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ORIGINAL PAPER

Azotosporobacter soli gen. nov., sp. nov., a novel nitrogen-fixing bacterium isolated from paddy soil

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Abstract A nitrogen-fixing strain designated SG130^T was isolated from paddy soil in Fujian Province, China. Strain SG130^T was Gram-staining-negative, rod-shaped, and strictly anaerobic. Strain SG130^T showed the highest 16S rRNA gene sequence similarities with the type strains *Dendrosporobacter quercicolus* DSM 1736^T (91.7%), *Anaeroarcus burkinensis* DSM 6283^T (91.0%) and *Anaerospora hongkongensis* HKU 15^T (90.9%). Furthermore, the phylogenetic and phylogenomic analysis also suggested strain SG130^T clustered with members of the family *Sporomusaceae* and was distinguished from other genera within this family. Growth of strain SG130^T was observed at 25–45 °C (optimum 30 °C), pH 6.0–9.5 (optimum 7.0) and 0–1% (w/v) NaCl (optimum 0.1%). The quinones were Q-8 and Q-9. The polar lipids were phosphatidylserine (PS), phosphatidylethanolamine (PE), glycolipid (GL), phospholipid (PL) and an unidentified lipid (UL). The major fatty acids (> 10%) were iso-$C_{13:0}$ 3OH (26.6%), iso-$C_{17:1}$ (15.6%) and iso-$C_{15:1}$ F (11.4%). The genomic DNA G+C content was 50.7%. The average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) values between strain SG130^T and the most closely related type strain *D. quercicolus* DSM 1736^T (ANI 68.0% and dDDH 20.3%) were both below the cut-off level for species delineation. The average

The GenBank accession numbers for 16S rRNA gene and genome sequence of strain SG130^T are OR142399 and JAUAOA000000000, respectively.

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amino acid identity (AAI) between strain SG130^T and the most closely related type strain *D. quercicolus* DSM 1736^T was 63.2%, which was below the cut-off value for bacterial genus delineation (65%). Strain SG130^T possessed core genes (*nifHDK*) involved in nitrogen fixation, and nitrogenase activity (106.38 μmol C_2H_4 g^{-1} protein h^{-1}) was examined using the acetylene reduction assay. Based on the above results, strain SG130^T is confirmed to represent a novel genus of the family *Sporomusaceae*, for which the name *Azotosporobacter soli* gen. nov., sp. nov. is proposed. The type strain is SG130^T (=GDMCC 1.3312^T=JCM 35641^T).

Keywords Polyphasic taxonomy · Nitrogen fixation · Paddy soil

Abbreviations

ANI	Average Nucleotide Identity
AAI	Average Amino acid Identity
dDDH	Digital DNA-DNA Hybridization
POCP	Percentage Of Conserved Proteins
PS	Phosphatidylserine
PE	Phosphatidylethanolamine
UL	Unidentified Lipid
GL	Glycolipid
PL	Phospholipid

Introduction

The family *Sporomusaceae*, proposed by Campbell et al. (2015), belongs to the phylum *Bacillota*, class *Negativicutes*, order *Selenomonadales*, and comprises 15 validated genera. All members of the family *Sporomusaceae* are Gram-staining-negative, rod-shaped, motile and they have been isolated from various anaerobic environments, including blood culture (Woo et al. 2005), rice field soils (Ouattara et al. 1992; Strömpl et al. 1999), and living oak trees (Strömpl et al. 2000). Currently, there are 27 validly and 1 invalidly published species (https://lpsn.dsmz.de/family/sporomusaceae) (Parte et al. 2020) within this family.

