

Azoarcus taiwanensis sp. nov., a denitrifying species isolated from a hot spring

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Abstract The strain NSC3^T, a novel, facultative, chemolithotrophic, denitrifying, alkaliphilic, sulfide-oxidizing bacterium isolated from a hot spring in Yang-Ming Mountain, Taiwan, was Gram negative, rod shaped, and motile by single polar flagella and grew facultatively by adopting a denitrifying metabolism. The 16S rRNA sequence analysis revealed that strain NSC3^T belongs to beta subclass of the Proteobacteria and most closely related to *Azoarcus evansii* KB740^T (95.44 %), *Azoarcus toluvorans* Td-21^T (95.21 %), *Azoarcus toluyticus* Tol-4^T (95.08 %), and *Azoarcus toluclasticus* MF63^T (94.94 %). The phylogenetic analyses based on 16S rRNA gene sequences indicated that the strain NSC3^T formed a distinct lineage in the Betaproteobacteria and that it exhibited the highest level of sequence similarity with species of the genera *Azoarcus* (95.28–93.13 %). The major fatty acids of the type strain were C_{16:0} (26.9 %), C_{16:1w7c} (28.9 %), C_{18:0} (9.6 %), and C_{18:1w7c/w6c} (29.9 %). The DNA G+C content of genomic DNA was 63.7 mol%. On the basis of the 16S rRNA sequence similarity, phenotypic and genotypic characteristics, and chemotaxonomic data, the strain NSC3^T could be differentiated from other species of the genus *Azoarcus*. Therefore, strain NSC3^T (equal to BCRC 80111^T and DSM 24109^T) is proposed as a novel species in genus *Azoarcus*, for which the name *Azoarcus taiwanensis* sp. nov. is proposed. The strain NSC3^T is deposited in

Bioresource Collection and Research Center, Taiwan, under the reference number BCRC 80111^T, and German Collection of Microorganisms and Cell Cultures, Germany (DSMZ), with DSM 24109^T.

Keywords Sulfide oxidizing · Facultative · Denitrification · Isolation · Identification

Introduction

Eutrophication of rivers and deterioration of water sources, which are hazards to human health, have been reported due to discharge of nitrogenous compound containing waste into the environment. The World Health Organization established the limit for nitrates in drinking water at 50 mg NO₃⁻/L (11.3 mg NO₃⁻-N/L), while the US Environmental Protection Agency has set this limit at 10 mg NO₃⁻ (2.3 mg NO₃⁻-N/L) (Directive 98/83/EC).

Oxidation of sulfide by chemolithoautotrophic denitrifying bacteria can lead to the formation of elemental sulfur or sulfate, depending on the physiological conditions. So far, genus *Azoarcus* is represented by validly published seven species. The genus *Azoarcus* primarily can be divided into two ecologically different groups of bacteria. The first group of bacteria is associated with grass roots which apparently does not survive well in root-free soil. Based on the nitrogen fixing potential, strains isolated from the rhizosphere microflora of Kallar grass were named as *Azoarcus indigenus* and *Azoarcus communis* (Reinhold-Hurek et al. 1993). Second group could be soil-borne strains that can degrade aromatic hydrocarbons under denitrifying condition. These include aromatic compound degrading strain such as *Azoarcus evansii* (Anders et al. 1995), toluene-degrading denitrifiers as *Azoarcus toluyticus* (Zhou et al. 1995), and resorcinol-degrading obligate denitrifying bacterium as *Azoarcus anaerobius* (Springer et al. 1998). The more detailed

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taxonomic examination and reclassification resulted to proposal of two additional species, *Azoarcus toluvorans* and *Azoarcus toluclasticus* (Song et al. 1999). In this study, a new member of genus *Azoarcus*, isolated from a hot spring in Yang-Ming Mountain, Taipei, Taiwan, is described and proposed as a novel member of the genus *Azoarcus* on the basis of the recommendations for the description of a novel species provided by Kampfer et al. (2003).

In this study, we demonstrate the sulfide-oxidizing ability under denitrifying condition by a new strain, *Azoarcus* sp. NSC3 isolated from Yang-Ming Mountain, Taipei County, Taiwan.

