Constrictibacter antarcticus gen. nov., sp. nov., a cryptoendolithic micro-organism from Antarctic white rock

Kozo Yamada,¹ Wakao Fukuda,¹ Yuka Kondo,² Yuki Miyoshi,² Haruyuki Atomi² and Tadayuki Imanaka¹

Correspondence
Tadayuki Imanaka
imanaka@sk.ritsumeikai.ac.jp

¹Department of Biotechnology, College of Life Sciences, Ritsumeikan University, 1-1-1 Nojihigashi, Kusatsu, Shiga 525-8577, Japan

²Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Katsura, Nishikyo-ku, Kyoto 615-8510, Japan

A Gram-negative, non-spore-forming, ovoid to rod-shaped aerobic or microaerobic bacterium, strain 262-8^T, was isolated from a cavity within white rock collected in Antarctica. Strain 262-8^T grew at 5–30 °C (optimum 25 °C), at pH 6–8 (optimum approximately pH 7) and with 0.1–2.0 % (w/v) NaCl (optimum 0.5 % NaCl). The addition of tryptone or yeast extract was essential for growth. Strain 262-8^T was able to utilize organic compounds such as ribose, pyruvate and succinate in the presence of a low concentration of tryptone. Ubiquinone 10 was the major respiratory quinone. The major fatty acids were C_{18:1}, C_{16:0} and C_{18:0}. The G + C content of the genomic DNA was 69.8 mol%. Comparative analyses of 16S rRNA gene sequences and physiological characteristics indicated that strain 262-8^T was a phylogenetically novel bacterium that should be classified in a new genus of the family *Rhodospirillaceae*, for which the name *Constrictibacter antarcticus* gen. nov., sp. nov. is proposed. The type strain of the type species is 262-8^T (=JCM 16422^T=ATCC BAA-1906^T).

The climate in Antarctica, with its extreme cold and low concentration of organic materials, makes life difficult for most organisms. Moreover, the land surface is seasonally exposed to strong UV radiation (Bargagli, 2005; Hughes & Lawley, 2003). Therefore, the diversity of plants and animals in Antarctica is limited. However, a great diversity of micro-organisms has been found in Antarctica's icy environments, such as permafrost, polar oceans, snow, sea ice and a cryoconite hole (Prisco, 2007; Tindall, 2004). For example, micro-organisms, including cyanobacteria, have been found in perennial Antarctic lake ice in the McMurdo Dry Valleys (Prisco *et al.*, 1998) and methanogenic archaea have been found in a frozen lake, Lake Fryxell (Karr *et al.*, 2006). In addition, cryptoendolithic communities have also been found in the pore space of rock (de la Torre *et al.*, 2003). Inhabiting the interior of rock is considered to be one alternative for survival in the harsh climate. Continuous summertime sunlight can raise the internal temperature of rocks above freezing point, up to 10 °C above the ambient temperature.

A white rock sample from the Skallen region in Antarctica was collected by the summer party of the 46th Japanese

Antarctic Research Expedition in 2004–2005. Interestingly, the interior of the white rock was colourful (green, pink, yellow and brown; Supplementary Fig. S1, available in IJSEM Online), which suggested the existence of a cryptoendolithic community containing cyanobacteria. Indeed, more than ten different bacterial species, including strain 262-8^T, were obtained from the rock sample. In this study, the identification of strain 262-8^T was performed. Analysis based on 16S rRNA gene sequences indicated that strain 262-8^T belonged to the order *Rhodospirillales*. The order *Rhodospirillales* in the class *Alphaproteobacteria* contains two families, *Rhodospirillaceae* and *Acetobacteraceae*, and the order is morphologically, metabolically and ecologically diverse. Most genera in family *Rhodospirillaceae* grow photoheterotrophically under anoxic conditions in the light and chemotrophically in the dark (Garrity *et al.*, 2005). Members of the family *Acetobacteraceae* are aerobic acetic acid bacteria that produce acetic acid during their respiration (Sievers & Swings, 2005a). Detailed comparisons of strain 262-8^T with members of these families made it clear that the new strain represented a novel genus in the family *Rhodospirillaceae*.

