Jannaschia pohangensis sp. nov., isolated from seashore sand in Korea

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A Gram-negative, aerobic bacterium, H1-M8^T, was isolated from seashore sand in Korea and then characterized using a polyphasic approach. Cells were short rods (0.7–1.0×1.5–2.0 µm) and were motile (each cell having at least one flagellum). Colonies were light-brown, non-pigmented, circular and convex with clear margins. Growth of the strain was observed at 5–35 °C, pH 6.0–9.0 and NaCl concentrations up to 8.4 % (w/v). Phylogenetic analysis of the 16S rRNA gene sequence revealed a clear affiliation between the novel strain and members of the genus *Jannaschia*. The sequence similarities between H1-M8^T and type strains of the genus *Jannaschia* ranged from 97.0 to 97.8 %. However, DNA–DNA hybridizations between the isolate and type strains of other related species produced low values (21–38 %). The major isoprenoid quinone was Q-10 and the predominant cellular fatty acids were 18:1 ω 7c (68.2 %) and 18:0 (10.5 %). The G+C content of the DNA was 63.6 mol%. On the basis of physiological, biochemical and chemotaxonomic traits and data from the comparative 16S rRNA sequence analysis, strain H1-M8^T represents a novel species of the genus *Jannaschia*, for which the name *Jannaschia pohangensis* sp. nov. is proposed. The type strain is H1-M8^T (=KACC 11609^T =DSM 19073^T).

The genus *Jannaschia* was proposed for Gram-negative, strictly aerobic, non-fermentative heterotrophs (Wagner-Döbler *et al.*, 2003). This genus is a member of the *Alphaproteobacteria*. At the time of writing, there are four accepted species in the genus *Jannaschia*: *Jannaschia helgolandensis*, *J. rubra*, *J. seosinensis* and *J. donghaensis* (Wagner-Döbler *et al.*, 2003; Macián *et al.*, 2005; Choi *et al.*, 2006; Yoon *et al.*, 2007). The type species, *J. helgolandensis*, contains Q-10 as the major respiratory quinone and 18:1 ω 7c as the major fatty acid. Recently, *Jannaschia cystaugens* (Adachi *et al.*, 2004) was transferred to the genus *Thalassobacter* as a synonym of *Thalassobacter stenotrophicus* (Pujalte *et al.*, 2005).

Strain H1-M8^T was isolated from seashore sand, using marine agar 2216 (MA; Difco). The seashore sand sample was collected from Homi Cape in Pohang, Republic of Korea. Serially diluted samples were spread on MA and incubated at 28 °C for 5 days. The strain did not grow on nutrient agar (Difco), R2A agar (Difco), trypticase soy agar (Difco) or MacConkey agar (Difco). The cell morphology was studied using transmission electron microscopy (model 912AB; LEO) and phase-contrast microscopy (Axio; Zeiss), with cells grown for 3 days at 28 °C on MA. Gram staining, catalase and oxidase activity and hydrolysis of casein, DNA, starch and Tween 80 were investigated according to the methods of Smibert & Krieg (1994). The temperature range for growth was tested on MA from 5 to 40 °C, in increments of 5 °C. The pH range for growth was determined in the range pH 5–10, in increments of 1 pH unit, in marine broth buffered with citrate/phosphate buffer or Tris/HCl (Breznak & Costilow, 1994). Halotolerance was tested at 28 °C for 10 days in tryptic soy broth (Difco) containing 0–20 % (w/v) NaCl and in marine broth (Difco) supplemented with 0–10 % NaCl (w/v). Anaerobic growth was also tested on MA

Abbreviation: ECL, equivalent chain length.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain H1-M8^T is DQ643999.

A transmission electron micrograph of a cell of strain H1-M8^T and a table showing the cellular fatty acid compositions of strain H1-M8^T and type strains of *Jannaschia* species are available as supplementary material with the online version of this paper.

incubated in an anaerobic jar (BBL). Hydrolysis of alginic acid (0.5 %, w/v), CM-cellulose (Sigma) (0.1 %), chitin (1 %, w/v), pectin (0.5 %, w/v) and tyrosine (0.5 %, w/v) was also tested on MA. Carbon utilization was tested on basal medium agar [Tris/HCl, 50 mM, pH 7.5; NH₄Cl, 190 mM; K₂HPO₄·3H₂O, 0.33 mM; FeSO₄·7H₂O, 0.1 mM and 1.5 % Bacto agar (Difco) in half-strength artificial seawater (Baumann & Baumann, 1981)] containing 0.2 % carbon source. The basal medium agar was supplemented with 0.01 % yeast extract. Tests in the commercial systems API 20NE and API ZYM (bioMérieux) were generally performed according to the manufacturer's instructions.

