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ORIGINAL PAPER

Thiopseudomonas acetoxidans sp. nov., an aerobic acetic and butyric acids oxidizer isolated from anaerobic fermentation liquid of food waste

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Abstract A Gram-stain-negative, oxidase-negative, rod-shaped, motile, facultatively anaerobic bacterial strain, designated as CY1220^T, was isolated from an anaerobic fermentation liquid of food waste treatment plant. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the strain CY1220^T belongs to the genus *Thiopseudomonas*, with the highest sequence similarity to *Thiopseudomonas alkaliphila* B4199^T (95.91%), followed by *Thiopseudomonas denitrificans* X2^T (95.56%). The genomic DNA G + C content of strain CY1220^T was 48.6 mol%. The average nucleotide identity values and digital DNA–DNA hybridization values between strain CY1220^T and the type species of *T. alkaliphila* and *T. denitrificans* were in the range of 70.8–71.6% and 19.2–20.0%, respectively, below the thresholds for species delineation. The strain was able to grow utilizing acetic acid and butyric acid (AABA) as the sole carbon source in aerobic conditions. Genomic analysis predicted that the strain could synthesize vitamin B_{12} and ectoine. The predominant cellular fatty acids were $C_{18:1}$ $\omega7c$ and/or $C_{18:1}$ $\omega6c$, $C_{16:0}$, $C_{16:1}$ $\omega7c$ and/or $C_{16:1}$ $\omega6c$ and $C_{12:0}$. The polar lipids comprised diphosphatidylglycerol, unknown polar lipid, phosphatidylethanolamine, phosphatidylglycerol, and phospholipid. Q-8 (2.1%) and Q-9 (97.9%) were detected as the respiratory quinones. Based on its phenotypic, genotypic and genomic characteristics, strain CY1220^T represents a novel species in the genus *Thiopseudomonas*, for which the name *Thiopseudomonas acetoxidans* sp. nov. is proposed. The type strain is CY1220^T (= GDMCC 1.3503 ^{T} = JCM 35747 ^{T}).

Keywords *Pseudomonadaceae* · Polyphasic taxonomy · High-throughput cultivation · Alkali-tolerant · Ectoine

Introduction

The genus *Thiopseudomonas* within family *Pseudomonadaceae* and class *Gammaproteobacteria* was first proposed by Tan et al. in 2015, with *Thiopseudomonas denitrificans* as the type species (Tan et al. 2015), and emended by Rudra et al. (2021). At the time of writing this manuscript, the genus *Thiopseudomonas* includes 2 species with validly published names, which are *T. denitrificans* and *T. alkaliphila* (https://lpsn.dsmz.de/genus/thiopseudomonas).

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Species within this genus have been described as Gram-stain-negative, rod-shaped, motile or non-motile, aerobic or facultatively anaerobic, and alkali tolerant. They were isolated from sludge of anaerobic bioreactor (Tan et al. 2015) and patients (Drobish et al. 2016). *T. denitrificans* is a type of denitrifying bacterium and exhibits the capacity of biological nitrogen removal (Tan et al. 2015). It indicates the significant role of *Thiopseudomonas* members in the natural nitrogen cycle.

At present, anaerobic digestion is the main processes of large-scale centralized food and food waste treatment in cities. The stability and gas-producing efficiency of anaerobic fermentation system are determined by microbial species and diversity. During an investigation of the microbial community structure in anaerobic fermentation process of food waste, strain CY1220^T was isolated from food waste anaerobic fermentation liquid (AFL) of a waste treatment plant, in Beijing, China. It was identified as a potential novel species within the genus *Thiopseudomonas* based on 16S rRNA gene sequence. Culture experiment showed that strain CY1220^T had the capability to utilize acetic acid and butyric acid (AABA) for growth. This study will further investigate its taxonomic status by the polyphasic taxonomic approaches including phenotypic, phylogenetic and genotypic analysis. It is expected to provide a reference for exploring the diversity of facultative anaerobic bacteria in anaerobic fermentation system of food waste, facilitating the mining of bacterial resources, and to offer new insights into the function, ecology, and distribution of *Thiopseudomonas* members.

