International Journal of Systematic and Evolutionary Microbiology (2016), **66**, 1807–1812 **DOI** 10.1099/ijsem.0.000946

Terasakiella brassicae sp. nov., isolated from the wastewater of a pickle-processing factory, and emended descriptions of *Terasakiella pusilla* and the genus *Terasakiella*

Shuai-Bo Han,[1] Yue Su,[2] Jing Hu,[1] Rui-Jun Wang,[2] Cong Sun,[1] Dildar Wu,[3] Xu-Fen Zhu[1] and Min Wu[1]

[1]College of Life Sciences, Zhejiang University, Hangzhou 310058, PR China

[2]Ocean College, Zhejiang University, Hangzhou 310058, PR China

[3]Department of Biology, Xinjiang Normal University, Urumqi 830054, PR China

Correspondence
Min Wu
wumin@zju.edu.cn
Xu-Fen Zhu
xufenzhu@zju.edu.cn

A Gram-stain-negative, motile, polyhydroxybutyrate-accumulating, aerobic, S-shaped bacterium, designated $B3^T$, was isolated from the wastewater of a pickle-processing factory. 16S rRNA gene sequence similarity analysis showed that it was most closely related to the type strain, *Terasakiella pusilla* (96.6 % similarity). Strain $B3^T$ was able to grow at 4–40 °C (optimum 32–37 °C), pH 5.5–9.0 (optimum 6.5–7.5) and with 0.5–8 % (w/v) NaCl present (optimum 1–2 %, w/v). Chemotaxonomic analysis showed that the respiratory quinone was ubiquinone Q-10, the major fatty acids included $C_{16:0}$, $C_{18:1}\omega7c$ and $C_{16:1}\omega7c$ and/or iso-$C_{15:}$2-OH. The major polar lipids included diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylserine, aminophospholipid and three uncharacterized phospholipids. The genomic DNA G+C content of strain $B3^T$ was 42.3 mol%. The DNA–DNA relatedness value between $B3^T$ and *T. pusilla* DSM 9263^T was 23.9 %. On the basis of the phenotypic, chemotaxonomic and genotypic characteristics of strain $B3^T$, it represents a novel species of the genus *Terasakiella*, for which the name *Terasakiella brassicae* sp. nov. is proposed. The type strain is $B3^T$ (=KCTC 42652^T=CGMCC 1.15254^T). Emended descriptions of *T. pusilla* and the genus *Terasakiella* are also presented.

The genus *Terasakiella*, belonging to the family *Methylocystaceae*, was first proposed by Satomi *et al.* (2002). The type species *Terasakiella pusilla* was isolated from a putrid infusion of a marine shellfish (Terasaki, 1973). The names '*Spirillum pusillum*' (Terasaki, 1973) and *Oceanospirillum pusillum* (Terasaki, 1979) have been used successively to describe this species. After that, the name was transferred to *Terasakiella pusilla* based on phylogenetic analysis and in honour of Terasaki by Satomi *et al.* (2002). At the time of writing, the genus *Terasakiella* contains only one species with a validly published name, *T. pusilla*.

Abbreviations: APL, aminophospholipid; DPG, diphosphatidylglycerol; L, lipid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PHB, polyhydoxybutyrate; PL, phospholipid; PS, phosphatidylserine.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain $B3^T$ is KP994391.

Two supplementary figures are available with the online Supplementary Material.

In this study, we focus on the description of strain $B3^T$, isolated from the wastewater of a pickle-processing factory in Zhejiang province, PR China. The pH of the wastewater was 7.26 and the salinity was 2.72 % (w/v). *T. pusilla* DSM 6293^T was used as a reference strain. The new isolate represented a novel species of the genus *Terasakiella* based on the phenotypic and phylogenetic data presented in this study.

The novel isolate was obtained by the following procedure. The wastewater was diluted and spread onto marine agar 2216 (MA) using a tenfold series dilution method. Obvious colonies formed after 2 days' incubation at 30 °C. Distinctive colonies were picked out and purified by repeated restreaking. Purity was confirmed by the uniformity of cell morphology. The isolate was routinely cultured on marine broth 2216 (MB) medium (BD) and maintained at −80 °C with 20 % (v/v) glycerol.

Cell morphology and motility were determined by using optical microscopy (Olympus; BX40) and transmission electron microscopy (JEOL; JEM-1230) (Huo *et al.*, 2010). Cells grown on plates were suspended and stained

