Psychromonas hadalis sp. nov., a novel piezophilic bacterium isolated from the bottom of the Japan Trench

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An obligately piezophilic bacterium was isolated from sediment collected from the bottom of the Japan Trench at a depth of 7542 m. The isolated strain, designated K41G^T, was closely affiliated with members of the genus *Psychromonas* on the basis of 16S rRNA gene sequence analysis. Levels of DNA–DNA relatedness between strain K41G^T and *Psychromonas* reference strains were significantly lower than that accepted as the phylogenetic definition of a species. The optimal temperature and pressure for growth of strain K41G^T were 6 °C and 60 MPa, respectively. The DNA G + C content was 39.1 mol%. Whole-cell fatty acids consisted of significant amounts of unsaturated fatty acids C_{16:1} (37 %) and C_{14:1} (17 %), saturated fatty acid C_{16:0} (31 %) and polyunsaturated fatty acid C_{22:6} (8 %). Based on the taxonomic differences observed, strain K41G^T is considered to represent a novel obligately piezophilic *Psychromonas* species. The name *Psychromonas hadalis* (type strain, K41G^T = JCM 11830^T = ATCC BAA-638^T) is proposed. This is the second species of obligately piezophilic bacteria to be proposed in the genus *Psychromonas*.

Psychrophilic and piezophilic bacteria have been isolated from deep-sea environments. They are interesting because of their physiological adaptations, which enable them to survive at high pressure and low temperatures, and because they contribute to our understanding of the interactions between the deep-sea environment and its microbial inhabitants (Kato *et al.*, 1995, 1998; Nogi *et al.*, 1998, 2004; Yayanos *et al.*, 1979). All piezophilic bacteria isolated to date fall into the class *Gammaproteobacteria*, according to phylogenetic classifications based on 16S rRNA gene sequence information (DeLong *et al.*, 1997; Nogi & Kato, 1999; Nogi *et al.*, 2002). Margesin & Nogi (2004) reported that 11 cultivated psychrophilic and piezophilic deep-sea species were affiliated with genera within the *Gammaproteobacteria*: *Shewanella*, *Photobacterium*, *Colwellia*, *Moritella* and *Psychromonas*. Members of the genus *Psychromonas* were isolated from the polar circles and deep-sea environments. Most of these isolates are not piezophilic strains. *Psychromonas kaikoe* JT7304^T (Nogi *et al.*, 2002) and *Psychromonas profunda* 2825^T (Xu *et al.*, 2003) were reported to be piezophilic strains within this group. In this study, a novel obligately piezophilic, C_{22:6} (docosahexaenoic acid)-producing bacterium of the genus *Psychromonas* is proposed on the basis of polyphasic studies.

Strain K41G^T was isolated from deep-sea sediment samples collected in November 2000 using sterilized mud samplers on the unmanned submersible *KAIKO* in the Japan Trench (38° 0.5' N 143° 59.9' E), at a depth of 7542 m. This piezophilic strain was maintained on marine agar 2216 (Difco) and was grown at 6 °C and 60 MPa. High-pressure cultivation was achieved using a liquid hydraulic system. Piezophilic bacteria were cultivated in a plastic bag containing liquid medium in a pressure vessel made of stainless steel (SUS304). If necessary, oxygen-saturated fluorinert (FC-72; Sumitomo-3M) was added to supply oxygen to the cultures (20 % of total volume). This was performed according to the procedure reported previously (Kato *et al.*, 1994; Yanagibayashi *et al.*, 1999). Optimal pressure and temperature for growth were measured by monitoring the optical density. These tests were performed anaerobically in marine broth 2216 under various pressure and temperature conditions.

Cells of the isolated deep-sea strain K41G^T were found to be Gram-negative rods, 1.5–2 µm long and 0.8–1.0 µm wide, and motile by means of a single unsheathed polar flagellum. The strain was unable to grow at atmospheric pressure at 2–15 °C, although it grew well in pressure vessels under hydrostatic pressures of 30–90 MPa at 6 °C and 60–90 MPa at 10 °C. No growth occurred at 15 °C under the pressures examined. The most rapid growth rate (about 0.14 h⁻¹) was observed at 60 MPa and 6 °C (Fig. 1).

Physiological tests were performed under high-pressure conditions to determine acid production from sugars and

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The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain K41G^T is AB094413.

Fig. 1. Pressure-dependent growth rate of strain K41G^T at 2 °C (▲), 6 °C (●) and 10 °C (○). Growth rates were determined based on optical density. t_d , Doubling time (h).

fermentation test cultures were grown using modified OF medium (Nogi *et al.*, 2002) to examine hydrogen sulfide production from thiosulfate, production of indole, fermentation and oxidization, gelatinase activity and oxidase and catalase production, according to methods described previously (Nogi & Kato, 1999; Nogi *et al.*, 2002).