Cells of strain H1-M8^T were aerobic, Gram-negative, short rods, each having one or more polar flagella (Supplementary Fig. S1, available in IJSEM Online). The strain was positive for oxidase and catalase activities. It did not grow anaerobically. Strain H1-M8^T could be differentiated from its closest phylogenetic relative, *J. helgolandensis* DSM 14858^T, on the basis of motility, the hydrolysis of gelatin

and aesculin, the temperature range for growth and assimilation patterns for several carbon sources (see Table 1 and the species description).

The 16S rRNA gene was analysed as described by Kwon *et al.* (2003). 16S rRNA gene sequences were aligned using CLUSTAL W software (Thompson *et al.*, 1994). Nucleotide similarity values were calculated from the alignment. Phylogenetic trees for the datasets were inferred using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods with MEGA, version 3.0 (Kumar *et al.*, 2004). The stability of relationships was assessed by performing bootstrap analyses based on 1000 resamplings. Strain H1-M8^T showed the highest sequence similarities (97.0–97.8 %) with respect to the type strains of *J. helgolandensis* (97.8 %), *J. rubra* (97.3 %), *J. seosinensis* (97.3 %) and *J. donghaensis* (97.0 %). Lower sequence similarities (<96.0 %) were found with respect to described species from other genera. Furthermore, the four *Jannaschia* type strains and H1-M8^T formed a compact cluster that is most closely related to *Thalassobacter*

Table 1. Phenotypic characteristics that serve to differentiate H1-M8^T from type strains of *Jannaschia* species

Strains: 1, H1-M8^T; 2, *J. helgolandensis* DSM 14858^T (data from Wagner-Döbler *et al.*, 2003); 3, *J. rubra* DSM 16279^T (Macián *et al.*, 2005); 4, *J. seosinensis* CL-SP26^T (Choi *et al.*, 2006); 5, *J. donghaensis* DSW-17^T (Yoon *et al.*, 2007). API 20NE data were obtained from Choi *et al.* (2006), Yoon *et al.* (2007) and this study. Utilization data for all strains except DSW-17^T were obtained from this study. None of the strains utilized cellobiose, maltose, sucrose or trehalose. +, Positive; (+), weakly positive; –, negative; NA, data not available.

Characteristic	1	2	3	4	5
Colony colour	Light brown	White	Dark red	Dark red	Strong orange
Motility	+	–	+	+	–
Temperature range for growth (°C)	5–35	15–30	4–25	5–35	4–32
Oxidase/catalase	+/+	(+)/+	+/+	–/+	+ /NA
Hydrolysis of:					
DNA	–	–*	+	–	NA
Gelatin	+	–	–	+	–
Starch	–	–	–	+	–
API 20NE results					
Nitrate reduction	–	–	+	–	–
Aesculin hydrolysis	+	–	+	+	–
Utilization of:					
L-Arabinose	–	+	–	–	–
L-Aspartate	–	–	+	–	NA
Citrate	+	+	–	–	–
Glycerol	+	+	+	–	NA
β-Hydroxybutyrate	–	–	+	–	NA
L-Leucine	–	–	+	–	NA
D-Mannitol	+	+	+	–	NA
Melibiose	+	–	–	–	NA
Propionate	+	–	–	–	NA
Pyruvate	+	–	+	+	–
L-Rhamnose	+	+	–	–	NA
D-Ribose	–	+	–	–	NA
L-Tyrosine	+	–	–	+	NA
D-Xylose	+	+	+	–	NA
DNA G + C content (mol%)	63.6	63.0–63.1	64.6	63	65.2

*Data from Macián *et al.* (2005).