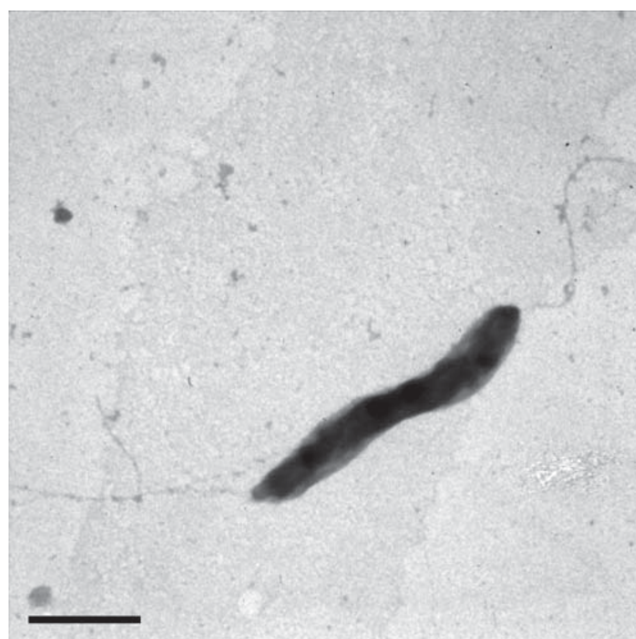
Downloaded from www.microbiologyresearch.org by
IP: 183.157.162.61
On: Tue, 14 Jun 2016 13:39:23

with uranyl acetate and then fixed on copper mesh before being observed by TEM. Optimal conditions for growth were determined in marine broth 2216 medium containing various NaCl concentrations (0.5 and 1.0–11.0 %, w/v, at increments of 1 %). The temperature range for growth was tested by incubating cells at various temperatures (4, 15, 20, 25, 28, 32, 37, 40, 42, 45 50 °C). The pH range (from pH 5.5 to 10.0, at intervals of 0.5 pH units) was determined in marine broth 2216 medium with the addition of 30 mM buffering agents, including: MES (pH 5.5–6.5), PIPES (pH 6.5–7.5), Tricine buffer (pH 7.5–8.5) and CAPSO (pH 9.0–10.0).

The utilization of single carbon sources was performed with modified BM medium (Farmer & Hickman-Brenner, 2006). The medium contained (l^{-1} distilled water): 1.0 g NH_4Cl, 0.075 g $K_2HPO_4 . 7H_2O$, 0.028 g $FeSO_4 . 7H_2O$, 11.7 g NaCl, 12.3 g $MgSO_4 . 7H_2O$, 0.75 g KCl, 1.45 g $CaCl_2$, 10 ml Tris/HCl (10 mM, pH 8.0), and 25 mM PIPES, pH 7.0. API ZYM, 20NE and 50CH kits (Bio Mérieux) were used according to the manufacturers' instructions. Other biochemical tests were performed using the methods described by Mata *et al.* (2002). Polyhydroxybutyrate (PHB) production was investigated based on the methods of Zhu *et al.* (2011). Antibiotic susceptibility tests were determined on MA plates for 3 days at 30 °C using antibiotic discs containing the following (μg per disc, unless indicated): amoxicillin (10), ampicillin(10), bacitracin (0.04 IU), carbenicillin (100), cefoxitin (30), chloramphenicol (30), erythromycin (15), gentamicin (10), kanamycin (30), neomycin (30), norfloxacin (10), novobiocin (30), penicillin G (10 IU), polymyxin (300 IU), rifampicin (5), streptomycin (10), sulfamethoxazole (300) and tetracycline (30). The strains were considered susceptible, intermediate and resistant, respectively, when the diameter of the inhibition zone was >5 mm, 2–5 mm and <2 mm, according to Nokhal & Schlegel (1983).

Cells of strain $B3^T$ and *T. pusilla* DSM 6293^T, which were grown on marine broth 2216 medium for 24 h at 30 °C, were used for polar lipid and isoprenoid quinone analysis. Polar lipids were extracted and separated on silica gel 60 F_{254} aluminium-backed thin-layer plates (10 × 10 cm; Merk 5554) which had dried for 30 min at 55 °C and further analysed according to Minnikin *et al.* (1984) and Cui *et al.* (2011). The first dimension of the solvent system was chloroform/methanol/water (65 : 24 : 4, by vol.) and the second dimension was chloroform/glacial acetic acid/methanol/water (80 : 15 : 12 : 4, by vol.). Then the plates were sprayed with sulfuric acid/ethanol (1 : 1, v/v) and heated at 120 °C for 10 min to reveal total lipids. Other reagents such as α-naphthol, ninhydrin and molybdenum blue (Sigma) were used to detect glycolipids, aminolipids and phospholipids, according to Tindall (1990). Phosphomolybdic acid (5 %, w/v, dissolved in alcohol) was sprayed and then heated at 160 °C for 10–15 min to identify total lipids. Ethanol/sulfuric acid/glacial acetic acid/anisaldehyde (135 : 5: 1.5 : 3.7, by vol.) was also sprayed and heated at 120 °C for 5 min to assist with total polar lipid analysis. Isoprenoid quinones were extracted from freeze-dried cells with chloroform/methanol (2 : 1, v/v) and analysed by reversed-phase HPLC. For the preparation of cellular fatty acid methyl esters (FAMEs), the two strains were harvested and freeze-dried at the exponential stage of growth according to Kuykendall *et al.* (1988). Identification and quantification of FAMEs were performed by the Sherlock Microbial Identification System (MIDI) with standard MIS Library Generation software (Microbial ID).

