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ORIGINAL PAPER

Alsobacter ponti sp. nov., a novel denitrification and sulfate reduction bacterium isolated from Pearl River sediment

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Abstract A Gram-staining negative, aerobic, motile, and short rods strain, designated SYSU M60028^T, was isolated from a Pearl River sediment sample in Guangzhou, Guangdong, China. The isolate could be able to grow at pH 6.0–8.0 (optimum, pH 7.0), 25–37 °C (optimum, 28 °C) and in the presence of 0–2% (w/v) NaCl (optimum, 0% NaCl). The cellular polar lipids of this strain were phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, one unidentified aminolipid and three unidentified lipids. The respiratory quinone of SYSU M60028^T was found to be Q-10. The major fatty acids (> 5% of total) were summed feature 8, $C_{16:0}$, and $C_{18:1}$ $\omega 7c$ 11-methy1. The genomic DNA G + C content was 69.9%. Phylogenetic analyses based on 16S rRNA gene sequences and core genes indicated that strain SYSU M60028^T belonged to the genus *Alsobacter* and had the highest sequences similarities to *Alsobacter metallidurans* SK200a-9^T (96.87%) and *Alsobacter soli* SH9^T (96.87%). Based on the phenotypic, genotypic, and phylogenetic data, strain SYSU M0028^T should be considered to represent a novel species of the genus *Alsobacter*, for which the name *Alsobacter ponti* sp. nov. is proposed. The type strain is SYSU M60028^T (= CGMCC 1.19341^T = KCTC 92046^T).

Keywords *Alsobacter ponti* sp. nov. · *Alsobacter* · Pearl River · Sediment · Polyphasic taxonomy

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Introduction

The genus *Alsobacter* of the family *Alsobacteraceae*, class *Alphaproteobacteria* was first proposed by Bao et al. (2014). At the time of writing, the genus *Alsobacter* comprises two species with validly published names. Species of this genus have been reported from a garden soil sample (Bao et al. 2014) and a paddy soil sample (Sun et al. 2018). According to the former studies, *Alsobacter* species are Gram-staining negative, aerobic and rod-shape, containing Q-10 as the respiratory ubiquinone (Bao et al. 2014; Sun et al. 2018). In this study, we describe a new isolate which

represents a novel species of this genus based on the analysis of morphological, phenotypic, genotypic, and phylogenetic characteristics.

Estuaries are the interaction zones between oceans and terrestrials, whose eco-environment health are essential to the sustainable development of human habitation and social economy. In order to investigate the cultivable bacteria community in the Pearl River estuary, surface sediment samples were collected from Pearl River estuary, Guangzhou, Guangdong, China. During this study, one novel isolate SYSU M60028^T was cultivated from a sediment sample.

Material and method

Sample collection and isolation

The surface sediment samples (10 cm) were collected at the depth of 3 m from the Pearl River, Guangdong, China, and transferred by a sterile homogeneous bag. The temperature and pH of the samples were 28 °C and pH 7.5, respectively. The sediment samples were diluted 10^3- fold with sterile distilled water and 0.2 mL final suspension was plated on Reasoner's 2A (R2A; BD) agar (Yeast Extract 0.5 g, Proteose Peptone 0.5 g, Glucose 0.5 g, Soluble Starch 0.5 g, Casamino Acids 0.5 g, Sodium Pyruvate 0.3 g, K_2HPO_4 0.3 g, $MgSO_4$-$7H_2O$ 0.05 g, Distilled Water 1000 ml, Agar 18 g, pH 7.0). The isolation plates were incubated at 28℃ for 7 days. Single colonies were selected and purified on the same medium. The purified strains were preserved as glycerol suspensions (20%, v/v) at − 80 °C for further use. Among the purified isolates, one off-white strain SYSU M60028^T showed low 16S rRNA sequences similarities to *Alsobacter metallidurans* (96.87%) and *Alsobacter soli* (96.87%), and was selected for further novel species characterization. The basal growth conditions of the strain SYSU M60028^T for all experiments were maintained at pH 7.0 and 28℃, unless otherwise stated.

