs at 10–42°C (optimum 30 °C), at pH 6.0–11.0 (optimum 8.0), and with 0–6.0% (w/v) NaCl

Table 2 Cellular fatty acid profiles of strain GSS15^T and its related species

Fatty acids (%)	1	2	3	4	5
Saturated straight-chain					
$C_{12:0}$3-OH	3.9	–	–	–	–
$C_{14:0}$	2.4	–	–	–	–
$C_{16:0}$	9.8	–	2.5	8.0	12.7
Saturated branched-chain					
iso-$C_{11:0}$	3.3	4.6	–	2.6	2.7
iso-$C_{11:0}$3-OH	–	7.6	2.2	5.8	–
iso-$C_{13:0}$3-OH	–	–	5.8	–	–
iso-$C_{15:0}$	18.7	33.6	39.8	26.9	19.6
iso-$C_{16:0}$	8.8	11.6	–	3.4	3.3
iso-$C_{17:0}$	2.5	14.6	6.0	2.3	13.9
Anteiso-$C_{15:0}$	13.2	3.6	10.9	20.7	2.7
Anteiso-$C_{17:0}$	–	–	–	2.4	22.0
Unsaturated branched-chain					
iso-$C_{17:1}\omega 7c$	–	–	–	7.3	–
iso-$C_{17:1}\omega 9c$	3.3	7.9	11.2	4.4	–
$C_{16:1}\omega 7c$	18.6	5.4	4.3	3.0	–

Taxa: 1, strain GSS15^T; 2, *P. helianthi* roo10^T; 3, *S. panacihumi* JCM 16536^T; 4, *P. broegbernensis* B1616/1^T; 5, *P. spadix* IMMIB AFH-5^T. Values were percentages of the total fatty acids; –, fatty acids represents < 2.0% of the total. Data for all strains were obtained from this study

(optimum 0.5%). Positive for catalase but negative for oxidase reaction. Nitrate is not reduced. The predominant quinone is Q-8. The major cellular fatty acids are iso-$C_{15:0}$, $C_{16:1}\omega 7c$, anteiso-$C_{15:0}$, $C_{16:0}$, and iso-$C_{16:0}$. The polar lipids consist of phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol. The DNA G + C content of the type strain is 69.7 mol%.

The type strain is GSS15^T (= MCCC 1K03334^T = KCTC 52974^T), which was isolated from compost in Guangzhou, China. The draft genome accession number of the type strain is SAWZ00000000 at DDBJ/EMBL/GenBank.

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Author contributions ZY and LZ designed research and project outline and drafted the manuscript. JL, GY performed isolation, deposition and identification. JT and ZL performed genome analysis. All authors read and approved the final manuscript.

Conflict of interest The authors declare that they have no direct or indirect conflict of interest.

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