Nitrogen (N) is crucial for the growth and yield of rice, playing an indispensable role in its development. To maintain the high yields, substantial quantities of nitrogen chemical fertilizer are administered to rice paddies. However, a mere 30–50% of this nitrogen fertilizer is assimilated by the plants, with the remaining 50–70% either consumed by microorganisms or leached into the soil, which causes a host of environmental concerns, emerges as a critical challenge in agricultural management (Tyagi et al. 2022). Biological nitrogen fixation (BNF) offers a viable, eco-friendly alternative to chemical fertilizers, capable of providing the necessary nitrogen without the associated environmental degradation or compromise in crop yield. This approach holds significant promise and potential advantages for sustainable agriculture (Bhattacharjee et al. 2008; Mus et al. 2016). During the exploration of diverse bacteria able to fix N_2 in paddy soils, a nitrogen-fixing bacterium SG130^T was isolated. This strain showed high 16S rRNA gene sequence similarities to members of the family *Sporomusaceae*, but also exhibited distinct differences. Consequently, the present study used polyphasic characterization to confirm the taxonomic status of strain SG130^T.

Materials and Methods

Isolation, culture and preservation

Strain SG130^T was isolated from paddy soil of Fujian Agriculture and Forestry University, Fuzhou City, Fujian Province, China (26°56′42.00″ N 119°22′37.56″ E). After removing debris, soils were put in a 100 mL serum bottle with 45 mL sterile water, mixed and exposed to mixed N_2:CO_2 (v/v, 80:20) gas for 30 min. Then, a standard soil dilution suspension was spread on modified Reasoner's 2A (R2A) medium plates [R2A medium (Hopebio, China) containing 40 mM disodium fumarate (Macklin, China)]. The plates were cultured at 30 °C for a week in a Whitley DG250 anaerobic workstation (Don Whitley Scientific, UK). The obtained single colonies were repeatedly streaked on modified R2A medium plates and incubated anaerobically at 30 °C for a week until pure colonies were obtained and stored as glycerol suspensions (20%, w/v) at -80 °C. Additionally, they were preserved at -80 °C in modified R2A broth with 10–15% (v/v) dimethyl sulfoxide (DMSO). All the above procedures were performed under the anaerobic conditions. The reference strain *Dendrosporobacter quercicolus* DSM 1736^T was

ordered from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen).

16S rRNA gene amplification and phylogeny

Genomic DNA was extracted using a genomic DNA extraction kit (Shanghai Generay Biotech Co., Ltd, China) according to the manufacturer's instructions. The 16S rRNA gene was amplified and sequenced using the primers 27F (5′-AGAGTTTGATCMTGG CTCAG-3′) and 1492R (5′-GGTTACCTTGTTACG ACTT-3′). The obtained 16S rRNA gene sequence was compared with similar sequences in the EZBioCloud server (Yoon et al. 2017a). Phylogenetic trees were constructed using the neighbor-joining (NJ) (Saitou and Nei 1987), maximum-likelihood (ML) (Felsenstein 1981) and maximum-parsimony (MP) (Fitch 1971) methods implemented with MEGA version X (Kumar et al. 2018) after multiple alignments of the data with ClustalW program (Thompson et al. 1994) using the Kimura two-parameter model (Kimura 1980). The reliability of each branch was evaluated by bootstrap analysis based on 1000 replications (Felsenstein 1985).

Morphological, physiological and biochemical characteristics

Gram-staining was performed using a Gram Staining kit (Solarbio Life Science, China) according to the manufacturer's instructions. Colony morphology was observed on modified R2A medium plates after three days' incubation under optimal growth conditions. Cell morphology was determined by transmission electron microscopy (Hitachi HT7700, Japan) using the negative staining method using 1% phosphotungstic acid. Endospores were examined according to Schaeffer-Fulton staining method (Kamlage 1996). Catalase activity was determined by observing bubble production after the application of 3% (v/v) hydrogen peroxide solution, a positive reaction being indicated by the production of bubbles. Oxidase activity was evaluated using 1% (w/v) tetramethyl-p-phenylenediamine (Kovacs 1956). Aerobic growth of strain SG130^T was tested using modified R2A agar plates for a week under air.