White rock was sampled in the Skallen region, Antarctica, and stored at 4 °C for 6 months. To screen for autotrophic bacteria, rock samples were crushed, added to BG-11 liquid medium (ATCC medium 616) and incubated at 25 °C in

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 262-8^T is AB510913.

Two supplementary figures are available with the online version of this paper.

the light. The resulting culture was transferred onto BG-11 solid medium. A green filamentous bacterium (strain 262-1), covered with several heterotrophic bacteria, was found by microscopic observation. Multiple attempts to purify single colonies were made but complete removal of the contaminant strains was not achieved. Therefore, an unpurified culture of strain 262-1 was inoculated into $0.10 \times \text{LB}$ agar (1^{-1} tap water: 1.0 g tryptone, 0.5 g yeast extract, 5.0 g NaCl, 20 g agar) and incubated at 25°C in the dark. Although strain 262-1 could not grow, several bacterial colonies were formed. The bacterium forming the smallest colony was designated strain 262-8^T.

The standard medium for cultivation of strain 262-8^T was $0.25 \times \text{LB/MA}$ medium [1^{-1} deionized water: 2.5 g tryptone, 1.25 g yeast extract, 6.3 g Marine Art SF-1 (Tomita Pharmaceutical)]. Strain 262-8^T could also grow in $0.25 \times \text{LB/ASW}$ medium [1^{-1} tap water: 2.5 g tryptone, 1.25 g yeast extract, 5.0 g NaCl, 0.75 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 1.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.25 g $(\text{NH}_4)_2\text{SO}_4$, 0.05 g NaHCO_3 , 0.075 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.125 g KCl, 0.105 g KH_2PO_4 , 0.0125 g NaBr, 0.005 g $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$, 0.025 g $\text{Fe}(\text{NH}_4)_3$ citrate] and marine broth 2216 (BD Difco). For solid media, 20 g agar 1^{-1} was added. Strain 262-8^T could be stored as a 20% (v/v) glycerol suspension at -80°C for at least 2 years.

Growth of strain 262-8^T at $5\text{--}37^{\circ}\text{C}$, at pH 4–10 and with 0–100 g NaCl 1^{-1} was determined in $0.25 \times \text{LB}$ medium (1^{-1} deionized water: 2.5 g tryptone, 1.25 g yeast extract, 5.0 g NaCl). To measure growth rate, the isolate was cultivated in $0.25 \times \text{LB/MA}$ medium under optimal growth conditions until OD_{660} reached 0.1, the preculture was inoculated into the same medium and the increase in OD_{660} was measured. Catalase and oxidase activity was analysed with standard methods (Smibert & Krieg, 1994). Hydrogen sulfide production and motility were determined using a modified semi-solid agar medium

[1^{-1} deionized water: 0.6 g peptone, 2.0 g tryptone, 0.2 g $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$, 0.2 g $\text{Na}_2\text{S}_2\text{O}_3$, 5.0 g NaCl, 3.5 g agar] (Holdeman *et al.*, 1977). To observe spore formation, cells in death phase and after heat treatment were observed by phase-contrast microscopy. Biochemical characteristics were analysed using the API 20 NE and API ZYM systems and sensitivity to antibiotics was examined using the ATB VET system (bioMérieux). Substrate utilization was also studied by comparing growth in a basal medium (1.0 g tryptone, 6.3 g Marine Art SF-1) with and without a carbon source (1%). The organic compounds tested were: sugars (arabinose, fructose, galactose, glucose, lactose, maltose, mannose, raffinose, ribose, sucrose, trehalose and xylose), hydroxyl carbons (erythritol, ethanol, glycerol, mannitol and methanol), organic acids (acetate, adipate, benzoate, butyrate, caproate, caprylate, citrate, fumarate, formate, glycolate, malate, propionate, pyruvate, succinate and valerate) and L-amino acids (alanine, arginine, aspartate, glutamate and glycine). Strain 262-8^T was cultivated microaerobically using the AnaeroPack MicroAero system (Mitsubishi Gas Chemical) and anaerobically in $0.25 \times \text{LB/MA}$ medium under light and dark conditions. CaCO_3 was used to test acetic acid production (Yamada *et al.*, 2000). Growth of strain 262-8^T was also tested on $0.25 \times \text{LB/ASW}$ medium containing (1^{-1}) 5.0 g CaCO_3 and 15.0 g agar for 3 weeks in aerobic, microaerobic and anaerobic conditions. To determine whether strain 262-8^T possessed pigments, the spectra of methanol extracts from cells grown on $0.25 \times \text{LB/MA}$ agar for 2 weeks were analysed by spectrometry (U-0080D; Hitachi).