Materials and methods

Medium, isolation, and characterization

A sample of soil mixed with water was collected from hot sulfur spring in Yang-Ming Mountain, New Taipei City, Taiwan. To enrich culture in alkaliphilic condition, samples were mixed with nitrate, sulfide, and acetate containing medium under suitable stoichiometry (per liter): 0.755 g of KNO_3 , 1.5 g of $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$, 0.25 g of NH_4Cl , 0.05 g of K_2HPO_4 , 1.5 CU of NaHCO_3 , 0.44 g of $\text{CH}_3\text{COONa}\cdot 3\text{H}_2\text{O}$, and 1 mL of trace element solution (Pfennig and Lippert 1966). The pH value was 9.0. After several enrichment of the culture, it forms aggregate of elemental sulfur granules. The enrichment samples were diluted and spread onto the solidified agar plates containing sulfide and nitrate (NS medium) for isolating autotrophic bacteria. The cell morphology of the strain was observed by a phase-contrast microscope and transmission electron microscopy (Hitachi H-7100) by staining with 1 % (w/v) phosphotungstic acid. Cells of isolates grown before and after in NS medium were used in microscopic characterization. The widths and lengths given represent the averages of measurements of several cells.

Growth test

The strain growths were tested in temperature between 4 and 50 °C and pH between 6 and 11. NaCl tolerance of the strains was tested with supplement with 1 to 6 % (w/v) NaCl. Kinetic test and stoichiometry for the isolated were investigated for autotrophic growth. The tests described above were carried out at 37 °C under anoxic/anaerobic condition, flushed with argon for 20 min. For heterotrophic growth, mineral medium was supplemented with acetate instead of sulfide.

Cell characterization

Cell morphology and motility of exponentially grown cultures were observed by phase-contrast confocal laser

scanning microscope (Leica TCS SP2 Confocal Spectral Microscope Imaging System, GmbH, Wetzlar, Germany). The presence of flagellum and surface morphology of the exponentially grown cultures were observed using transmission electron microscopy by staining with 1 % (w/v) phosphotungstic acid. Growth of strain NSC3^T under denitrifying condition was tested using acetate as a carbon source or sulfide as electron donor. The strain NSC3^T and its related type strains were characterized for its optimum growth at the temperature range of 0–50 °C, pH 4.5–11, requirement of NaCl, and its tolerance in minimal medium containing nitrate, acetate, and bicarbonate. Gram staining and oxidase and catalase activities were determined using standard methods described by Smibert and Krieg (1994), while catalase activity was also confirmed using hydrogen peroxide. The biochemical testing of strain NSC3^T was carried out using API 20E and API 50 CH test strips, API 50 CHB/E medium, and the Biolog identification system. The carbon utilization capabilities of strain NSC3^T was determined using Biolog EcoPlate™ (Biolog Inc., Hayward, CA) according to the manufacturer's instructions and also confirmed using traditional cultivation methods using minimal medium supplemented with test substrates at 37 °C. The Biolog EcoPlate™ 96 (microwell plate) in triplicate was inoculated with test strains, incubated at 37 °C for 48–72 h, and absorbance was recorded using microplate reader. Antibiotic susceptibility of strain NSC3^T was tested using disc diffusion technique using antibiotic discs (Oxoid, Cambridge, UK) containing penicillin G (10 U) and streptomycin (25 µg). The sensitivities to gentamicin (5 µg), streptomycin (10 µg), vancomycin (10 µg), rifampicin (5 µg), and amoxicillin (25 µg) were tested in minimal medium using traditional method. The fatty acids were extracted, saponified, and methylated according to the protocol of the Sherlock Microbial Identification System. The DNA G+C content was determined as described by Manaia et al. (2003).

Analytical methods

Thiosulfate concentrations were analyzed by cyanolysis (Kelly et al. 1969); sulfide concentrations were measured by methylene blue method according to the Standard Method (APHA 1998). Nitrate and nitrite were detected by high-performance liquid chromatography (HPLC) as described by Zecca et al. (1998). Samples before sulfur precipitation were filtered through glass fiber filter paper. The biomass protein was calculated according to the Standard Method (APHA 1998). The N_2 , CO_2 , and N_2O gas contents in the headspace samples were analyzed by gas chromatography (GC-9A; Shimadzu, Tokyo, Japan) equipped with a thermal conductivity detector and column packed with molecular sieve 5A 80/100 mesh. Oven temperature was set at 70 °C, and helium was the carrier gas flowing at 30 ml min⁻¹.