The temperature range for growth on modified R2A agar plates was determined at 6, 10, 16, 20, 25, 30, 35, 37, 40, 42, 45 and 50 °C for 7 days. Tolerance to NaCl concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1%, w/v) and pH (5.0–10.0 with an increment of 1.0 pH unit) were confirmed on modified R2Aagar plates for 7 days. The pH of the modified R2A medium was adjusted using a buffer system (20 mM MES for adjusting pH 5.0–6.5, 20 mM HEPES for adjusting pH 7.0–8.0 and 20 mM Tricine for adjusting pH 8.5–12.0) which was described by Liu et al. (2022). Other biochemical characteristics of strains SG130^T and *D. quercicolus* DSM 1736^T were examined using API ZYM, API 20E and API 50CH strips (bioMérieux, France) following the manufacturer's instructions. Fermentation growth using different carbon sources (20 mM) as substrates i.e. lactate, fumarate, pyruvate, formate and acetate was examined in liquid medium A (Choi et al. 2016) with 100% N_2 headspace.

Chemotaxonomic analysis

Chemotaxonomic characteristics of strain SG130^T were observed using several standard methods under similar conditions. The polar lipids were extracted as described by Minnikin et al. (1979) and analyzed by two-dimensional thin layer chromatography (Collins and Jones 1980). For the determination of cellular fatty acids, biomass was collected after cultivation at 30 °C for 3 days. The cellular fatty acids were extracted and analyzed according to the standard protocol of the Microbial Identification System (MIDI) on a GC system (model 6890, Agilent) (Sasser 1990). Quinones of strains SG130^T and *D. quercicolus* DSM 1736^T were extracted and purified as described by Collins et al. (1977) and analyzed by HPLC (The mobile phase was set to methanol:ethanol = 2:1 (v/v), the flow rate was 1.00 mL·min^{-1} and the column temperature was 40 °C) (Kroppenstedt 1982).

Genome sequencing and analysis

The genome sequence of strain SG130^T was sequenced by Illumina NovaSeq PE150 at the Beijing Novogene Bioinformatics Technology Co., Ltd (Beijing, China). A library was reconstructed with Illumina NovaSeq PE150. GeneMarks was utilized to retrieve the correlated coding genes (Besemer et al. 2001). KEGG (Kyoto Encyclopedia of Genes and Genomes) database was used to perform gene functions predictability (Kanehisa et al. 2004,

2006). Transfer RNA (tRNA) and ribosomal RNA (rRNA) genes were predicted and analyzed with tRNAscan-SE version 1.3.1 and rRNAmmer (Lowe and Eddy 1997; Lagesen et al. 2007). The genomic relatedness was estimated based on the average nucleotide identity (ANI), average amino acid identity (AAI) and digital DNA-DNA hybridization (dDDH), which were calculated using the EzBioCloud platform (Yoon et al. 2017b), the AAI calculator (Rodriguez-R and Konstantinidis 2014) and Genome-to-Genome Distance Calculator version 3.0 (Meier-Kolthoff et al. 2022), respectively. The percentage of conserved proteins (POCP) was calculated using a Python script (https://github.com/2015qyliang/POCP) with the formula $\frac{C1+C2}{T1+T2} \times 100\%$, where C1 and C2 represent the conserved number of proteins in the two genomes being compared, respectively, and T1 and T2 represent the total number of proteins in the two genomes being compared, respectively (Qin et al. 2014). The phylogenomic tree was constructed by UBCG2 based on 81 bacterial core genes (Kim et al. 2021) and GTDB-Tk v.1.5.1 using a concatenated alignment of 120 conserved bacterial single-copy genes (Parks et al. 2018; Chaumeil et al. 2019).

Nitrogen fixation activity

The nitrogen-fixing activity was determined by the acetylene reduction activity (ARA) method based on the C_2H_2 reduction to C_2H_4 by nitrogenase (Postgate 1972). C_2H_4 production in the gaseous phase was measured by gas chromatography equipped with a fused silica column (Porapak; Hychrom) as described previously (Nakajima et al. 2012). The protein content was determined by Pierce BCA protein assay kit (Thermo Scientific; America) according to the manufacturer's instructions. Strain SG130^T was grown in 20 mL of modified R2A liquid medium at 30 °C for 3 days under anaerobic conditions. Then cells of strain SG130^T were washed three times using sterilized ammonium-free liquid medium under sterile conditions. The washed cells were resuspended in 20 mL volumes sterilized ammonium-free medium in a 60 mL bottle and incubated at 30 °C after being sealed under mixed gas He:C_2H_2 (90:10, v/v). The negative control was set up using pure He gas in replacement of He/C_2H_2 gas.