Cell size and shape were examined by differential interference microscopy (BX51; Olympus) and cell ultra-structure was observed by scanning electron microscopy (S-4700; Hitachi) and atomic force microscopy (OLS3500; Olympus). For scanning electron microscopy,

Fig. 1. Micrographs of cells of strain 262-8^T, using differential interference microscopy (a), scanning electron microscopy (b), confocal laser scanning microscopy and nucleic acid staining with fluorescence reagent (c) and atomic force microscopy (d). Bars, 20 μm (a), 2 μm (b–d).

stationary-phase broth was used and the sample was prepared according to the manufacturer's instructions: the sample was freeze-dried with ES-2020 and metallized with a metal evaporator (E-1030). For observation by atomic force microscopy, cells growing in $0.25 \times \text{LB/MA}$ medium were collected by centrifugation for 5 min, treated with 4% paraformaldehyde phosphate buffer solution and resuspended. The sample was spread on slides and dried overnight at room temperature. Nucleic acids were visualized by confocal laser scanning microscopy (FV1000;

Olympus) after staining with SYTO 9 dye (Invitrogen), according to the manufacturer's instructions.

To analyse the cellular fatty acid composition, cells of strain 262-8^T were cultivated in $0.25 \times \text{LB/MA}$ medium at 25 °C until late exponential phase. Cellular fatty acid methyl esters were extracted and purified using a fatty acid methylation kit (Nacalai Tesque). The methyl esters were analysed with a Thermo Scientific GC/MS ITQ900 equipped with a TR-5ms SQC capillary column (30 m $\times$

Fig. 2. Neighbour-joining phylogenetic tree of strain 262-8^T and members of the order *Rhodospirillales* based on 16S rRNA gene sequences. Bootstrap values (>50%) based on 1000 resamplings are shown at branch nodes. Circles indicate that the corresponding nodes were recovered using the maximum-likelihood method (filled circles, >50% bootstrap values; open circles, >70% bootstrap values). *Escherichia coli* (ATCC 11775^T) was used as an outgroup. Bar, 10 substitutions per 100 nucleotide positions.

Table 1. Nucleotides unique to the 16S rRNA gene of strain 262-8^T

Gene sequences listed in Fig. 2 were compared. Positions are according to the *Escherichia coli* numbering system.

Position in <i>E. coli</i>	Strain 262-8 ^T	<i>Rhodospirillaceae</i>	<i>Acetobacteraceae</i>
108	A	C or G	G
262	G	A	A
630	T	A	G or A
835	G	T or C	T
852	C	G or A	A
1011	T	G or A	A or G
1214	A	G	G

0.25 mm; Thermo Fisher Scientific). Quinones were extracted from freeze-dried cells and purified by TLC on Kieselgel 60 F254 plates (Merck) with petroleum ether/diethyl ether (85:15, v/v). The recovered extract of quinones was analysed using a HPTLC RP-18 F254 (Merck) with acetone/acetonitrile (80:20, v/v). Q-10 and

Q-8 were used as standards. The G+C content was analysed using a GC content of DNA kit (Yamasa Syoyu) and HPLC (LC-10A VP; Shimadzu) equipped with a Cosmosil 5C₁₈ column (4.6 × 250 mm; Nacalai Tesque).