Column, injector port, and detector temperatures were 70, 120, and 120 °C, respectively. Concentrations of ammonium ions were determined using Nessler's method (APHA 1998). Sulfide concentrations were analyzed calorimetrically using the methylene blue scheme (Moest 1975). The VFA contents were analyzed by HPLC (LCP 4100 Pump, LCD 2083 Detector; ECOM, Prague) with an autosampler (HTA HT3000L; ECOM, Prague). The mobile phase was 25 mM phosphate buffer (pH 2.5) at a flowrate of 1 ml min⁻¹. Biologically produced elemental sulfur was extracted into chloroform and analyzed using HPLC with wavelength of 263 nm and mobile phase of chloroform–methanol ratio of 1:9. To verify the existence of NO or NOS in the culture, a cell-permeable NO specific probe 4,5-diaminofluorescein diacetate (DAF-2DA) was used. Culture was withdrawn by syringe and centrifuged at 10,000 rpm to remove the supernatant. Ten micrometers of DAF-2DA was added and incubated for 60 min at 37 °C.

16S rDNA sequence determination and phylogenetic analysis

The 16S rRNA gene sequence was determined after PCR amplification using primers F27 (5'-CCA GAG TTT GAT CMT GGC TCA G-3') and R1492 (5'-TAC CTT GTT ACG ACT T-3). The amplified PCR product was checked by electrophoresis and purified by GeneSpin™ (Protect Technology Co., Ltd., USA). The purified PCR-amplified 16S rDNA was sequenced using the ABI Prism model 3730 (version 3.2) DNA sequencer. This generated 1,406 bp gene sequence. The closest relatives were identified by comparing the 16S rRNA gene of strain with those of previously reported strains using the Ez Taxon (Chun et al. 2007) and NCBI BLAST program. The sequences were aligned by the multiple alignment ClustalW, and phylogenetic trees were generated by neighbor joining, maximum likelihood, minimum evolution, and unweighted pair group with arithmetic mean (UPGMA) by using the MEGA 4.0.2 software (Kumar et al. 2004) using bootstrap resampling methods with 1,000 replications (Tamura et al. 2007). The topology of phylogenetic tree obtained by these methods were further confirmed using different out-groups.

The 16S rRNA sequence was deposited to GenBank under accession number GQ389714.

Results

Strain identification

The strain (NSC3^T) was isolated from the soil sample from a hot sulfur spring in Yang-Ming Mountain, New Taipei City,