Materials and methods

Isolation and ecology

Strain CY1220^T was isolated from AFL (pH = 7.86 ± 0.05) collected from an anaerobic fermentation reactor that continuously operated at medium temperature (37 °C) to treat food waste in July 2020 (Haidian District, Beijing, China). To isolate low abundance, oligotrophic, and slow-growing microorganisms, AFL of food waste was diluted using TSB liquid medium (Tryptic soy broth, Oxoid, Thermo Fisher Scientific) and strains were isolated using the high-throughput cultivation method in 96-well cell culture plates, based on Poisson distribution (Zhang et al. 2021). The 96-well cell culture plates were incubated aerobically in the dark at 25 °C for 14 days. The wells showing visible bacterial growth was selected and streaked three times on TSB agar medium at 28 °C for 5–7 days in order to obtain a pure culture. Finally, seventy strains with distinct colonies were obtained, and they were identified via 16S rRNA gene sequencing, respectively. Following a preliminary comparative analysis, approximately 12 strains were identified to have low abundance in the general environment, making them challenging to isolate and cultivate using conventional methods. Based on the results of 16S rRNA gene sequencing, strain CY1220^T was identified as a potential novel species of the genus *Thiopseudomonas* and was deposited in the Guangdong Microbial Culture Collection Center (GDMCC) and the Japan Collection of Microorganisms (JCM). Strain CY1220^T was maintained on TSA plates at 4 °C and preserved as aqueous glycerol suspensions (20%, v/v) at − 80 °C for short-term and long-term storage, respectively. The reference type strains *T. alkaliphila* DSM 100830 ^{T} and *T. denitrificans* KCTC 42076 ^{T} were obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) and Korean Collection for Type Cultures (KCTC), respectively.

16S rRNA phylogeny

Genomic DNA extraction and amplification of 16S rRNA gene with universal bacterial primers (27F and 1492R) were performed as previously reported (Weisburg et al. 1991). The 16S rRNA gene sequence of the strain CY1220^T was obtained by TA clone (vector pCloneEZ-TOPO, CloneSmarter) and sequenced using primers on T vector (M13F and M13R). The 16S rRNA gene sequence was compared with available sequences in the NCBI database (https://www.ncbi.nlm.nih.gov) and the EzBioCloud (https://www.ezbiocloud.net) data libraries (Yoon et al. 2017a, b). Multiple alignment of the related sequences and the construction of phylogenetic consensus trees with three algorithms: neighbour-joining (NJ) (Saitou and Nei 1987), minimum-evolution (ME) and maximum-likelihood (ML) (Fitch 1971), were performed using the software package MEGA X (Kumar et al. 2018). The genetic distances were calculated using the two-parameter method (Kimura 1980), and the topologies

of the phylogenetic trees were evaluated with bootstrapping of 1000 replications (Felsenstein 1985).

Genome features

The draft genome of strain CY1220^T was sequenced using the Illumina HiSeq X Ten platform at the Guangdong Microbial Culture Collection Center (GDMCC), China. Library preparation was performed using the NEXTflex™ Rapid DNA-Seq kit (Bioo Scientific). Quality control of raw sequencing data was conducted using fastp 0.20.0 (https://github.com/OpenGene/fastp) (Chen et al. 2018). Sequence assembly was performed with the SOAPdenovo2 (Luo et al. 2012). Completeness and contamination of the genome data were evaluated using CheckM (v1.2.2) (Parks et al. 2015). The average nucleotide identity (ANI) and the DNA G+C content were estimated by using an online ANI calculator available on the EzBioCloud web server (http://www.ezbiocloud.net/tools/ani) (Yoon et al. 2017a, b), and the average amino acid identity (AAI) was estimated by using the Kostas lab AAI calculator online service (http://enve-omics.ce.gatech.edu/aai/) (Rodriguez-R and Konstantinidis 2014). Digital DNA–DNA hybridization (dDDH) values for whole genome sequences between the strain CY1220^T and closely related type strains were calculated using formula 2 in DSMZ's online service (http://ggdc.dsmz.de) (Meier-Kolthoff et al. 2013). The genome annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP; https://www.ncbi.nlm.nih.gov/genome/annotation_prok) (Tatusova et al. 2016) and Rapid Annotation using Subsystem Technology server (RAST; https://rast.nmpdr.org/) (Aziz et al. 2008). Prediction of tRNAs and rRNAs was performed using tRNAscan-SE (Chan and Lowe 2019) and RNAmmer (Lagesen et al. 2007), respectively. Annotation of gene functions were carried out using eggNOG 5.0 (http://eggnog-mapper.embl.de/) (Huerta-Cepas et al. 2019). Antibiotic resistance genes were identified using the comprehensive antibiotic resistance database (CARD; http://arpcard.mcmaster.ca) (Jia et al. 2017). Genomic islands (GIs; https://www.pathogenomics.sfu.ca/islandviewer/) were predicted by the IslandViewer 4 webserver (Bertelli et al. 2017). The identification of biosynthetic gene clusters for various secondary metabolites were predicted by the antiSMASH 7.0 database with strict detection level (https://antismash.secondarymetabolites.org/) (Blin et al. 2023). Based on a concatenated alignment of 120 conserved bacterial marker genes with the Genome Taxonomy Database Toolkit (GTDB-Tk v. 1.5.1), the phylogenomic tree was constructed using neighbour-joining (NJ) algorithm (Chaumeil et al. 2019). To further estimate the phylogenetic position of strain CY1220^T, a multilocus sequence analysis (MLSA) study based on whole genome sequences was carried out. Phylogenetic trees based on the concatenation of 83 housekeeping genes were built using the automated Multi-Locus Species Tree (MLST) server (https://automlst.ziemertlab.com/) (Alanjary et al. 2019). The 83 housekeeping genes are listed in Table S1. Bacterial genome framework map was drawn using CGView Server (https://proksee.ca/) (Grant and Stothard 2008). The genome sequences of strain CY1220^T were submitted to the National Center for Biotechnology Information database (accession number: JAUCDY000000000).