The 16S rRNA gene of strain 262-8^T was amplified by PCR with KOD-plus DNA polymerase (Toyobo) and two universal primers: 5'-GAGTTTGATCCTGGCTCA-3' and 5'-AAGGAGGTGATCCAGCC-3' (corresponding to positions 9F and 1541R of the *Escherichia coli* 16S rRNA gene; Brosius *et al.*, 1978). The amplified PCR product was sequenced using an ABI Prism 3100 genetic analyser (Applied Biosystems). To examine the phylogenetic position of strain 262-8^T, a rooted phylogenetic tree was constructed with 16S rRNA gene sequences from bacteria belonging to the order *Rhodospirillales* using CLUSTAL W (Thompson *et al.*, 1994).

Strain 262-8^T was able to grow in 0.25 × LB/MA, 0.25 × LB/ASW and marine broth 2216. The isolate grew at 5–30 °C (optimum 25 °C), but not at 37 °C, at pH 6.0–8.0 (optimum approximately pH 7.0) and with 0.10–2.0 % NaCl (optimum 0.5 % NaCl). The maximum OD₆₆₀ was

Table 2. Characteristics that differentiate strain 262-8^T from members of the order *Rhodospirillales*

Taxa: 1, strain 262-8^T; 2, *Azospirillum* (Zhang *et al.*, 2008); 3, *Magnetospirillum* (Schüler & Schleifer, 2005; Sizova *et al.*, 2007); 4, *Phaeospirillum* (Imhoff, 2005c); 5, *Rhodocista* (Imhoff, 2005d); 6, *Rhodospira* (Pfennig *et al.*, 1997); 7, *Rhodospirillum* (Imhoff, 2005a); 8, *Rhodovibrio* (Imhoff, 2005e); 9, *Roseospira* (Guyoneaud *et al.*, 2002; Imhoff, 2005b); 10, *Skermanella* (Sly & Stackebrandt, 1999; Zhang, *et al.*, 2008; Weon *et al.*, 2007); 11, *Stella* (Vasilyeva, 2005); 12, *Thalassobaculum* (Zhang, *et al.*, 2008); 13, *Acetobacter* (Sievers & Swings, 2005b); 14, *Acidiphilium* (Kishimoto *et al.*, 1995); 15, *Acidisphaera* (Hiraishi *et al.*, 2000); 16, *Acidocella* (Kishimoto *et al.*, 1995); 17, *Acidomonas* (Urakami *et al.*, 1989); 18, *Asaia* (Loganathan & Nair, 2004; Yamada *et al.*, 2000); 19, *Craurococcus* (Saitoh *et al.*, 1998); 20, *Gluconacetobacter* (Sievers & Swings, 2005d); 21, *Gluconobacter* (Sievers & Swings, 2005c); 22, *Paracraurococcus* (Saitoh *et al.*, 1998); 23, *Rhodopila* (Madigan & Imhoff, 2005); 24, *Roseococcus* (Alarico *et al.*, 2002; Yurkov *et al.*, 1994); 25, *Roseomonas* (Gallego *et al.*, 2006; Weyant & Whitney, 2005); 26, *Swaminathania* (Loganathan & Nair, 2004). +, Positive; +/–, some strains positive; –, negative; NA, not applicable; ND, no data available.