Results and Discussion

16S rRNA gene sequence and phylogenetic analysis

Strain SG130^T shared high 16S rRNA gene sequence similarities with the type strains *D. quercicolus* DSM 1736^T (91.7%), *Anaeroarcus burkinensis* DSM 6283^T (91.0%) and *Anaerospora hongkongensis* HKU 15 ^{T} (90.9%) in the family *Sporomusaceae*. The maximum-likelihood tree (Fig. S1) showed that strain SG130^T clustered with members of the family *Sporomusaceae*. The cluster was further found to be robust when the trees were reconstructed using the neighbor-joining (Fig. S2) and maximum-parsimony methods (Fig. S3).

Morphological and biochemical characterization

Cells of strain SG130^T were Gram-staining-negative, motile, endospore-forming (Fig. S4), and strictly anaerobic. Under TEM, cells of strain SG130^T appeared to be rod-shaped and showing the presence of flagella (Fig. 1). On modified R2A medium plates, colonies were light green, circular, raised, smooth and transparent. Strain SG130^T grew optimally at pH 7.0 and 30 °C, while *D. quercicolus* DSM 1736^T grew optimally at pH 7.3 and 25–30 °C. Strain SG130^T and *D. quercicolus* DSM 1736^T were both catalase and oxidase negative (Strömpl et al. 2000). Strain SG130^T could use glucose, mannitol, inositol, saccharose, amygdalin and arabinose as the sole carbon sources. In API 50CH strip, strain SG130^T could use most carbon sources as substrates to produce acids, e.g., glycerol, erythritol, L-arabinose, ribose, *D*-xylose, galactose, fructose, mannose, sorbose, α-Methyl-*D*-glucoside, arbutin, esculin, cellobiose, maltose, lactose, trehalose (Table S1). Additionally, fumarate and lactate could be fermented by strain SG130^T. Comparison of characteristics of strain SG130^T and closely related *Sporomusaceae* members are listed in Table 1, and different characteristics between strain SG130^T and its closest reference strain *D. quercicolus* DSM 1736^T was showed in Table S1.

Chemotaxonomy

Major quinones of strain SG130^T were Q-8 and Q-9, which was consistent with the type strain *D. quercicolus* DSM 1736^T, as there were no menaquinones