The characteristics of strain K41G^T and reference strains are shown in Table 1. Strain K41G^T was a facultatively anaerobic, chemo-organotroph, displaying both respiratory and fermentative types of metabolism. Strain K41G^T shared many physiological characteristics with members of the genus *Psychromonas*. However, unlike the reference strains of *Psychromonas*, strain K41G^T did not utilize cellobiose or sucrose and reduced nitrite, and strain K41G^T and *Psychromonas kaikoe* were unable to grow at atmospheric pressure.

To determine the phylogenetic relationships, 16S rRNA gene sequences were obtained by direct sequencing of PCR-amplified DNA, as described previously (Kato *et al.*, 1998). Nucleotide substitution rates (K_{nuc} ; Kimura, 1980) were determined and a distance matrix tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) with the program CLUSTAL_X (Thompson *et al.*, 1997). Alignment gaps and unidentified base positions were not taken into consideration in the calculations. The topology of the phylogenetic tree was evaluated by performing bootstrap analysis with 1000 bootstrapped trials. The results of phylogenetic analyses based on 16S rRNA gene sequence information support the conclusions described below and further clarify the taxonomic and phylogenetic position of the novel isolate among members of the genus *Psychromonas* and related genera. The results of the phylogenetic analyses are shown in Fig. 2. Strain K41G^T falls into the genus *Psychromonas* and is closely related to the psychrotrophic

species *Psychromonas arctica* (96.1 %) and the piezophilic species *Psychromonas profunda* (97.0 %). The generally recommended and accepted criteria for delineating bacterial species state that strains with 16S rRNA gene sequence dissimilarity greater than 3 % are considered to belong to separate species (Stackebrandt & Goebel, 1994; Stackebrandt *et al.*, 2002). For analysis of relatedness, DNA–DNA hybridization was carried out at 40 °C for 3 h and was measured fluorometrically using the method of Ezaki *et al.* (1989). The DNA–DNA hybridization value between strain K41G^T and the reference strain *Psychromonas profunda* was less than 40 %. This level of relatedness is significantly lower than that accepted as the phylogenetic definition of a species (Wayne *et al.*, 1987). This and other results in Table 1 and Fig. 2 suggest that strain K41G^T represents a novel *Psychromonas* species.

The whole-cell fatty acid compositions of the piezophilic strain K41G^T and reference strains are shown in Table 2. The major fatty acids of strain K41G^T were C_{14:1} (tetradecenoic acid), C_{16:0} (hexadecanoic acid), C_{16:1} (hexadecenoic acid) and C_{22:6}. There was a low level of similarity between this fatty acid profile and those of the reference strains. For example, the predominant components in the fatty acid profiles of the reference strains differed from those in the profile of strain K41G^T, which contained substantial amounts of C_{14:1} and C_{22:6} and does not have the long-chain unsaturated fatty acid C_{18:1} (octadecenoic acid).

On the basis of the phenotypic, genotypic and phylogenetic data, we conclude that the isolate is a member of the genus *Psychromonas* and that it represents a novel species within this genus, for which the name *Psychromonas hadalis* sp. nov. is proposed.

Table 1. Phenotypic characteristics of strain K41G^T and other members of the genus *Psychromonas*

Strains: 1, strain K41G^T (*Psychromonas hadalis* sp. nov.); 2, *Psychromonas antarctica* DSM 10704^T; 3, *Psychromonas arctica* Pull 5.3^T; 4, *Psychromonas ingrahamii* 37^T; 5, *Psychromonas kaikoe* JT7304^T; 6, *Psychromonas marina* 4-22^T; 7, *Psychromonas profunda* 2825^T. All strains were Gram-negative, oxidase-positive and catalase-positive, and were able to use D-fructose, D-glucose, maltose and D-mannitol. Data for reference species are from Mountfort *et al.* (1998), Groudieva *et al.* (2003), Auman *et al.* (2006), Nogi *et al.* (2002), Kawasaki *et al.* (2002) and Xu *et al.* (2003). +, Positive; –, negative; ND, no data available.