Phenotypic and physiological properties analysis

The cross section and morphology of cells were examined using transmission electron microscope (JEM-1010, JEOL, Tokyo Japan) and scanning electron microscope (SU8010, HITACHI, Tokyo Japan) with cells grown at 30 °C for 3 days on TSB solid medium. Gram staining was performed using a Solarbio Gram staining kit, following the manufacturer's instructions. Motility was observed by optical microscopy. Strain CY1220^T was inoculated into a serum bottle containing TSB broth bubbled with N_2, followed by shaking cultivation at 30 °C to test its anaerobic growth ability. The temperature and pH range for growth as well as salt tolerance were examined by measuring turbidity at a wavelength of 600 nm using the microplate reader (Infinite® 200 PRO, TECAN) in TSB liquid medium. The growth capacity was determined in TSB broth at 4, 12, 16, 20, 25, 28, 30, 37 and 40 °C. The pH range for growth was investigated between pH 4.0 and 12.0 in increments of 1 pH unit by using the buffer systems (pH 4.0–7.0, 0.2 M citric acid/0.4 M Na_2HPO_4; pH 8.0–9.0, 0.3 M Tris/HCl; pH 10.0–12.0, 0.3 M glycine/0.2 M NaOH) with TSB broth as basal medium. The components of the basal medium for NaCl tolerance test were as follows ($g \cdot L^{-1}$): tryptone, 17.0; neutralised soya peptone, 3.0; dipotassium hydrogen phosphate (K_2HPO_4),

2.5; glucose 2.5; and 0–15% (w/v) sodium chloride was supplemented in 0.5% increments, respectively. Catalase activity was determined by bubble formation in 3% (v/v) H_2O_2, while cytochrome c oxidase activity was analyzed with *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine. Other phenotypic characteristics such as hydrolysis of Tween-80, casein, and starch were investigated as previously described (Tindall et al. 2007). The capacity of anaerobic sulfide oxidation with nitrate were tested as described by Tan et al. (2015). Enzyme activities were determined using the API ZYM system (bioMérieux). GEN III MicroPlates (Biolog) and API 20NE were used for detecting the utilization of carbon sources, acid production, assimilation tests, and other conventional tests. Filter-paper disk diffusion method was used to examine the antibiotic sensitivity. Minimal Salt Medium (MSM, Coolaber Technology) with supplemented AABA as the only carbon sources, respectively, was used to investigate the AABA utilization capability of strain CY1220^T (Liu et al. 2023). 0.1% (v/v) vitamin solution was added to the medium with AABA as the sole carbon source to evaluate the potential role of vitamins. The components of vitamin solution were as follows (mg/L): thiamine hydrochloride (vitamin B_1) 5.0, pantothenic acid 5.0, riboflavin (vitamin B_2) 5.0, biotin 2.0, folate 2.0, vitamin B_{12} 5.0, niacin 5.0, p-aminobenzoic acid 5.0, vitamin B_6 10.0. The 24 h broth culture of strain CY1220^T was inoculated into the above medium (5%, v/v) and shaken at 180 rpm, 30 °C for 72 h, and the optical density at 600 nm (OD_{600}) was measured.