Characteristic	<i>Rhodospirillaceae</i>											
	1	2	3	4	5	6	7	8	9	10	11	12
Cell morphology*	O, R	PV, STR	S	V, S	V, S	V, S	V, S	V, S	V, S	R, MC	FSPS	SCSR
Cell characteristics												
Width/diameter (µm)	0.8–1	0.6–0.9	0.2–0.7	0.5–1	1–2	1.5–3	0.8–1.5	0.6–0.9	0.6–1	1–1.5	0.7–3	0.3–0.5
Length (µm)	1.5–2	2–30	1–20	3.5–6	3	1.5–3	NA	1–3.5	1.5–6.5	2–3	NA	1.3–1.5
Flagellation	+	+	+	+	+	+	+	+	+	+	–	+
pH for growth												
Range	6–8	5–8.5	ND	6–8.5	5.7–7	7–7.8	6–8.5	7.5–8	5.3–8.5	4–9	Neutral	7–9
Optimum	7	5–7.2	7	7.3	6.8	7.3–7.5	6.8–7	ND	6.7–7	6–7	ND	8
Major quinone†	Q-10	Q-10	Q-10	Q-9, MK-9	Q-9	Q-7, MK-7	Q-10, RQ-10	Q-10, MK-10	ND	Q-10, Q-8	ND	Q-10
Bacteriochlorophyll <i>a</i>	–	–	–	+	+	–‡	+	+	+	–	ND	–
Anoxic photosynthetic growth	–	–	–	+	+	+	+	+	+	–	–	ND
DNA G+C content (mol%)	69.8	64–71	63–64	60–66	69.9	65.7	63–66	65–69	65–68	65–67	69.3–73.5	68.0
Major fatty acids	C _{18:1} , C _{16:0} , C _{18:0}	C _{18:1} , C _{16:1}	C _{18:1} , C _{16:1} , C _{16:0}	C _{18:1} , C _{16:1}	ND	C _{18:1} , C _{16:0}	C _{18:1} , C _{16:1}	C _{18:1} , C _{18:0}	ND	C _{18:1} , C _{16:0}	ND	C _{18:1} , C _{17:0} , C _{16:0}

*C, coccoid; E, ellipsoid; FSPS, flat, six-pronged stars; MC, multicellular conglomerate; O, ovoid; PCR, plump coccoid rod; PV, plump vibrioid; R, rod; RRE, rod with rounded end; S, spiral; SCSR, slightly curved and straight rods; SPH, spherical; SR, short rod; STR, straight rod; V, vibrioid.

†MK, menaquinone; Q, ubiquinone; RQ, rhodoquinone.

‡Bacteriochlorophyll *b* has been detected.

0.120, after about 200 h incubation (doubling time about 36 h; Supplementary Fig. S2). After growth on 0.25 × LB/MA medium for 2 weeks, colonies were white and circular, with a diameter of <0.2 mm. Strain 262-8^T was able to form colonies microaerobically, but anaerobic growth could not be observed after 2 weeks in either light or dark conditions. Cells were Gram-negative-staining, ovoid to rod-shaped (0.8–1.0 × 1.5–2.0 µm) and often occurred in pairs or chains (Fig. 1a and b). When the location of nucleic acids was observed by confocal laser scanning microscopy, nucleic acids were not observed at the cell constrictions (Fig. 1c). The cytoplasm of each cell was clearly divided, although complete separation of cells was not observed. Motility and flagella could not be observed by scanning electron microscopy (Fig. 1b); however, flagella were observed by atomic force microscopy (Fig. 1d) and motility was observed in semi-solid agar. Strain 262-8^T was catalase-positive and oxidase-negative. Production of hydrogen sulfide, acetic acid and spores was not observed. The results of the substrate utilization tests and the API ZYM and ATB VET systems are given in the species description. Although strain 262-8^T was resistant to β-lactam antibiotics, strain 262-8^T was sensitive to amoxicillin/clavulanic acid, which suggested that strain 262-8^T produced β-lactamase. Cell growth was not observed for any of the assimilation tests in the API 20 NE system. It seems that the basal medium provided with the kit lacks unknown essential nutrients for the growth of strain 262-8^T; indeed, complex nutrients such as tryptone or yeast extract are essential. Because no growth was observed in synthetic media, the organic compounds supporting cell growth were determined. Thirteen organic compounds, given in the species description, supported cell growth of strain 262-8^T.