Characteristic	1	2	3	4	5	6	7
Growth temperature (°C):							
Range	2–12	2–17	0–25	–12 to 10	4–15	0–25	2–14
Optimum	6	12	20	5	10	14–16	10
Optimum pressure (MPa)	60	0.1–10	ND	ND	50	0.1	25
Growth at atmospheric pressure	–	+	+	+	–	+	+
Motility	+	+	+	–	+	+	+
DNA G+C content (mol%)	39.1	43	40.1	40	43.8	43.5	38.1
Quinone	Q-8	Q-8	ND	ND	Q-8	Q-8	Q-8
Gelatinase	–	+	–	–	+	–	–
Amylase	–	–	+	–	–	+	+
Production of H ₂ S	–	–	ND	ND	–	+	+
Production of indole	–	–	ND	–	–	–	+
Reduction of nitrate	+	–	–	+	+	+	+
Reduction of nitrite	+	–	ND	ND	–	ND	–
Gas production from glucose	–	+	+	–	–	–	–
Acid produced from:							
L-Arabinose	–	–	ND	–	–	–	–
Cellobiose	–	+	ND	+	+	+	+
D-Galactose	+	+	ND	+	+	+	+
Glycerol	+	–	+	+	+	+	+
<i>myo</i> -Inositol	–	–	ND	–	–	–	+
D-Lactose	–	–	+	+	–	+	+
D-Mannose	+	–	+	–	+	–	+
D-Raffinose	–	–	ND	ND	–	–	–
L-Rhamnose	–	–	ND	ND	–	–	+
D-Sorbitol	–	–	ND	–	–	–	–
Sucrose	–	+	+	+	+	+	+
D-Trehalose	+	+	ND	+	+	–	+
Xylose	–	–	–	–	–	+	+

Description of *Psychromonas hadalis* sp. nov.

Psychromonas hadalis [ha.da'lis. N.L. fem. adj. *hadalis* (from Greek *Haidēs*), *hadal* of or relating to the deepest regions of the ocean].

Cells are Gram-negative rods, 1.5–2 µm long and 0.8–1.0 µm wide, and motile by means of a single unsheathed polar flagellum. Halophilic, psychrophilic and piezophilic. Optimal growth occurs at a NaCl concentration of about 3 %. No growth occurs in the absence of NaCl.

Fig. 2. Phylogenetic tree constructed using the neighbour-joining method and based on 16S rRNA gene sequences showing the relationships of strain K41G^T within the genus *Psychromonas*. Percentage bootstrap values were calculated from multiple resamplings of the sequence dataset. Bar, 0.01 (mean) nucleotide substitutions per site.

Table 2. Whole-cell fatty acid content of strain K41G^T (*Psychromonas hadalis* sp. nov.) and other members of the genus *Psychromonas*

Strains: 1, strain K41G^T (*Psychromonas hadalis* sp. nov.); 2, *Psychromonas antarctica* DSM 10704^T; 3, *Psychromonas arctica* Pull 5.3^T; 4, *Psychromonas ingrahamii* 37^T; 5, *Psychromonas kaikoe* JT7304^T; 6, *Psychromonas marina* 4-22^T; 7, *Psychromonas profunda* 2825^T. Values are percentages of total fatty acids. Values below 1% are not shown. Data are from this study, Nogi *et al.* (2002), Groudieva *et al.* (2003), Auman *et al.* (2006), Kawasaki *et al.* (2002) and Xu *et al.* (2003).

Fatty acid	1	2	3	4	5	6	7
C _{12:0}	1	1	2.7–5.2	2.5	1		
C _{14:0}	1				6		
C _{15:0}					1		
C _{16:0}	31	24	7.0–16.2	18.7	15	46.6	31
iso-C _{16:0}						2.7	
C _{14:1}	17	8	2.7–5.2		10	3.2	15
C _{16:1}	37	58	~50 (ω 7c), 7.0–16.2 (ω 7t)	67	54	39.4	44
C _{18:1}		3	7.0–16.2	3.6	2	3.1	
C _{20:5}					2		
C _{22:6}	8				2	1.6	
3-OH-C _{12:0}					2		
3-OH-C _{14:0}	3	6			4		10
C _{12:0} alde, iso-C _{16:1} or 3-OH-C _{14:0}				4.5			

Optimal temperature and pressure for growth are 6 °C and 60 MPa, respectively. No growth occurs at atmospheric pressure. Facultatively anaerobic, chemo-organotroph, having both respiratory and fermentative types of metabolism. Catalase- and cytochrome oxidase-positive, nitrate is reduced to nitrite and nitrite is reduced to nitrogen. Gelatin is not hydrolysed, and amylase, H₂S production and indole production are negative. The DNA G + C content of the type strain is 39.1 mol%. The major isoprenoid quinone is Q-8. Predominant cellular fatty acids are C_{14:1}, C_{16:0}, C_{16:1} and C_{22:6}. Other characteristics are shown in Table 1.

The type strain is strain K41G^T (=JCM 11830^T=ATCC BAA-638^T), which was isolated from sediment collected from the bottom of the Japan Trench.

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