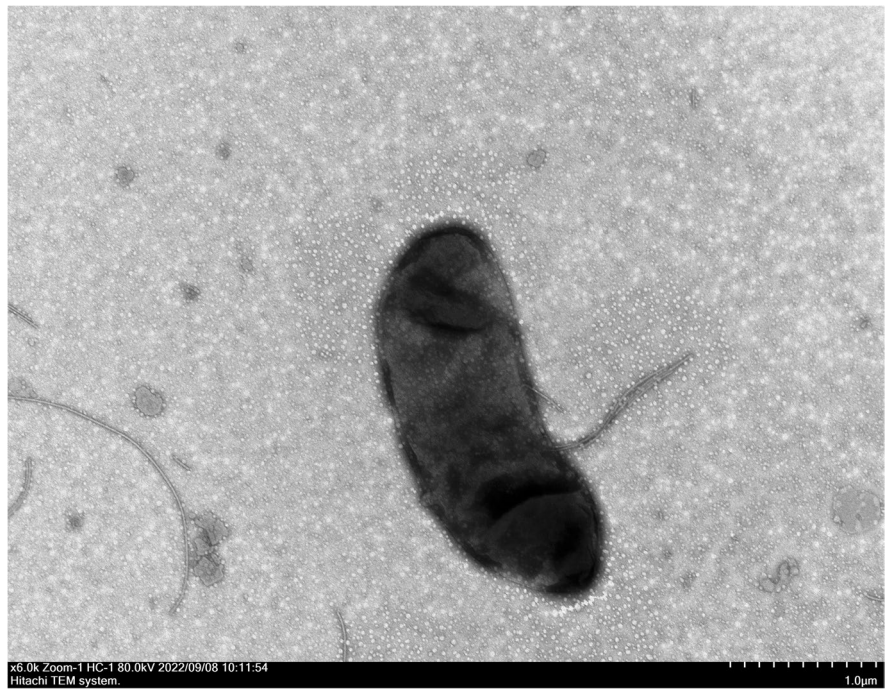

Fig. 1 Transmission electron micrograph of strain SG130^T grown on modified R2A agar at 30 °C for 3 days

present. The major fatty acids (>5.0%) of strain SG130^T were iso-$C_{13:0}$ 3OH (26.6%), iso-$C_{17:1}$ (15.6%), iso-$C_{15:1}$ F (11.4%), iso-$C_{11:0}$ (9.3%), $C_{15:1}$ (9.1%), $C_{16:0}$ (7.9%) and iso-$C_{15:0}$ (7.0%), which were a bit different from those of its type strains *D. quercicolus* DSM 1736^T and *A. burkinensis* DSM 6283^T. It was indicated that iso-$C_{13:0}$ 3OH, iso-$C_{15:1}$ F, iso-$C_{15:0}$, $C_{16:0}$ and iso-$C_{17:1}$ of stain SG130^T were significantly higher than those of *D. quercicolus* DSM 1736^T and *A. burkinensis* DSM 6283^T. However, $C_{15:0}$, $C_{15:1}$, $C_{16:1}$ and $C_{17:1}$ of stain SG130^T were much lower than those of *D. quercicolus* DSM 1736^T and *A. burkinensis* DSM 6283^T. Detailed differences

Table 1 Differential characteristics of present studied strains and its closely related species of the family *Sporomusaceae*

Characteristics	1	2	3	4
Length (μm)	2.0–3.0	1.2–2.7	1.5–3.0	3.1–14.3
Width (μm)	0.4–0.5	0.4–0.6	0.5	0.4–0.6
Temperature (ºC) (Optimum)	25–45 (30)	20–45 (25–30)	13–43 (35)	ND (37)
pH (Optimum)	6.0–9.5(7.0)	6.5–9.0 (7.3)	5.3–8.4 (6.8)	ND (ND)
NaCl (%) (Optimum)	0–1 (0.1)	0–1.1 (0)	0–1.2 (0)	ND
DNA G+C (%)	50.7	52.0–54.0	44.1	46.8
Cell shape	straight or slightly curved rods	straight rods	curved or spiral-shaped rods	straight or slightly curved rods
Spore formation	+	+	-	+
Catalase	-	-	ND	-
Oxidase	-	-	ND	-

1, *Azotosporobacter soli* SG130^T; 2, *Dendrosporobacter quercicolus* DSM 1736^T (Strömpl et al. 2000); 3, *Anaeroarcus burkinensis* DSM 6283^T (Strömpl et al. 1999); 4, *Anaerospora hongkongensis* HKU 15^T (Woo et al. 2005). All strains had motility. Note: +, positive; -, negative; ND, not detected