Comparative analysis of 16S rRNA gene sequences indicated that strain 262-8^T belonged to the order *Rhodospirillales*. The most closely related neighbours of strain 262-8^T were *Stella vacuolata* DSM 5901^T (AJ535711), *Stella humosa* DSM 5900^T and *Tistrella mobilis* IAM 14872^T, with which the isolate shared only 90.2, 90.2 and 89.7% 16S rRNA gene sequence similarity, respectively (Fritz *et al.*, 2004; Shi *et al.*, 2002). The low similarity values suggested that it would be difficult to analyse the phylogenetic position of strain 262-8^T by DNA–DNA hybridization. In the neighbour-joining tree (Fig. 2), strain 262-8^T was located in the family *Rhodospirillaceae* and in the same cluster as the genus *Stella* (Fritz *et al.*, 2004). However, comparison of 16S rRNA gene sequences from the families *Rhodospirillaceae* and *Acetobacteraceae* revealed that some nucleotides were unique to strain 262-8^T: at *E. coli* positions 108, 262, 630, 835, 852, 1011 and 1214, only strain 262-8^T possessed A, G, T, G, C, T and A, respectively (Table 1). Therefore, it was concluded that strain 262-8^T belonged to the family *Rhodospirillaceae* but should be distinguished from the recognized bacteria belonging to the family.

The 16S rRNA gene sequence of strain 262-8^T indicated high sequence similarities (99–90%) with sequences of uncultured bacteria found in environmental samples worldwide (EF157109, EF157242, EU617826, DQ001629, DQ378260, GQ263761, GQ263791, GQ263953 and GQ263966). However, there has been no report of the isolation of these bacteria. In the case of strain 262-8^T, colonies were very small (<0.2 mm) and the growth rate was low (doubling time 36 h). The isolation of strain 262-8^T from the white rock sample was made possible by long-term cultivation and

<i>Acetobacteraceae</i>													
13	14	15	16	17	18	19	20	21	22	23	24	25	26
E, R	R	C, SR	R	RRE	R	C	R	E, R	C	SPH, O	C	PCR	R
0.6–0.9	0.3–1.2	0.7–0.9	0.5–0.8	0.8–1	0.4–1	0.8–2	0.6–1.2	0.5–1	0.8–1.5	1–2.5	0.9–1.3	ND	0.7–0.9
1–4	1–4	0.9–1.6	1–1.8	1.5–3	0.8–2	NA	1–3	2.6–4.2	NA	NA	1.3–1.6	ND	1.9–3.1
+/-	+/-	–	+	+/-	+	–	+/-	+/-	–	+	+	+	+
ND	2.5–6	3.5–6	3–6	2–5.5	ND	ND	ND	ND	ND	4.2–6.5	ND	5–9	ND
4–6	ND	4.5–5	ND	3–5	3	7.5	ND	5–6	6.6–6.8	4.8–5	ND	7	ND
Q-9	Q-10	Q-10	Q-10	Q-10, Q-9, Q-11	Q-10	Q-10	Q-10	Q-10	Q-10	Q-9/10, MK-9/10, RQ-9/10	Q-9	Q-10	Q-10
–	+	+	–	–	–	+	–	–	+	+	+	–	ND
–	–	–	–	–	–	–	–	–	–	+	–	–	ND
50.5–60.3	63.2–67.5	69.1–69.8	58.7–64.4	63–65	59.3–61.0	70.5	54.9–66.4	52–64	70.3–71.0	66.3	70.4	65–71	57.6–59.9
C _{18:1}	C _{18:1}	ND	C _{18:1}	C _{18:1}	C _{18:1} , C _{16:0} , C _{16:0} 2-OH, C _{14:0} 2-OH	C _{18:1}	C _{18:1} , C _{16:0}	ND	C _{18:1}	C _{18:1}	C _{18:1} , C _{18:1} 2-OH	C _{18:1} , C _{18:1} 2-OH, C _{15:0} 2-OH and/or C _{16:1} , C _{16:0}	C _{18:1} , C _{19:0}

selection of small colonies. These methods will help to isolate novel bacteria from the environment in the future.