Phenotypic characterization

Cultural characteristics were performed on Luria–Bertani (LB) agar, R2A agar, tryptic soy agar (TSA; Difco), and marine agar 2216E (MA; BD Difco) at 28 °C for 5 days. The microscopic morphological characteristics of the strain SYSU M60028^T were observed by transmission electron microscope (JEM–1400FLASH, JEOL, JAPAN). The Gram-reaction was performed by the standard Gram reaction and was confirmed by the non-staining method using 3% KOH (Buck 1982). Growth temperature (4, 10, 15, 20, 25, 28, 30, 37, 45, 55, 60 and 65 °C), pH range (pH 4.0–10.0, at intervals of 1 unit, adjusted by using the buffer system) (Xu et al. 2005), and NaCl tolerance (0, 1, 2, 3, 4, 6, 9, 12 and 15%, w/v) were tested on R2A agar. Oxidase and catalase activities were determined by assessing the oxidation of 1% (w/v) tetramethyl-p-phenylenediamine (Kovacs 1956) and the formation of bubble on addition of 3% H_2O_2, respectively. Tweens (20, 40, 60, and 80) degradation, H_2S production, milk coagulation and peptonization, cellulose, starch, gelatin hydrolysis, anaerobic growth, and urease activity were tested according to the methods previously described (Gonzalez et al. 1978; Smibert Krieg 1994). Other physiological and biochemical characteristics of strain SYSU M60028^T were identified by GEN III Microplates system (Biolog, USA), API 20NE and API ZYM kits (bioMérieux) according to the manufacturer's instructions.

Chemotaxonomy characterisation

Biomass for chemical and molecular studies of strain SYSU M60028^T was obtained on R2A agar during the logarithmic growth period (3 days). For polar lipids, strain SYSU M60028^T was extracted and identified by two-dimensional TLC on silica gel G60 plates (Merck) as previously described (Collins Jones 1980; Minnikin 1979). The respiratory ubiquinone was extracted from lyophilized cells as described by Collins et al. (1977), purified, and analysed by HPLC as described by Tamaoka et al. (1983). The cellular fatty acid methyl esters were extracted and analysed according to the standard protocol of the Microbial Identification System (Sherlock version 6.1; MIDI database: TSBA6) (Athalye et al. 1985).

Phylogenetic analysis

Genomic DNA extraction and PCR amplification of 16S rRNA gene were carried out according to the method described by Li et al. (2007). The obtained sequences were compared with the corresponding validly published species in the EzBioCloud database

(Yoon et al. 2017). The 16S rRNA gene sequences of close related type strains were aligned using CLUSTAL X software package (Thompson et al. 1997) and were used for constructing the phylogenetic trees by MEGA X software (Kumar et al. 2018). Algorithms based on neighbour-joining (Saitou Nei 1987), maximum-likelihood (Felsenstein 1981), and maximum-parsimony (Fitch 1971) methods were used for generation of the phylogenetic trees. Kimura's two-parameter model was used to calculate evolutionary distance matrices of the phylogenetic trees (Kimura 1980). Stability of the phylogenetic trees was evaluated by bootstrap analysis with 1000 replications (Felsenstein 1985). *Chelatococcus reniformis* B2974^T was used as an outgroup.