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA sequences, showing the positions of strain H1-M8^T and its relatives. Numbers at nodes indicate levels of bootstrap support (based on analysis of 1000 resampled datasets); bootstrap percentages below 50 % are not shown. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. Bar, 0.01 nucleotide substitutions per site.

stenotrophicus (98 % bootstrap support) (Fig. 1). The tree constructed using maximum parsimony also supported the affiliation of strain H1-M8^T with the genus *Jannaschia*.

For quantitative analysis of the whole-cell fatty acids of H1-M8^T, the strain was cultivated on MA at 28 °C for 4 days. The preparation and analysis of the fatty acid methyl esters were performed according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The fatty acid pattern of strain H1-M8^T was largely similar to those of *Jannaschia* species. Strain H1-M8^T contained 18:1 ω 7c (68.2 %) as the predominant fatty acid, which is a characteristic shared by the species of the genus *Jannaschia*. However, H1-M8^T could be unique in possessing a moderate amount (8.0 %) of 18:1 ω 7c 11-methyl and lacking 16:0, 19:0, 19:0 10-methyl, 19:0 ω 8c cyclo and summed feature 2 (14:0 3-OH/16:1 iso). The fatty acid profiles of the isolate and the type strains of *Jannaschia* species are detailed in Supplementary Table S1.

Isoprenoid quinones were extracted from dry cell mass and analysed by HPLC (Minnikin *et al.*, 1984; Groth *et al.*, 1996); the DNA G + C content (mol%) was also measured by HPLC (Mesbah *et al.*, 1989). The predominant isoprenoid quinone in strain H1-M8^T was Q-10 and the G + C content of the genomic DNA of strain H1-M8^T was 63.6 mol%.

DNA–DNA hybridization experiments were performed with strain H1-M8^T and type strains of three *Jannaschia* species by using the filter hybridization method described by Seldin & Dubnau (1985). Probe labelling was conducted by using the non-radioactive DIG-High Prime system (Roche) and hybridized DNA was visualized using the DIG luminescent detection kit (Roche). DNA–DNA relatedness was quantified by using a densitometer (Bio-Rad). Strain H1-M8^T showed low values for hybridization with *J. helgolandensis* DSM 14858^T (21 %), *J. rubra* DSM 16279^T (38 %) and *J. seosinensis* KCCM 42114^T (24 %). These values are below the 70 % threshold suggested for species delineation (Stackebrandt & Goebel, 1994), indicating that strain H1-M8^T is separate at the species level.

In conclusion, the 16S rRNA sequence, physiological characteristics and fatty acid profile of strain H1-M8^T differed from those of its phylogenetic neighbours.

Furthermore, the low levels of DNA–DNA relatedness supported the novelty of the isolate. On the basis of the data presented, therefore, strain H1-M8^T represents a novel species of the genus *Jannaschia*, for which the name *Jannaschia pohangensis* sp. nov. is proposed.

Description of *Jannaschia pohangensis* sp. nov.

Jannaschia pohangensis (po.hang.en'sis. N.L. fem. adj. *pohangensis* referring to Pohang Region in the Republic of Korea, where the type strain was found).

Cells are Gram-negative, strictly aerobic rods, approximately 0.7–1.0 μ m wide and 1.5–2.0 μ m long. Motile (one or more polar flagella). Colonies are light brown, non-pigmented, circular and convex with clear margins. Grows at 5–35 °C (optimally at 30 °C) and pH 6–9 (optimally at pH 7). Growth occurs at NaCl concentrations up to 8.4 %. No growth occurs in tryptic soy broth containing NaCl as the only salt. Does not grow under anaerobic conditions. Catalase- and oxidase-positive. Hydrolyses tyrosine but not alginate, CM-cellulose, casein, chitin, DNA, pectin, starch or Tween 80. Utilizes citrate, D-fructose, D-galactose, D-glucose, L-glutamate, glycerol, D-mannitol, D-mannose, melibiose, propionate, pyruvate, L-rhamnose, L-serine, L-tyrosine and D-xylose, but not L-alanine, L-arabinose, L-aspartate, cellobiose, glycine, L-histidine, β -hydroxybutyrate, lactose, L-leucine, maltose, D-ribose, sucrose, L-threonine or trehalose. Positive for β -galactosidase and for hydrolysis of gelatin, urea and aesculin, but negative for nitrate reduction, indole production, arginine dihydrolase and acid production from glucose (API 20NE test strips). Positive for alkaline phosphatase, esterase (C4 and C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and β -galactosidase, but negative for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase (API ZYM test strips). The fatty acids are 18:1 ω 7c (68.2 %), 18:0 (10.5 %), 18:1 ω 7c 11-methyl (8.0 %), 10:0 3-OH (4.4 %), an unknown fatty acid with an equivalent chain length (ECL) of 11.799 (3.9 %) and summed feature 7 [unknown (ECL 18.846)/19:1 ω 6c; 2.8 %]. The major quinone is Q-10. The DNA G + C content of the type strain is 63.6 mol% (HPLC).