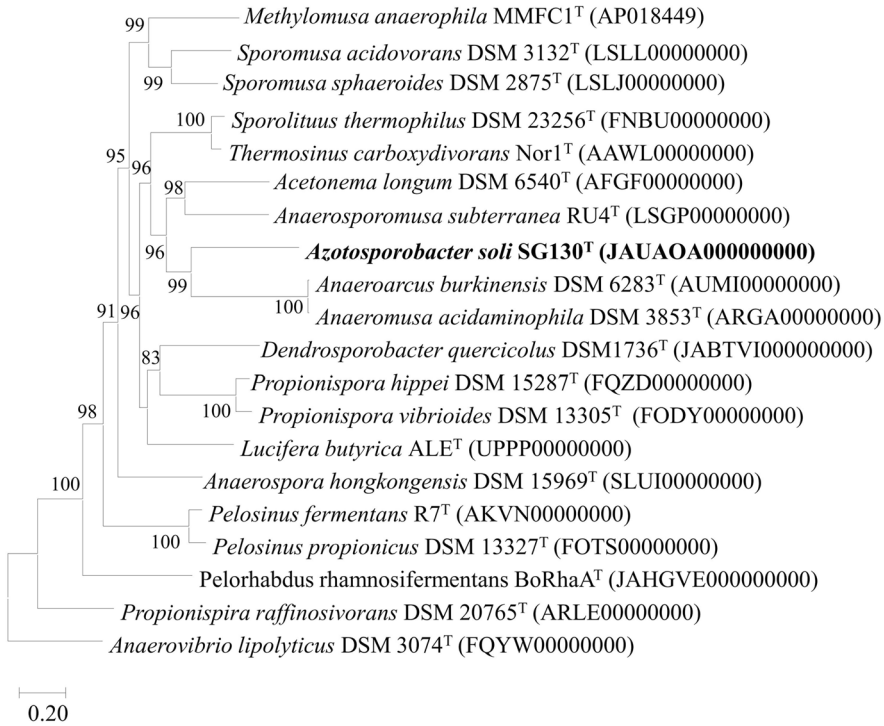

Fig. 2 Phylogenomic tree based on the core gene sequence by UBCG2 showing the relationship between strain SG130^T and closely related type species. Bootstrap values based on 1000 replications are listed as percentages at branch points. Bar, 0.2 substitutions per site. *Anaerovibrio lipolyticus* DSM 3074^T was used as an outgroup

of fatty acid (> 5.0%) profiles in strain SG130^T are mentioned in Table S2. The polar lipids of strain SG130^T were phosphatidylserine (PS), phosphatidylethanolamine (PE), glycolipid (GL), phospholipid (PL) and an unidentified lipid (UL) (Fig. S5). There was no amino-phospholipid (APL) detected in strain SG130^T, while *D. quercicolus* DSM 1736^T and *A. burkinensis* DSM 6283^T possessed APL (Strömpl et al. 1999, 2000).

Genome analysis

The genome size of strain SG130^T was 3.77 Mbp and containing 40 contigs. A total of 74 tRNAs and 11 rRNAs were predicted for strain SG130^T (Table S3). In a phylogenomic tree, strain SG130^T clustered with members of the family *Sporomusaceae* (Figs. 2 and S6). The genomic DNA G+C content was 50.7%. The ANI values between strain SG130^T and its most closely related species *D. quercicolus* DSM 1736^T, *A. burkinensis* DSM 6283^T and *A. hongkongensis* HKU 15^T were 68.0%, 67.8% and 67.7%, respectively, which were much lower than the ANI cut-off value for species definition (95–96%) (Meier-Kolthoff et al. 2022; Richter and Rosselló-Móra 2009). The dDDH values between strain SG130^T and its most closely related species *D. quercicolus* DSM 1736^T, *A. burkinensis* DSM 6283^T and *A. hongkongensis* HKU 15^T were 20.3%, 18.1% and 21.2%, respectively, which were all lower than the standard cut-off value for species delineation (70%) (Meier-Kolthoff et al. 2013; Goris et al. 2007). The AAI values between strain SG130^T and members of the family *Sporomusaceae* were 60.9–63.0% (Table S4), which were all lower than the recommended threshold value of 65% for a bacterial genus (Rodriguez-R and Konstantinidis 2014). The POCP values between strain SG130^T and closely related taxa were 43.3–56.4%, which were higher than the threshold of the original POCP for delineation of prokaryotic genera, but many recent studies have shown that strains may still belong to a different genus when the POCP values between strains were higher than 50% and lower than 65% (Wirth and Whitman 2018). So, it is suggested that strain SG130^T is a novel genus within the family *Sporomusaceae*.