Genome sequencing, annotation, and analysis

The whole genome sequencing of strain SYSU M60028^T was performed by using Illumina NovaSeq platform at the Guangdong Microbial Culture Collection Center (GDMCC, Guangdong, China). Reads of each data sets were filtered, and high quality paired-end reads were assembled using readfq (version 10), SOAP denovo (version 2.04), and SPAdes (Bankevich et al. 2012; Li et al. 2008, 2010). The quality of the genomes was examined using CheckM v.1.0.7 (Parks et al. 2015). The COG database (Galperin et al. 2014) and KEGG database (Kanehisa Sato 2020; Kanehisa et al. 2022) were used to annotate the genome sequences. The secondary metabolite biosynthetic gene clusters were annotated by antiSMASH (Blin et al. 2021). The Average nucleotide identity (ANI) was calculated using JSpecies (http://jspecies.ribohost.com/jspeciesws/) (Richter et al. 2016). The digital DNA-DNA hybridization analysis was performed by using the DSMZ Genome-to-Genome Distance Calculator platform (http://ggdc.dsmz.de/distcalc2.php) (Goris et al. 2007). The phylogenomic tree was constructed according to the method described by Jiao et al. (2021). The multiple sequence alignments (MSAs) were generated by GTDB-Tk software (Chaumeil et al. 2019). The Maximum-likelihood phylogenetic tree was calculated by IQ-Tree with parameters (alrt 1000 bb 1000 nt AUTO) (Nguyen et al. 2015). The best-fit model (LG + F + R4) determined by ModelFinder was well supported by Akaike information criterion (AIC), corrected AIC (AICc), and Bayesian information criterion (Kalyaanamoorthy et al. 2017). The phylogenomic tree was visualized using the online Interactive Tree of Life program version 4.2 finally (Letunic Bork 2016).

Result and discussion

Phenotypic characteristics

Cells of strain SYSU M60028^T were Gram-staining negative, aerobic, and had the ability to move by flagella (Fig. S4). Colonies were circular, punctiform and off-white in colour. Cells were short rods with size measuring 0.6–0.7 × 0.9–1.0 μm (Fig. S4). The isolate could be able to grow at pH 6.0–8.0 (optimum, pH 7.0), 25–37 °C (optimum, 28 °C) and in the presence of 0–2% (w/v) NaCl (optimum, 0% NaCl). Strain SYSU M60028^T was positive for oxidase activity, α-glucosidase, H_2S production, nitrate reduction and trypsin, but negative for milk coagulation and peptonization, Tweens (20, 40, 60, and 80), or catalase activity. The characteristics distinguishing strain SYSU M60028^T from closely related strains are listed in Table 1. The detailed physiological characteristics of strain SYSU M60028^T are given in the species descriptions below.

Chemotaxonomical characteristics

The respiratory quinone of strain SYSU M60028^T was identified as ubiquinone Q-10. The major cellular fatty acids (> 5%) detected of strain SYSU M60028^T were summed feature 8 ($C_{18:1}\omega7c$/ $C_{18:1}\omega6c$, 55.3%), $C_{16:0}$ (9.0%), and $C_{18:1}$ ω7c 11-methy1 (5.8%). The cellular polar lipids were phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, one unidentified aminolipid and three unidentified lipids (Fig. S5). The detailed fatty acid profile of strain SYSU M60028^T was provided in Table S4.

Phylogenic and phylogenomic analysis

The 16S rRNA gene sequences of strain SYSU M60028^T was submitted to GenBank with accession number ON881906. On pairwise comparison with the 16S rRNA gene sequences available in the EzBioCloud database, strain SYSU M60028^T showed highest 16S rRNA gene sequences similarities to

Table 1 Differential phenotypic and chemotaxonomical characteristics between strain SYSU M60028^T and its closely related type strains in the genus *Alsobacter*

Characteristics	1	2	3
Cell morphology	Off white	White to cream*	Cream white*
Growth at10℃	–	+	–
Growth at 35℃	–	+	+
Growth at 40℃	–	+	–
Growth at 2% (w/v) NaCl	+	–	+
API 20NE results			
Reduction of nitrates to nitrites	+	–	–
Fermentation of N-Acetyl-Glucosamine	–	+	–
Fermentation of maltose	–	+	–
Fermentation of potassium gluconate	–	–	+
Fermentation of capric acid	–	+	+
Fermentation of adipic acid	–	+	–
Fermentation of malic acid	–	+	–
API ZYM results			
Valine arylamidase	+	–	+
Cystine arylamidase	+	–	+
α-glucosidase	+	–	–
Polar lipids	DPG, PE, PC, PG	DPG, PE, PC, PG*	DPG, PE, PC, PG, PME, PGL*
Major fatty acids (> 5%)	$C_{16:0}$, $C_{18:1}$ $\omega 7c$ 11–methy1 and summed feature 8	$C_{16:0}$, $C_{18:1}$ $\omega 7c$ 11–methy1, summed feature 3 and 8	$C_{16:0}$, summed feature 3 and 8
DNA genomic G + C (%)	69.9	64.8*	68.5*