Chemotaxonomic characteristics

For chemotaxonomic analysis of cellular fatty acid, polar lipids and respiratory quinones, strain CY1220^T and closely related *T. denitrificans* KCTC 42076 ^{T} and *T. alkaliphila* DSM 100830 ^{T} were grown on TSB medium at 30 °C and harvested at the late exponential phase after 48 h. Cellular fatty acids were methylated and analyzed by the gas chromatography (HP 6890 Series GC System; Hewlett Packard), using Sherlock Microbial Identification System (version 6.0, MIDI Inc., Newark, DE, US) (Kellogg et al. 2001). Polar lipids of strain CY1220^T were extracted using a chloroform, methanol system and analyzed by two-dimensional thin-layer chromatography (TLC), according to the method of Tindall (1990). Respiratory quinones were extracted from freeze-dried cells (200 mg) with chloroform–methanol (2:1, v/v), and separated from other components by thin-layer chromatography. The purified quinones were identified by HPLC as previously described (Jiang et al. 2008).

Results and discussion

Phylogenetic analysis

The 16S rRNA gene sequence of strain CY1220^T was 1510 bp length and was deposited in the GenBank database (accession number: OQ842236). The comparison of 16S rRNA gene sequences using EzBioCloud showed that strain CY1220^T was phylogenetically related to the members of *Thiopseudomonas*, and was most closely related to strain *T. alkaliphila* B4199^T (95.91%) (Drobish et al. 2016), followed by *T. denitrificans* X2^T (95.56%) (Tan et al. 2015). None of the reference taxa displayed sequence identity to the new isolate above the recommended threshold of 98.7% for delineating bacterial species (Stackebrandt and Ebers 2006). The phylogenetic tree revealed that strain CY1220^T was positioned within the genus *Thiopseudomonas*, forming a separate and stable clade with *T. denitrificans* X2^T and *T. alkaliphila* B4199^T (Fig. 1). These results strongly suggested that strain CY1220^T represented a distinct *Thiopseudomonas* species.

Genome analysis

The final assembled genome sequences of CY1220^T contained 28 contigs, with a size of 2,536,766 bp, an N50 value of 185,885 bp and a sequencing depth of 200×, and the genomic DNA G+C content was 48.6 mol%. The genome comprises 2400 predicted genes, mainly including 2311 protein-encoding genes, 38 tRNA genes, and 3 rRNA genes (5S, 16S, 23S). The genome was estimated at 82.34% completeness and 0.73% contamination. The 16S rRNA gene sequence obtained from genomic data showed 100% identity with the sequence obtained through polymerase chain reaction amplification, confirming the authenticity of the final genome assembly (Chun et al. 2018). The average nucleotide identity (ANI) between CY1220^T and *T. denitrificans* X2^T was 71.6%, and that between CY1220^T and *T. alkaliphila*