Table 2 shows the chemotaxonomic characteristics of strain 262-8^T and those of other representatives of genera in the order *Rhodospirillales*. The major respiratory quinone of strain 262-8^T was ubiquinone 10 and the minor quinone was ubiquinone 8. The cellular fatty acids of strain 262-8^T were C_{18:1} (42.7%), C_{16:0} (32.1%), C_{18:0} (19.4%), C_{17:0} cyclo (3.2%) and C_{16:1} (2.7%). The G+C content of genomic DNA was 69.8 mol%. These chemotaxonomic characteristics are in agreement with those for other representatives of genera in the order *Rhodospirillales* (Table 2). However, a few physiological characteristics of strain 262-8^T were different from those of the family *Rhodospirillaceae*. Strain 262-8^T could not grow photoheterotrophically under anoxic conditions, contrary to most genera in the family *Rhodospirillaceae* (Garrity *et al.*, 2005; Vasilyeva, 2005). No spectrum corresponding to the presence of pigments could be observed (data not shown). The result indicated that strain 262-8^T contained no bacteriochlorophyll *a* or other pigments, which agreed with the result that strain 262-8^T could not grow photoheterotrophically under anoxic conditions. In addition, strain 262-8^T could not grow under acidic conditions and no acid production was observed, as opposed to members of the order *Acetobacteraceae* (Sievers & Swings, 2005a).

The order *Rhodospirillales* of the class *Alphaproteobacteria* has been divided into two families, *Rhodospirillaceae* and *Acetobacteraceae*, by phylogenetic analysis of 16S rRNA gene sequences (Garrity *et al.*, 2005). According to phylogenetic analysis and physiological characteristics, strain 262-8^T is a novel bacterium belonging to family *Rhodospirillaceae*. Therefore, a novel genus and species, *Constrictibacter antarcticus* gen. nov., sp. nov., is proposed.

Description of *Constrictibacter* gen. nov.

Constrictibacter (Cons.tric.ti.bac'ter. L. adj. *constrictus* compressed, contracted; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Constrictibacter* rod with compressed parts).

A member of the class *Alphaproteobacteria*, order *Rhodospirillales*, family *Rhodospirillaceae*. Cells are ovoid to rod-shaped and often occur in pairs or chains. Cells are motile and do not form spores. Grows aerobically or micro-aerobically. The respiratory quinones are Q-10 and Q-8. The major cellular fatty acids are C_{18:1}, C_{16:0} and C_{18:0}. The DNA G+C content of the type strain of the type species is 69.8 mol%.

The type species is *Constrictibacter antarcticus*.

Description of *Constrictibacter antarcticus* sp. nov.

Constrictibacter antarcticus (ant.arc'ti.cus. L. masc. adj. *antarcticus* pertaining to Antarctica).

Cells are 0.8–1.0 µm in diameter and 1.5–2.0 µm in length. Colonies are white and circular, <0.2 mm in diameter on

0.25 × LB/MA agar. Catalase is produced, but oxidase is not produced. Produces acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase (C4), esterase lipase (C8), β-glucosidase, leucine arylamidase, lipase (C14), naphthol-AS-BI-phosphohydrolase, trypsin and valine arylamidase. Reduces nitrate. Complex nutrients (tryptone or yeast extract) are essential for growth. Grows at 5–30 °C (optimum 25 °C) and pH 6.0–8.0 (optimum approximately pH 7.0). Doubling time is about 36 h in 0.25 × LB/MA medium under optimal growth conditions. Ribose, fructose, lactose, sucrose, trehalose, glycerol, glycolate, malate, acetate, succinate, pyruvate, aspartate and glutamate support cell growth. The type strain is resistant to erythromycin, lincomycin, pristinamycin, tylosin, cotrimoxazole, sulfamethizole, nitrofurantoin, fusidic acid, metronidazole, penicillin, amoxicillin, oxacillin, cephalothin, cefoperazone, chloramphenicol and tetracycline, but sensitive to colistin, flumequine, oxolinic acid, enrofloxacin, rifampicin, amoxicillin/clavulanic acid, streptomycin, spectinomycin, kanamycin, gentamicin, apramycin and doxycycline.

The type strain, 262-8^T (=JCM 16422^T=ATCC BAA-1906^T), was isolated from a cavity within white rock collected in the Skallen region of Antarctica. The DNA G+C content of the type strain is 69.8 mol%.

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