There were 2931 protein-encoding genes in the KEGG database, 2637 protein-encoding genes in the GO database and 2636 protein-encoding genes in the COG database, respectively (Table S2). Metabolism

encompassed the highest number of genes (1278 genes) among the six classifications of KEGG pathways (Fig S7), followed by genetic information processing (154 genes). The GO analysis revealed that the predicted genes could be categorized into three groups: molecular function, cellular component, and biological process (Fig. S8). Among these, the four main pathways identified were metabolic process (1596 genes), cellular process (1568 genes), catalytic activity (1530 genes) and binding (1312 genes).

In the rice field ecosystem, biological nitrogen fixation (BFN) into ammonium by microorganisms is an important process, which has significant implications for agricultural production and environmental protection (Pandey et al. 2019). A number of studies have reported that *nifHDK* encoding nitrogenase, play a key role in microbial nitrogen fixation (Dos Santos et al. 2012). Genome analysis showed strain SG130^T possessed a *nif* core gene cluster (*nifHDKENBVUJ*). Therefore, it was inferred that strain SG130^T had the potential ability to fix N_2. In addition, *D. quercicolus* DSM 1736^T possessed the genes *nifBESUHDKJ*, suggesting it is also capable to perform nitrogen fixation.

The biochemical process of nitrate reduction has closely been correlated to the paddy field environment, which may affect crop yield and provide ecological benefits (Nojiri et al. 2020). The KEGG pathway predicted that strain SG130^T lacks *nrtABCD*, *napAB*, *nasABC* and *nar* gene clusters, preventing strain SG130^T transporting nitrate into the cell and undergoing assimilatory and dissimilatory nitrate reduction (Maeda and Omata 2009, 1997; Blasco et al. 1990; Ogawa et al. 1995). In the genome of *D. quercicolus* DSM 1736^T, the aforementioned gene clusters were also not identified, suggesting that *D. quercicolus* DSM 1736^T does not possess the capacity to reduce nitrate. However, the operon *nrfAH* was identified on the genome indicating that strain SG130^T has the potential to reduce nitrite (Simon et al. 2000). This was in contrast to strain *D. quercicolus* DSM 1736^T, which did not contain the *nrfAH* gene cluster.

For sulfur metabolism, KEGG pathway indicated that the genome of strain SG130^T lacked the gene *cysW* (Green et al. 1989), thus it was unable to transfer extracellular sulfate into the cell, whereas *D. quercicolus* DSM 1736^T possessed the gene *cysW* and may have the potential to transfer extracellular sulfate. Both strains SG130^T and *D. quercicolus* DSM 1736^T lacked the *soe* gene cluster (*soeABC*) (Dahl et al. 2013), rendering them unable to complete the process of sulfate reduction.

Nitrogen fixation

BNF driven by diazotrophs occurred frequently in flooded paddy soil (anaerobic condition), which was considered as an important nitrogen-fixing site (Guo et al. 2023). Previous studies have shown that there is a close relationship between anaerobic nitrogen-fixing bacteria and rice yield, their colonization in flooded paddy soil could significantly promote rice growth (Govindarajan et al. 2008). Up to now, there has been no study about the nitrogen-fixing potential of members of the family *Sporomusaceae*. Our findings therefore provide new insights into novel function of the family *Sporomusaceae*. To further confirm the nitrogen-fixing ability of strain SG130^T, the nitrogenase activity was tested using the acetylene reduction assay (ARA). The nitrogenase activity of strain SG130^T reached 106.38 μmol C_2H_4 g^{-1} protein h^{-1} (Fig S9).

Based on phenotypic, phylogenetic, biochemical, chemotaxonomic and genome analysis, strain SG130^T represents a novel species of a novel genus of the family *Sporomusaceae*, for which the name *Azotosporobacter soli* gen. nov., sp. nov. is proposed.