Strains: 1, SYSU M60031^T; 2, *Alsobacter metallidurans* SK200a-9^T; 3, *Alsobacter soli* SH9^T

All data for SYSU M60028^T, SK200a-9^T and SH9^T are from this study (*except those relating to the morphology, polar lipids, and DNA G + C content of the reference strains. +, Positive; -, negative; PE, phosphatidylethanolamine; PME, phosphatidylmonomethylethanolamine; PC, phosphatidylcholine; PG, phosphatidylglycerol; PGL, phosphoglycolipid; DPG, diphosphatidylglycerol

*Data are retrieved from Bao et al. (2014) and Sun et al. (2018)

Alsobacter metallidurans SK200a-9^T (96.87%) and *Alsobacter soli* SH9^T (96.87%). The neighbour-joining tree based on 16S rRNA gene sequences showed that strain SYSU M60028^T formed a distant clade with *Alsobacter metallidurans* SK200a-9^T and *Alsobacter soli* SH9^T (Fig. 1). An apparently stable lineage was also supported by maximum-parsimony and maximum-likelihood phylogenetic trees (Fig. S1, Fig. S2). Phylogenomic tree (Fig. S3) based on the concatenated alignment of 120 marker genes provided further evidence of this topological structure. Based on the 16S rRNA gene sequences identity and the phylogenetic relationship, *Alsobacter metallidurans* SK200a-9^T and *Alsobacter soli* SH9^T were used as reference type strains.

Genome analysis

The genome properties of strain SYSU M60028^T included 56 contigs amounting to 4,727,519 bp. The G + C content based on DNA genomic was 69.9% (Table S1). The completeness and contamination of the genomic were 98.59% and 0.26%, respectively. The dDDH value comparing strain SYSU M60028^T with *Alsobacter soli* SH9^T and *Alsobacter metallidurans* SK200a-9^T were 21.2% and 20.8% (Table S2), which were below the cutoff (70%) recommended as the criterion for interspecies identity (Wayne 1988). The ANI values comparing SYSU M60031^T with *Alsobacter soli* SH9^T and *Alsobacter metallidurans* SK200a-9^T were 75.39% and 75.37%