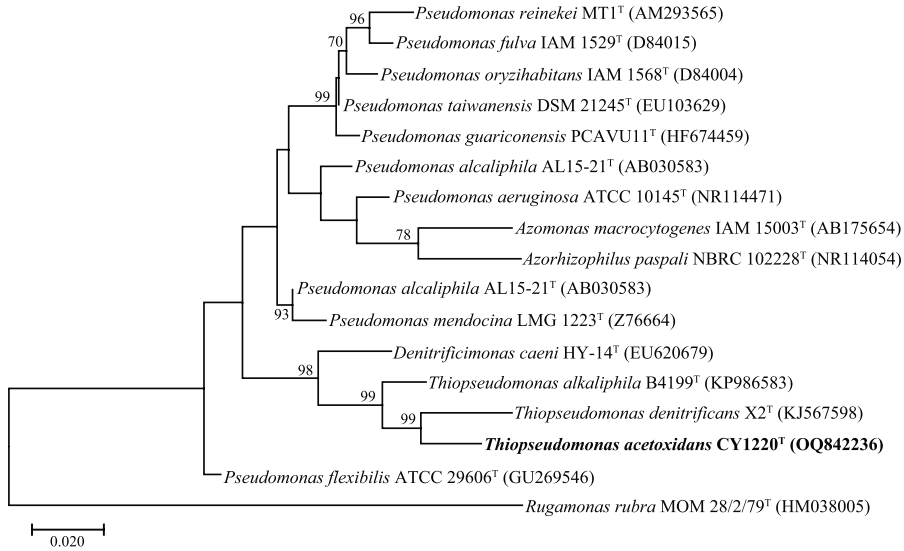

Fig. 1 Phylogenetic tree reconstructed using the maximum-likelihood method based on 16S rRNA gene sequences of strain CY1220^T and other related type strains of recognized species within the genus *Thiopseudomonas*. *Rugamonas rubra* MOM 28/2/79 ^{T} (HM038005) was used as an outgroup. Numbers at branch nodes represent confidence levels (values ≥ 70% are shown) based on 1000 replicates bootstrap samplings. GenBank accession numbers are given in parentheses. Bar, 0.02, represents the number of substitutions per nucleotide site

B4199^T was 70.8%, far below the 95–96% interspecies threshold (Thompson et al. 2013). The amino-acid identity (AAI) between CY1220^T and *T. denitrificans* X2^T was 71.6%, and that between CY1220^T and *T. alkaliphila* B4199^T was 66.1%, far below the 95% interspecies threshold (Luo et al. 2014). The digital DNA–DNA hybridization (dDDH) value between CY1220^T and *T. denitrificans* X2^T was 19.2%, between CY1220^T and *T. alkaliphila* B4199^T was 20.0%, which were significantly below the threshold of 70% for bacterial species classification (Richter and Rosselló-Móra 2009). The detailed comparison of genomic characterization between strain CY1220^T and closely related *Thiopseudomonas* type strains was summarized in Table S2. Whole-genome-based taxonomic analysis revealed that CY1220^T was closely related to *T. denitrificans* X2^T and *Denitrificimonas caeni* DSM 24390 ^{T}, and it was clustered in a separate clade in the GTDB-Tk (Fig. S1) and MLSA phylogenetic trees (Fig. S2). These results provide strong support for our proposal that strain CY1220^T represents a novel species within the genus *Thiopseudomonas*.

The genome of strain CY1220^T was annotated using the RAST. A total of 2,478 coding sequences were annotated and classified into 256 subsystems (Fig. S3), including 5 fatty acid metabolism gene clusters, 21 denitrification gene clusters, 15 phosphate metabolism gene clusters and 17 inorganic sulfur assimilation gene clusters. The presence of these genes showed that strain CY1220^T was closely related to the metabolic cycles of carbon, nitrogen, phosphorus and sulfur during anaerobic fermentation of food waste. A total of 2223 genes of strain CY1220^T was assigned to 20 COG (Cluster of Orthologous Groups) functional categories (Fig. S4) and 920 genes involved in the 5 KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways (Fig. S5). After conducting comparison analysis based on database, it was found that strain CY1220^T had carbon fixation (ko00720), propionate metabolic (ko00640), and butyrate metabolic (ko00650) pathways. The associated carbon fixation genes *accB*, *accC* and *accD* encoded acetyl-CoA carboxylase, which catalyzes CO_2 fixation in the 3-hydroxypropionate cycle (Liu et al. 2022). Genes involved in propionate metabolism, including *prpBCDE, acnB*, and *acnD*, encoded enzymes that convert propionate to pyruvate via the methylcitrate pathway (Suvorova et al. 2012). *scpC*, *phaJ*, *fadA* and *atoB* genes, which encoded enzymes associated with the fatty acid β-oxidation pathway were detected in

strain CY1220^T, and contribute to the degradation of butyrate to acetate (Becker et al. 2023).