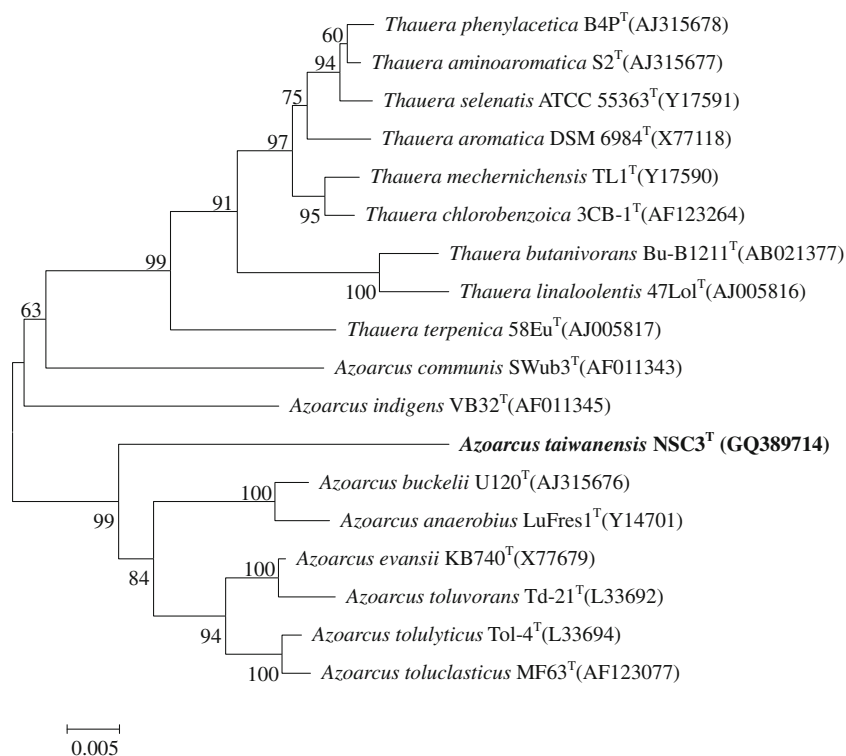
Taiwan. Based on 16S rRNA gene sequence, the closest relatives of the strain NSC3^T were *A. evansii* KB740^T (95.44 %), *A. toluvorans* Td-21^T (95.21 %), *A. toluclasticus* Tol-4^T (95.08 %), *A. toluclasticus* MF63^T (94.94 %), *Azoarcus buckelii* U120^T (94.58 %), *A. anaerobius* LuFres1^T (94.30 %), *Thauera butanivorans* Bu-B1211^T (93.41 %), *A. communis* SWub3^T (93.13 %), and *Thauera phenylacetica* B4P^T (92.949 %). The phylogenetic tree based on 16S rRNA revealed that the strain formed a distinct robust lineage among type strains of *Azoarcus* spp. within beta-subclass of Proteobacteria (Fig. 1 and Figs. S1–S4 in Electronic supplementary material). According to Stackebrandt and Goebel (1994), if rRNA sequence similarities are 97 % or above, DNA–DNA relatedness studies of novel and closely related type strains are required for species differentiation. However, in the case of strain NSC3^T, 16S rRNA sequence similarities below this level justify validity of 16S rRNA-based description of a new species of the genus *Azoarcus*. The isolate has been deposited in Bioresource Collection and Research Center, Taiwan, under the reference number BCRC 80111^T, and German Collection of Microorganisms and Cell Cultures, Germany (DSMZ), with DSM 24109^T.

Strain characterization

The major fatty acids of the strain NSC3^T were C_{16:0} (26.9 %), C_{16:1w7c} (28.9 %), C_{18:0} (9.6 %), and C_{18:1w7c/w6c} (29.9 %) (Table 1). The major fatty acid component C_{16:1w7c} was 28.9 % in strain NSC3^T, while it was 44.7 % in *A. toluclasticus* Tol-4^T, 51.0 % in *A. toluvorans* Td21^T, 51.8 % in *A. toluclasticus* MF63^T, and 46.9 % in *A. evansii* KB 740^T which distinguished strain NSC3^T from these type strains. The higher content of C_{16:1} in *A. toluvorans* Td21^T, *A. toluclasticus* MF63^T, *A. communis* SWub3^T, *A. indigenus* VB32^T, and *A. evansii* KB 740^T differentiated strain NSC3^T from these type strains.

The biochemical, physiological, and morphological characteristics of strain NSC3^T were summarized in Table 2. The denitrification and sulfide removal potential of strain is shown in Fig. S5. The strain grew well on minimal mineral media with NaCl (1–3 % w/v, optimum at 1 %), 1.5 g NaHCO₃ and with optimum growth at 35–37 °C. No growth was observed at 4 °C and above 55 °C. The strain grew between pH 6.5 and 10.5 with optimal growth occurring at pH 9.0. The strain can grow chemolithotrophically with sulfide as electron donor and nitrate as electron acceptor. The strain NSC3^T was positive to nitrate reductase, nitrite reductase, catalase, and oxidase; motile with single polar flagella (Fig. 2); Gram negative; and consistent with those of *Azoarcus* sp. The cells were sensitive to gentamicin (5 µg), streptomycin (10–25 µg), and vancomycin (10 µg) and resistant to penicillin G (10 U), rifampicin (5 µg), and amoxicillin (25 µg).