Description of Azotosporobacter gen. nov

Azotosporobacter (A.zo.to.spo.ro.bac.ter. N.L. neut. n. *azotum*, nitrogen; from French masc. n. *azote*, nitrogen; from Gr. pref. *a-*, not (inseparable prefix); from Gr. fem. n. *zôê*, life; from N.Gr. fem. n. *azôê*, not sustaining life; Gr. fem. n. *spora*, spore; N.L. masc. n. *bacter*, a rod; N.L.masc. n. *Azotosporobacter*, a spore-forming nitrogen rod).

Cells are Gram-staining-negative, curved-rod-shaped, flagellated, motile, strictly anaerobic. Oxidase and catalase are negative. The predominant respiratory quinones are Q-8 and Q-9. The major fatty acids are iso-$C_{13:0}$ 3OH, iso-$C_{17:1}$ and iso-$C_{15:1}$ F. Genomic DNA G + C content is 50.7%. The genus is part of the family *Sporomusaceae*. The type species is *Azotosporobacter soli*.

Description of Azotosporobacter soli gen. sp. nov

Azotosporobacter soli (so'li. L. gen. n. soli, of soil)

Cells are Gram-staining-negative and strictly anaerobic. The ellipsoidal endospore is located at subterminal or intermediate position. Colonies are light green, circular, raised, smooth and transparent. Growth is observed at 25–45 °C (optimum 30 °C), pH 6.0–9.5 (optimum pH 7.0) and 0–1% NaCl (w/v) (optimum 0.1%). Catalase, oxidase and nitrate reduction are negative. Tests for arginine dihydrolase, tryptophan deaminase, citrate, glucose, mannitol, inositol, sucrose, amygdalin and arabinose utilization, Voges–Proskauer reaction are positive, but tests for urease, ortho-nitrophenyl-*β*-galactoside, lysine decarboxylase, ornithine decarboxylase, H_2S and indole production, hydrolysis of gelatin, utilization of sorbitol, rhamnose and melibiose are negative. In API 50 CH system, it can produce acids from glycerol, erythritol, L-arabinose, ribose, *D*-xylose, *β*-methyl-*D*-xyloside, galactose, glucose, fructose, mannose, sorbose, inositol, mannitol, *α*-Methyl-*D*-glucoside, *N*-Acetylglucosamine, amygdalin, arbutin, esculin, salicin, cellobiose, maltose, lactose, saccharose, trehalose, inulin, melezitose, raffinose, amyloid, glycogen, gentiobiose, *D*-turanose, gluconate and 2-keto-gluconate. In API ZYM, alkaline phosphatase, esterase (C4), acid phosphatase and naphthol-AS-BI-phosphohydrolase are produced but esterase lipase (C8), leucine arylaminase, valine arylamidase, chymotrypsin, *α*-glucosidase, *N*-acetyl-*β*-glucosaminidase, lipase (C14), cystine arylamidase, trypsin, *α*-galactosidase, *β*-glucuronidase, *β*-glucosidase, *β*-galactosidase, *α*-mannosidase and *β*-fucosidase not. Fumarate and lactate can be used as fermentation substrates, but pyruvate, formate and acetate not. The major quinones present are Q-8 and Q-9. The polar lipids are phosphatidylserine (PS), phosphatidylethanolamine (PE), glycolipid (GL), phospholipid (PL) and an unidentified lipid (UL). The major fatty acids are iso-$C_{13:0}$ 3OH, iso-$C_{17:1}$ and iso-$C_{15:1}$ F. The genomic DNA G + C content is 50.7%.

The type strain, SG130^T (= GDMCC 1.3312^T = JCM 35641^T), was isolated from paddy soil in Fujian Province, China.

The GenBank accession numbers for the 16S rRNA gene and the genome sequence are OR142399 and JAUAOA000000000, respectively.

Author contributions SGZ and GHL designed research and project outline. CJX performed deposition and polyphasic taxonomy. LY performed isolation. RT, SH and SY performed genome analysis. CJX drafted the manuscript. SGZ, HA, CR and GHL revised the manuscript. All authors read and approved the final manuscript.

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Declarations

Competing interests The authors declare no competing interests.

Conflicts of interests The authors declare that they had no conflict of interest.

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