The genomic characteristics of strain CY1220^T were further analyzed based on the whole genome framework map (Fig. S6). The results indicated that the genome of strain CY1220^T contained *cobN* genes, which is essential for the biosynthesis of bacterial cobalamin (vitamin B_{12}), suggesting its potential ability to synthesize cobalamin (Antonov 2020). Strain CY1220^T also contained the *norB* gene, which encoded nitric oxide (NO) reductase responsible for the reduction of nitric oxide (NO) produced by bacterial denitrification to nitrogen (N_2) (Truchon et al. 2022). This indicated that strain CY1220^T may play a pivotal role in the nitrogen cycle of food waste anaerobic fermentation system. The *pilY1* gene of strain CY1220^T encoded the PilY1 protein, which is an essential, calcium-dependent regulator for bacterial motility (Orans et al. 2010). Besides, the *msck* gene of strain CY1220^T encoded the MscK protein, functioning as a bacterial mechanosensitive channel that protected cells from structural damage during osmoprotection (van den Berg et al. 2016), which likely contributed to the strain's adaptation to high-salt conditions in food waste. The *mexB*, *mdtB* and *mdtC* genes of strain CY1220^T encoded multidrug efflux transporter MexB and MdtABC efflux pump, which can transport a large variety of compounds, including antibiotics, contributing to multidrug resistance phenotype (Gervasoni et al. 2022; Nagakubo et al. 2002). Genome annotation by PGAP showed that strain CY1220^T contained *ectA* and *ectB* genes, which are involved in ectoine biosynthesis (Zhang et al. 2022). Ectoine is an amino acid derivative produced by bacteria living under high osmotic conditions, which serves as an osmoregulatory compatible solute that protect cells from damage (Fatollahi et al. 2021). The *nrdD* gene involved in anaerobic metabolism was also detected, encoding anaerobic ribonucleoside-triphosphate reductase, which catalyzes the reduction of ribonucleoside triphosphates to deoxyribonucleotides (Torrents et al. 2001). Under strict anaerobic conditions, strain CY1220^T relies on the activity of *nrdD* gene to grow.

Carbohydrate-Active Enzymes (CAZymes) database (http://www.cazy.org/) analysis of the genome sequences of strain CY1220^T revealed the presence of 22 genes encoding glycosyltransferases (GTs), glycoside hydrolases (GHs) and carbohydrate-binding modules (CBMs). RGI analysis in CARD showed that the genome of strain CY1220^T contained four antibiotic resistance genes (*mphG*, *sul2*, *rsmA*, and *aadA5*), which confer resistance to macrolide, sulfonamide, fluoroquinolone, diaminopyrimidine, phenicol, and aminoglycoside antibiotics, and the resistance mechanisms were antibiotic inactivation, target replacement and antibiotic efflux. Using IslandViewer, 30 GIs were identified by integrating islandPath-DIMOB and SIGI-HMM prediction algorithms. In the predicted GIs, no annotated genes associated with virulence, antibiotic resistance, or pathogenicity were identified. Genome mining analysis using antiSMASH database indicated that the strain CY1220^T contained an ectoine biosynthetic gene cluster with a similarity of 66% to known gene clusters in database, consistent with the genome annotation results. *T. denitrificans* X2^T also has an ectoine biosynthetic gene cluster with similarities of 75%.

Morphological, physiological, and biochemical characteristics.

Cells of strain CY1220^T were short rods, Gram-stain-negative, facultatively anaerobic and motile by means of a polar flagellum (Fig. 2). The differential enzyme activities, utilization of carbon sources, and

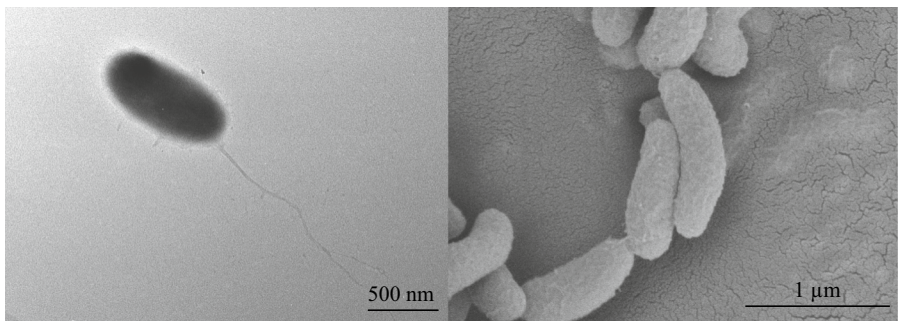

Fig. 2 Transmission electron micrograph (left) and scanning electron micrograph (right) of strain CY1220^T after growing on TSB agar medium for 3 days at 30℃