Fig 1 Neighbor-joining phylogenetic tree strain NSC3^T with closely related species. Bootstrap values (expressed as percentages of 1,000 replications) >50 % are given at nodes. The *bar* represents 1 substitution per 100 nucleotides



Strain description

The *Azoarcus taiwanensis* sp. nov. is a rod-shaped bacterium of 0.9–2.2 × 0.5–0.7 μm, Gram negative, appears as a single cell or in pairs, and motile by means of a single polar flagellum. The colonies are circular, smooth, convex, and

transparent; are positive to catalase, oxidase, nitrate reductase, and sulfide oxidase; and grew at 15–40 °C, optimally at a temperature of 37 °C, pH 6.5–10.5, with optimum growth at pH 9.0. In Biolog microplate test, the strain assimilated D-alanine, L-alanine, L-alanyl-glycine, D-glucosamine, L-glutamine, glycine, L-methionine, L-ornithine, L-phenylalanine,

Table 1 Major fatty acid compositions (percent) of the strain NSC3^T and closely related *Azoarcus* species

Fatty acid	NSC3 ^T	<i>Azoarcus toluyliticus</i> Tol-4 ^T	<i>Azoarcus. evansii</i> KB 740 ^T	<i>Azoarcus toluvorans</i> Td21 ^{Ta}	<i>Azoarcus toluclasticus</i> MF63 ^{Tb}	<i>Azoarcus communis</i> SWub3 ^{Tc}	<i>Azoarcus indigens</i> VB32 ^{Tc}
C _{10:0} 3-OH	2.5	7.7	8	3.1	5.5	6.5	5
C _{12:0}	5.5	9.5	6.9	4.7	5.6	Tr	6.5
C _{14:0}	1.1	1.5	0.8	0.8	0.4	4.1	Tr
C _{16:1w7c}	28.9	44.7	46.9	51.0	51.8	ND	ND
C _{16:1w9c}	Tr	Tr	Tr	ND	ND	51.4	33.5
C _{16:1w5c}	Tr	Tr	0.5	ND	0.8	ND	ND
C _{16:0}	26.9	31.0	27.5	29.7	25.6	25.8	34.6
C _{17:0} cyclo	Tr	1.2	11.3	ND	ND	Tr	11.3
C _{18:1}	2.7	2.6	8.2 ^d	10.3 ^d	9.3 ^d	9.7	8.3
C _{18:0}	9.6	0.5	ND	ND	ND	Tr	ND
C _{18:1w7c/w6c}	29.9	Tr	Tr	ND	ND	ND	ND

Tr trace quantities (less than 1 %), ND not determined

^a Data from Song et al. 1999

^b Data from Zhou et al. 1995

^c Data from Reinhold-Hurek et al. 1993

^d Mixture of 7c/9w/12w, 9c/12w/7c, adn 12w/9w/7c

Table 2 Phenotypic characteristics of strain NSC3^T and related species

Characteristic	NSC3 ^T	<i>Azoarcus tolyticus</i> Tol-4 ^T	<i>Azoarcus evansii</i> KB740 ^T	<i>Azoarcus toluvorans</i> Td-21 ^{Ta}	<i>Azoarcus toluclasticus</i> MF63 ^{Tb}	<i>Azoarcus communis</i> SWub3 ^{Tc}	<i>Azoarcus indigenus</i> VB32 ^{Tc}	<i>Azoarcus anaerobius</i> LuFres1 ^{Td}
Cell morphology	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Rods
Cell width (μm)	0.5–0.7	0.8–1	0.6–0.8	0.8–1.0	0.6–0.8	0.8–1.0	0.5–0.7	1.5
Cell length (μm)	0.5–2.2	1.4–2.8	1.5–3.0	1.4–2.8	1.7–4.0	1.5–3.0	2.0–4.0	2.7–3.3
Flagella	Single	Single	Single	Single	Single	Single	Single	ND
Catalase	+	+	+	+	–	+	+	–
Nitrite reductase	+	–	+	ND	ND	ND	ND	+
Chemolithotrophic growth with sulfide	+	–	–	ND	ND	ND	ND	–
Optimum growth temperature (°C)	37	30	37	30	30	37	40	28
Optimum growth (pH)	9.0	8.0	7.8	ND	ND	ND	ND	7.2
NaCl concentration for growth (% w/v)	1 %	0 %	0 %	ND	ND	0–2 %	0–1 %	0 %
DNA G+C content (mol%)	63.7	66.9	67.5	67.8	67.3	62.4	66.6	66.5
Acetate	+	–	+	ND	+	+	+	+
Benzoate	+	+	+	ND	+	–	–	+
Citrate	+	–	–	ND	ND	+	–	ND
D-Glucose	–	+	+	+	–	–	–	–
4-Hydroxybenzoate	+	+	–	ND	+ ^e	+	+	+
D-Malate	–	+	–	ND	ND	+	+	–
L-Malate	+	+	+	ND	ND	+	+	–
Phenol	+	–	+	–	+ ^e	ND	ND	ND
L-Phenylalanine	+	–	+	–	+	+	+	+
Pyruvate	+	–	+	ND	+	+	+	+
L-Serine	+	–	+	ND	ND	–	–	ND
Sucrose	+	+	+	ND	+	–	–	ND
Toluene	+ ^e	+ ^e	–	+ ^e	+	–	–	ND