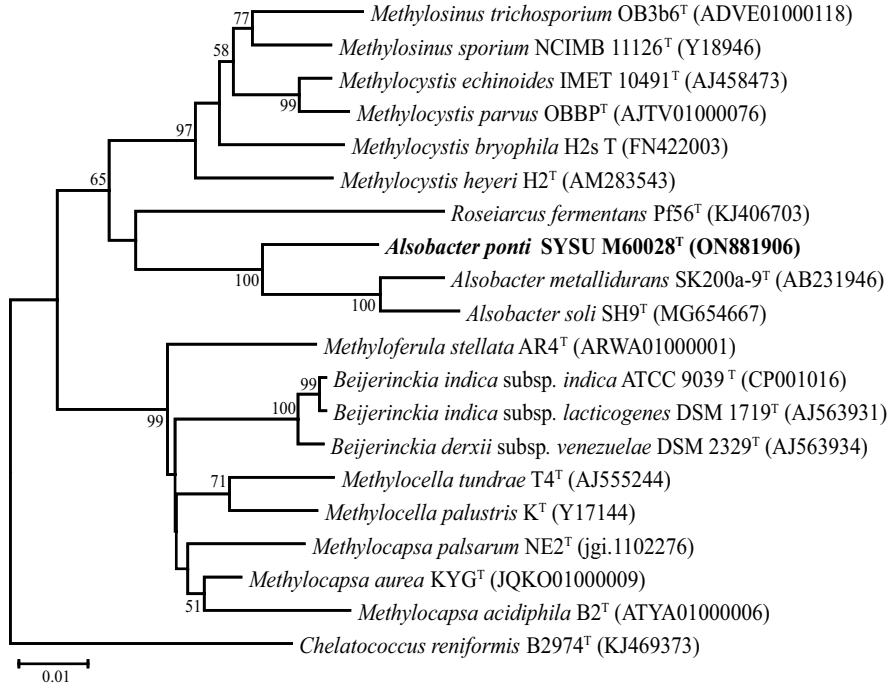

Fig. 1 Neighbour–joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain SYSU M60028^T and their closest relatives. Bootstrap values (> 50%) based on 1000 replications are given at the nodes. Bar represents 0.01 substitutions per nucleotide position. *Chelatococcus reniformis* B2974^T (KJ469373) was used as an outgroup

(Table S2), further suggesting the strain SYSU M60028^T represents a novel species belonging to the genus *Alsobacter*.

According to the genome annotation results, the genome of the strain SYSU M60028^T contained 4,264 protein-coding genes. The number of genes assigned to COG database and KEGG database were 3,304 and 3,732, respectively. COGs (Clusters of Orthologous Groups) categories distributions for the genes were presented in the Table S3. Amino acid transport and metabolism (COG category E) was the most abundant represented CDs (sequence coding for amino acids in protein) of strain SYSU M60028^T, followed by general function prediction only (COG category R), function unknown (COG category S), and carbohydrate transport and metabolism (COG category G). According to the antiSMASH database, T3PKS, NRPS, NRPS-like and terpene gene clusters were four main clusters of the strain SYSU M60028^T, which were responsible for the biosynthesis of secondary metabolites (Table S5). Strain SYSU M60028^T had the potential to produce terpene, which offered new insight into biosynthesis of terpene in bacteria.

According to the annotation results of KEGG analysis, strain SYSU M60028^T contained 14 genes which were relevant to the nitrogen cycle, including dissimilatory nitrate reduction, denitrification, and nitrification. Genome annotation results suggested that strain SYSU M60028^T contained 4 genes (*narGHI*, *nirK*, *norBC*, and *nosZ*), which formed a complete denitrification pathway. Compared with type strains, *Alsobacter soli* SH9^T also had the ability of denitrification, while *Alsobacter metallidurans* SK200a-9^T did not have this complete pathway. This result was identified by the positive result of nitrate reduction test in API 20NE kit. Ntr C (a DNA-binding transcriptional regulator) was predicted in strain SYSU M60028^T. The previous studies had indicated that Ntr C participated in nitrogenous compounds utilization, biological nitrogen fixation, the biosynthesis of biopolymer and maintenance on carbon–nitrogen balance (Hervas et al. 2008). Therefore, the strain might involve in nitrogen-transforming reactions and remove nitrogen, which meant this strain could play an important role in nitrogen cycle and reducing the eutrophication of nearshore waters (Falkowski et al. 2008). Strain SYSU M60028^T also contained a complete assimilatory sulfur pathway (*cysND*, *cysC*, *cysH*, and *cysJI*), while *Alsobacter metallidurans* SK200a-9^T and *Alsobacter soli* SH9^T did not have this complete pathway. This result was consistent with the positive result of

H_2S production test and suggested the strain might reduce sulfate to sulfide, use sulfate as a terminal electron acceptor for the degradation of organic compounds, resulting in the production of sulphide, and kept the dynamics of sulfur cycle in balance (Muyzer et al. 2008). In addition, the genome contained 25 genes related to flagellar assembly. The diffusion of cells in semi-solid medium, and the flagellar observed by transmission electron microscope (Table S3) further supported the result of genome analysis.