other physiological and biochemical characteristics of strain CY1220^T and other members of the genus *Thiopseudomonas* were provided in Table 1, and the negative traits of CY1220^T were listed in Table S3. Through experimental verification, strain CY1220^T was unable to grow on carbon-source-free medium and showed no growth with sodium carbonate, sodium bicarbonate and sodium propionate as the sole carbon source. Strain CY1220^T showed incapacity for anaerobic sulfide oxidation using nitrate as the electron acceptor. However, strain CY1220^T displayed some distinct physiological characteristics compared to other members of this genus. The strain CY1220^T can utilize AABA as the sole carbon source for growth (Fig. S7). The group supplemented with vitamins exhibited higher optical density values at 600 nm (OD_{600}), suggesting that vitamin supplementation can better promote the growth of strain, enhance its ability to utilize AABA. The strain CY1220^T also exhibited negative results for oxidase, could hydrolyze aesculin, tested positive for lipase (C14) and valine arylamidase, and weakly positive for cystine arylamidase and acid phosphatase. Additionally, unlike *T. denitrificans* X2^T (Tan et al. 2015), the strain CY1220^T showed negative results for nitrate reduction to nitrite and denitrification. After conducting a genome analysis, it was revealed that the relevant genes encoding nitrate reductases (*napA*/*narG*) were absent in the genome of CY1220^T, which is responsible for the reduction of nitrate to nitrite (Qin et al. 2021). Partially consistent with the results of RGI analysis, strain CY1220^T was resistance to erythromycin (15 μg) of the macrolide class and compound sulfamethoxazole (25 μg) of the sulfonamide class (per disc unless otherwise indicated, Table S4). In addition, strain CY1220^T also exhibited resistant to several other different types of antibiotics, including clindamycin (2 μg) and oxacillin (1 μg) of the penicillin class, vancomycin (30 μg) of the glycopeptide class, and cefradine (30 μg) of the cephalosporin class, which may be related to the existence of multidrug efflux transporter and efflux pump (Table S4).

Table 1 Differential phenotypic characteristics of CY1220^T and closely related members of the genus *Thiopseudomonas*

Characteristic	1[a]	2[b]	3[c]
Motility	+	+	–
Oxidase	–	+	+
Temperature range for growth (℃) (optimum)	20–40 (30)	10–37 (30)	10–42 (20–35)
NaCl tolerance (%) (optimum)	0–5 (2)	0–3 (0–1)	0–8 (4–6)
pH range for growth (optimum)	6–11 (8)	6–10 (8)	6–11 (7.5)
Utilization of:			
Malate	–	+	–
Acetate	+	–	–
Sodium butyrate	w	–	–
D-mannose	–	–	+
α-D-glucose	–	–	+
API 20E:			
Reduction of nitrate to nitrite	–	+	–
D-Glucose assimilation	–	–	+
Gelatin hydrolysis	–	+	–
Aesculin hydrolysis	+	–	–
Enzyme activity:			
Esterase lipase (C8)	+	w	–
Lipase (C14)	+	–	–
Valine arylamidase	+	–	–
Cystine arylamidase	w	–	–
Acid phosphatase	w	–	–
Naphtol-AS-BI-phosphohydrolase	+	+	–
DNA G + C content (mol%)	48.6†	59.0†	47.4†

Strains: 1, CY1220^T; 2, *T. denitrificans* KCTC 42076^T; 3, *T. alkaliphila* DSM 100830^T. All data were obtained in this study. +, positive; w, weakly positive; –, negative

†Data from whole-genome sequencing

The predominant fatty acids (>5%) of CY1220^T included $C_{18:1}$ $\omega7c$ and/or $C_{18:1}$ $\omega6c$ (summed feature 8, 36.0%), $C_{16:0}$ (27.1%), $C_{16:1}$ $\omega7c$ and/or $C_{16:1}$ $\omega6c$ (summed feature 3, 17.6%) and $C_{12:0}$ (5.9%). The cellular fatty acid components of CY1220^T were similar to other reference type strains in the genus *Thiopseudomonas*, but it can be differentiated from them by its distinct fatty acid contents (Table S5). Compared with *T. alkaliphila* DSM 100830 ^{T}, strain CY1220^T had lower proportion of $C_{14:0}$, and compared with *T. denitrificans* KCTC 42076 ^{T}, strain CY1220^T exhibited a greater proportion of $C_{14:0}$ and a lower proportion of summed feature 8. Similar to the type strain *T. alkaliphila* B4199^T, strain CY1220^T contained diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phospholipid (PL), whereas only the former contained unknown polar lipid (L) (Fig. S8) (Drobish et al. 2016). The strain CY1220^T can be differentiated from *T. denitrificans* X2^T by the presence of phosphatidylglycerol (PG) and unknown polar lipid (L), as well as the absence of phosphatidylinositol (PI) (Tan et al. 2015). The respiratory quinones of strain CY1220^T were Q-8 (2.1%) and Q-9 (97.9%), with Q-9 being the major respiratory quinone, similar to *T. alkaliphila* B4199^T (100%) (Drobish et al. 2016). However, in contrast, the major respiratory quinone of *T. denitrificans* X2^T was Q-8 (90.4%) (Tan et al. 2015).