The type strain, H1-M8^T (=KACC 11609^T =DSM 19073^T), was isolated from seashore sand in Pohang, Republic of Korea.

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References

- Adachi, M., Kanno, T., Okamoto, R., Shinozaki, A., Fujikawa-Adachi, K. & Nishijima, T. (2004). *Jannaschia cystaugens* sp. nov., an *Alexandrium* (Dinophyceae) cyst formation-promoting bacterium from Hiroshima Bay, Japan. *Int J Syst Evol Microbiol* **54**, 1687–1692.
- Baumann, P. & Baumann, L. (1981). The marine Gram-negative eubacteria: genera *Photobacterium*, *Beneckea*, *Alteromonas*, *Pseudomonas*, and *Alcaligenes*. In *The Prokaryotes*, pp. 1302–1330. Edited by M. P. Starr, H. Stolp, H. G. Trüper, A. Balows & H. G. Schlegel. Berlin: Springer.
- Breznak, J. A. & Costilow, R. N. (1994). Physicochemical factors in growth. In *Methods for General and Molecular Bacteriology*, pp. 137–154. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Choi, D. H., Yi, H., Chun, J. & Cho, B. C. (2006). *Jannaschia seosinensis* sp. nov., isolated from hypersaline water of a solar saltern in Korea. *Int J Syst Evol Microbiol* **56**, 45–49.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Groth, I., Schumann, P., Weiss, N., Martin, K. & Rainey, F. A. (1996). *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diamminobutyric acid in the cell wall. *Int J Syst Bacteriol* **46**, 234–239.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Kwon, S. W., Kim, J. S., Park, I. C., Yoon, S. H., Park, D. H., Lim, C. K. & Go, S. J. (2003). *Pseudomonas koreensis* sp. nov., *Pseudomonas umsongensis* sp. nov. and *Pseudomonas jinjuensis* sp. nov., novel species from farm soils in Korea. *Int J Syst Evol Microbiol* **53**, 21–27.
- Macián, M. C., Arahál, D. R., Garay, E., Ludwig, W., Schleifer, K. H. & Pujalte, M. J. (2005). *Jannaschia rubra* sp. nov., a red-pigmented bacterium isolated from sea water. *Int J Syst Evol Microbiol* **55**, 649–653.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaál, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Pujalte, M. J., Macián, M. C., Arahál, D. R. & Garay, E. (2005). *Thalassobacter stenotrophicus* Macián *et al.* 2005 is a later synonym of *Jannaschia cystaugens* Adachi *et al.* 2004, with emended description of the genus *Thalassobacter*. *Int J Syst Evol Microbiol* **55**, 1959–1963.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids. *USFCC Newsl* **20**, 16.
- Seldin, L. & Dubnau, D. (1985). Deoxyribonucleic acid homology among *Bacillus polymyxa*, *Bacillus macerans*, *Bacillus azotofixans*, and other nitrogen-fixing *Bacillus* strains. *Int J Syst Bacteriol* **35**, 151–154.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Wagner-Döbler, I., Rheims, H., Felske, A., Pukall, R. & Tindall, B. J. (2003). *Jannaschia helgolandensis* gen. nov., sp. nov., a novel abundant member of the marine *Roseobacter* clade from the North Sea. *Int J Syst Evol Microbiol* **53**, 731–738.
- Yoon, J.-H., Kang, S.-J., Park, S. & Oh, T.-K. (2007). *Jannaschia donghaensis* sp. nov., isolated from seawater of the East Sea, Korea. *Int J Syst Evol Microbiol* **57**, 2132–2136.