ND not determined

^aData from Song et al. 1999

^bData from Zhou et al. 1995

^cData from Reinhold-Hurek et al. 1993

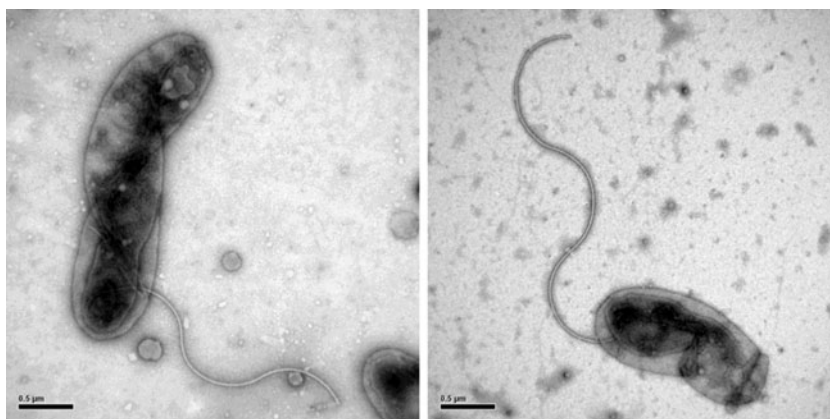
^dData from Springer et al. 1998

^eGrown under denitrifying condition

L-proline, D-serine, L-serine, L-valine, β-D-allose, D-arabinose, L-arabinose, cellulose, 2-deoxy-D-ribose, D-fructose, L-fucose, D-glucose-6-phosphate, L-lyxose, maltotriose, D-melibiose, palatinose, D-raffinose, D-ribose, stachyose, sucrose, D-tagatose, acetic acid, butyric acid, capric acid, citric acid, formic acid, fumaric acid, L-lactic acid, DL-malic acid, L-malic acid, propionic acid, pyruvic acid, succinic acid, acetoacetic acid, γ-aminobutyric acid, δ-aminovaleric acid, bromosuccinic acid, 3-0-β-D-galactopyranosyl-D-arabinose, D-galacturonic acid, D-gluconic acid, D-glucuronic acid, D-glucosaminic acid, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, α-hydroxyglutaric acid-γ-lactone, itaconic acid, α-ketobutyric acid, 5-keto-D-

gluconic acid, α-ketoglutaric acid, D-lactic acid methyl ester, 3-methylglucose, α-methyl-D-glucoside, α-methyl-D-mannoside, methyl pyruvate, β-methyl-D-xyloside, oxalomalic acid, L-pyroglutamic acid, sorbic acid, tricarballic acid, amygdalin, sec-butylamine, DL-carnitine, chondroitin sulfate C, α-cyclodextrin, dextrin, gelatin, inosine, inulin, laminarin, DL-octopamine, tryptophan, Tween 20, Tween 80, urea, xylan, adonitol, 2-aminoethanol, dulcitol, L-erythritol, isopropanol, lactitol, maltitol, propanol, xylitol, anthrone, and 2,3-butanone. Under anaerobic denitrifying condition, the strain NSC3^T utilized different reduced sulfur compounds such as sulfide, sulfite, tetrathionate, and thiosulfate and various aromatic compound included benzene, benzoic acid, o-cresol, p-cresol,

Fig. 2 Transmission electron micrographs of cells of strain NSC3^T showing a single polar flagellum



m-cresol, 4-hydroxybenzoic acid, *p*-hydroxyphenylacetic acid, quinoline, pyridine, terephthalic acid, toluene (denitrifying), phenol, and *m*-xylene. The G+C content was 63.7 %. The cells are sensitive to gentamicin, streptomycin, amoxycilin, and vancomycin and resistant to penicillin G, rifampicin, and amoxicillin. The major fatty acids of the strain NSC3^T were C_{16:0} (26.9 %), C_{16:1w7c} (28.9 %), C_{18:0} (9.6 %), and C_{18:1w7c/w6c} (29.9 %).