Conclusion

Based on the results of the phylogenetic, phenotypic, physiological, biochemical, chemotaxonomic and genomic data, strain CY1220^T belongs to the genus *Thiopseudomonas*. However, strain CY1220^T can be clearly differentiated from other members of this genus by several significant characteristics, including an obvious phylogenetic distance in 16S rRNA and genomic phylogeny, the absence of nitrate reduction capability, along with negative oxidase activities. In addition, the content of major fatty acids, the polar lipid profiles, the composition and content of respiratory quinones, and the metabolism characteristics including the existence of acetic acid and butyric acid metabolic pathways also showed the specificity of the strain CY1220^T. In conclusion, strain CY1220^T represents a novel species of the genus *Thiopseudomonas*, for which the name *Thiopseudomonas acetoxidans* sp. nov. is proposed. The type strain is CY1220^T (=GDMCC 1.3503 ^{T}=JCM 35747 ^{T}).

Description of *Thiopseudomonas acetoxidans* sp. nov.

Thiopseudomonas acetoxidans (a.cet.o′xi.dans. L. neut. n. *acetum*, vinegar; N.L. pres. part. *oxidans*, oxidizing, N.L. part. adj. *acetoxidans*, acetate-oxidizing, referring to the ability of the type strain to oxidize acetic acid).

Cells are short rods (0.3–0.5×1.0–1.2 μm), facultatively anaerobic, Gram-stain-negative and motile by means of a polar flagellum. Colonies are smooth, light yellow and opaque circular with entire margins and usually 0.5–1.0 mm in diameter after incubation for 5 days at 30 °C on TSA plates. Growth occurs at 20–40 °C (optimum, 30 °C), 0–5.0% (w/v) NaCl (optimum, 2.0%) and pH 6.0–11.0 (optimum, pH 8.0). Catalase is positive, whereas oxidase is negative. It is positive for aesculin hydrolysis but negative for aerobic nitrate reduction to nitrite or nitrogen, urease, indole production, gelatin hydrolysis, dextrin hydrolysis, D-glucose fermentation and D-glucose assimilation. Sulfide is not oxidized anaerobically with nitrate as the electron acceptor. Tests are positive for esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase and naphtol-AS-BI-phosphohydrolase, weakly positive for acid phosphatase and cystine arylamidase. The following carbon sources support growth: acetic acid, L-malic acid, D-galacturonic acid, L-galactonic acid lactone, D-glucuronic acid, glucuronamide. Weakly oxidizes the following substances: fusidic acid, D-serine, D-fructose-6-PO_4, guanidine HCl, tetrazolium violet, bromo-succinic acid, nalidixic acid, aztreonam, sodium butyrate. Strain CY1220^T could not grow on carbon-source-free medium and unable to utilize sodium carbonate, sodium bicarbonate and sodium propionate as sole carbon source. Predominant fatty acids are $C_{18:1}$ $\omega7c$ and/or $C_{18:1}$ $\omega6c$, $C_{16:0}$, $C_{16:1}$ $\omega7c$ and/or $C_{16:1}$ $\omega6c$. The polar lipids include DPG, L, PE, PG and PL. The predominant respiratory quinone is ubiquinone Q-9. The genome has a DNA G+C content of 48.6 mol% and a draft genome size of 2.54 Mb with 28 contigs and 2355 coding DNA sequences (CDS).

The type strain, CY1220^T (=GDMCC 1.3503^T=JCM 35747^T), was isolated from anaerobic fermentation liquid of food waste (37 °C).

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Author contributions MMA and GZZ conceived the study and drafted the manuscript. YJL and XXL collected the samples. MMA and RNL performed the experiment and analyzed the data.

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Data availability The 16S rRNA gene sequence of *Thiopseudomonas acetoxidans* CY1220^T has been assigned the accession number OQ842236 in the GenBank database of the NCBI. The draft genome sequences of CY1220^T have been deposited under the accession number JAUCDY000000000.

Declarations

Competing interests The authors declare no competing interests.

Conflict of interest The authors declare that there are no conflicts of interest.

Consent for publication The authors approved for the publication.

Ethical approval This article does not contain any studies with human participants or animals.

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