Conclusion

The results of the phylogenetic analysis showed the strain SYSU M60028^T was a member of *Alsobacter*. Combining the morphological and chemotaxonomic characteristics, the strain SYSU M60028^T could be distinguished from other *Alsobacter* species. For example, the proportions of fatty acids and polar lipid composition or the utilization of carbon and nitrogen source of strain SYSU M60028^T are significantly different from those of the related species. Compared with *Alsobacter metallidurans* SK200a-9^T (4630) and *Alsobacter soli* SH9^T (4751), Strain SYSU M60028^T contained the least number of protein-coding genes (4290). According to the annotation results of KEGG analysis, strain SYSU M60028^T contained the complete assimilatory sulfur pathway (*cysND*, *cysC*, *cysH*, and *cysJI*), while *Alsobacter metallidurans* SK200a-9^T and *Alsobacter soli* SH9^T did not have this complete pathway. Strain SYSU M60028^T contained 4 genes (*narGHI*, *nirK*, *norBC*, and *nosZ*), which were formed a complete denitrification pathway. Compared with type strains, *Alsobacter soli* SH9^T also had the ability of denitrification, while *Alsobacter metallidurans* SK200a-9^T did not have this complete pathway. Thus, based on above results, we propose the strain SYSU M60028^T as a novel species of the genus *Alsobacter*, for which the name *Alsobacter ponti* sp. nov. is proposed.

Description of *Alsobacter ponti* sp. nov

Alsobacter ponti (pon'ti. L. gen. masc. n. ponti, of the sea).

Cells are Gram-staining negative, aerobic, motile, and short rods (0.6–0.7 × 0.9–1.0 μm). Colonies on R2A agar are off-white, circular, and smooth. Able to grow at pH 6.0–8.0 (optimum, pH 7.0), 25–37 °C (optimum, 28 °C) and in the presence of 0–2% (w/v) NaCl (optimum, 0% NaCl). Positive for oxidase activity, but negative for milk coagulation and peptonization, Tweens (20, 40, 60, and 80), or catalase activity. Positive for sterase (C4), esterase lipase (C8), *α*-chymotrypsin, trypsin, alkaline phosphatase, cystine arylamidase, valine arylamidase and leucine arylamidase. Negative for hydrolysis of urea, cellulose and gelatin, reduction of nitrates to nitrites, lipase (C14), acid phosphatase, *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, *α*-glucosidase, *β*-glucosidase, and *α*-mannosidase. The predominant respiratory quinone is Q-10. The cellular polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, one unidentified aminolipid and three unidentified lipids. The major fatty acids (> 5% of total) are summed feature 8, $C_{16:0}$ and $C_{18:1}$ *ω7c* 11-methy1. The G + C content of the genome DNA is 69.9%.

The type strain, SYSU M60028^T (= CGMCC 1.19341^T = KCTC 92046^T) was isolated from a sediment sample collected from the Pearl River estuary, Guangdong, China. The 16S rRNA gene sequences of strain SYSU M60028^T has been submitted to GenBank with accession number ON881906. The accession numbers of raw data and assembly genome are SRR23021099 and JANCLU000000000, respectively.

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Author contributions WJL designed research and project outline. QQD, JLL and SHL performed isolation, deposition and polyphasic taxonomy. PDW, XQL and YY performed genome analysis. QQD, ZWY and WJL drafted the manuscript. All authors read and approved the final manuscript.

Data and materials availability The 16S rRNA gene sequences of strain SYSU M60028^T has been deposited in GenBank under the accession number ON881906. Whole Genome Shotgun project of strain SYSU M60028^T has been deposited at DDBJ/ENA/GenBank under accession number JANCLU000000000. The genome raw data of strain SYSU M60028^T has been deposited in GenBank under the accession

number SRR23021099. Five supplementary tables and five supplementary figures are included on the online supplementary file.

Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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