Discussion

Strain identity

In phenotypic characteristics, chemolithotrophic growth in presence of sulfide, high NaCl tolerance, and alkaliphilic nature could differentiate strain NSC3^T from *A. evansii* KB740^T, *A. toluvorans* Td-21^T, *A. toluyticus* Tol-4^T, *A. toluclasticus* MF63^T, *A. buckelii* U120^T, *A. anaerobius* LuFres1^T, and *A. communis* SWub3^T. Based on GC content, strain NSC3^T (63.7 %) could be distinguished from *A. toluyticus* Tol-4^T (66.9 %), *A. toluvorans* Td-21^T (67.8 %), *A. toluclasticus* MF63^T (67.3 %), *A. indigenes* VB32^T (66.6 %), *A. evansii* KB740^T (67.5 %), and *A. anaerobius* LuFres1^T (66.5 %). Strain NSC3^T could be distinguished from *A. toluyticus* Tol-4^T, *A. toluvorans* Td-21^T, and *A. toluclasticus* MF63^T by positive to nitrate reductase and citrate assimilation, while it can be differentiated from *A. communis* SWub3^T and *A. indigenes* VB32^T based on L-serine, sucrose, and toluene utilization potential. The antibiotic resistance to penicillin G (10 U), rifampicin (5 μg), and amoxicillin (25 μg) distinguished strain NSC3^T from other strains of genus *Azoarcus*. The cells were sensitive to gentamicin (5 μg), streptomycin (10–25 μg), and vancomycin (10 μg). Resistance of reported type strains of *Azoarcus* spp. to antibiotics has not been extensively tested. The 16S rRNA sequence similarities and phylogenetic analysis by neighbor joining, maximum parsimony, maximum likelihood,

and UPGMA suggested affiliation of strain NSC3^T to genus *Azoarcus* with a robust clad with members of *Azoarcus*. The phylogenetic tree topology and clustering of strain NSC3^T were further confirmed by selecting different out-groups. The strain NSC3^T was isolated from a hot spring, while strain *A. toluyticus* Tol-4^T was isolated from petroleum-contaminated soil, *A. evansii* KB740^T from a creek sediment, *A. indigenes* VB32^T from stem bases/root of Kallar grass, *A. communis* SWub3^T from root piece of Kallar grass, *A. toluvorans* Td-21^T from petroleum-contaminated soil, and *A. anaerobius* LuFres1^T from anoxic sewage sludge. Based on phenotypic features, biochemical tests, fatty acid composition comparisons, 16S rRNA gene sequence similarities, and phylogenetic analysis, the strain NSC3^T represents a novel member of genus *Azoarcus*, for which the name *A. taiwanensis* sp. nov. is proposed.

Facultative autotrophic denitrifiers

The denitrifying sulfide removal (DSR) process was proposed to achieve simultaneous conversion of nitrate to N₂, sulfide to elementary sulfur, and organic substances to CO₂ via balanced growth of both autotrophic and heterotrophic denitrifiers (Chen et al. 2008a, b). Chen et al. (2008c) noted that when concentrations of sulfide were too high to inhibit activities of heterotrophic denitrifiers (*Pseudomonas aeruginosa* and *Sulfurospirillum* sp. in that study), nitrite would be accumulated so the activities of autotrophic denitrifiers (*Sulfurovum* sp. and *Paracoccus denitrificans*) would be inhibited, hence leading to complete breakdown of the DSR process. The facultative autotrophic bacteria (FAB) that can grow mixotrophically and heterotrophically with oxidization of reduced sulfur compounds may be used to lift the major limitations of balanced growth of autotrophic and heterotrophic bacteria in the DSR reactor (Lee et al. 2013). A group of FAB were proposed, including *Thiobacillus delicatus*, *Thiobacillus pantotrophus*, *Pseudomonas stutzeri*, and *Paracoccus denitrificans* (Robertson and Kuenen 1983; Sorokin et al. 1996, 1999;

Gupta 1997; Nemati et al. 2001; Sorokin 2003). The strain NSC3^T represents a novel member of FAB that can have potential to be applied in DSR-related processes.

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