We used a quick bacteria genomic DNA extraction kit (DongSheng Biotech) to obtain a high quality template. An almost complete 16S rRNA gene sequence of this isolate was obtained by PCR using the primer pair 27F (5′-AGAGTTTGATCCTGGCTCAG- 3′) / 1492R (5′-GGTTACCTT



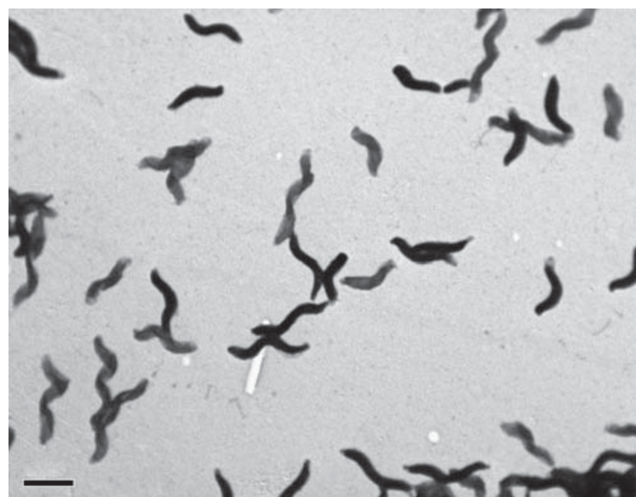


Fig. 1. (a) Transmission electron micrograph of a cell of strain $B3^T$ growing on MA plates at 30 °C for 24 h; bar, 1.0 μm. (b) TEM of cells of strain $B3^T$; bar, 2.0 μm.

Downloaded from www.microbiologyresearch.org by
IP: 183.157.162.61
On: Tue, 14 Jun 2016 13:39:23

GTTACGACTT-3′) and the PCR products were cloned into a pMD19-T vector (Takara) for sequencing (Xu *et al.*, 2007). The sequence was compared with closely related organisms provided by EzTaxon services (Kim *et al.*, 2012). Multiple sequences were aligned with CLUSTAL W 1.8 (Thompson *et al.*, 1994). Phylogenetic trees were reconstructed using the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and the maximum-parsimony (Fitch, 1971) methods with the MEGA5 program package. Evolutionary distances were calculated according to the algorithm of Kimura's two-parameter model (Kimura, 1980) for the neighbour-joining method. The DNA G+C content was determined by reversed-phase HPLC, as described by Mesbah & Whitman (1989). DNA–DNA hybridization experiments were performed with the thermal denaturation and renaturation method according to Zhang *et al.* (2010), using a Backman DU800 spectrophotometer.

Cells of strain $B3^T$ were Gram-stain-negative, PHB-accumulating, S-shaped, helical counter-clockwise (Fig S1, available in the online Supplementary Material) and motile by means of single bipolar flagellum (Fig. 1). Strain $B3^T$ grew at 4–40 °C (optimum 32–37 °C), pH 5.5–9.0 (optimum 6.5–7.5) and with 0.5–8 % NaCl (w/v, optimum 1–2 %). Detailed results of physiological and biochemical tests are given in the species description. The differential characteristics between strain $B3^T$ and the reference strain are summarized in Table 1.

The polar lipid profile of strain $B3^T$ included diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylserine (PS), aminophospholipid (APL), three uncharacterized phospholipids (PL1–3) and three uncharacterized lipids (L1–3), which differed from that of the reference strain, *T. pusilla* DSM 6293^T, in this study (Fig. S2). The major cellular fatty acids of strain $B3^T$ were $C_{16:0}$ (14.5 % of the total), $C_{18:1}\omega7c$ (28.8 %) and $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$2-OH (33.4 %) (Table 2). Ubiquinone Q-10 was detected as the sole respiratory quinone of strain $B3^T$, which is in accordance with the genus description.

An almost-complete 16S rRNA gene sequence (1461 nt) of strain $B3^T$ was obtained. Similarity analysis based on it and those of other representative bacteria with validly published names revealed that the strain belongs to the genus *Terasakiella* and the most closely strain was *T. pusilla* DSM 6293^T (96.6 %). Phylogenetic analysis, based on 16S rRNA gene sequences, showed that strain $B3^T$ clustered with *T. pusilla* DSM 6293^T in the neighbour-joining, maximum-likelihood and maximum-parsimony trees (Fig. 2). The level of DNA–DNA relatedness between strains $B3^T$ and *T. pusilla* DSM 6293^T was 23.9 %, lower than the threshold value of 70 % for separating species (Stackebrandt & Goebel, 1994). The DNA G+C content of strain $B3^T$ was 42.3 mol% (HPLC).

The phylogenetic analysis, similar cell morphology (S-shaped and bipolar single flagellum), same major fatty

Table 1. Differential phenotypic and genotypic characteristics of strain $B3^T$ and *T. pusilla* DSM 6293^T

Strains: 1. strain $B3^T$; 2. *T. pusilla* DSM 6293^T. +, Positive; −, negative; R, resistant; S, susceptible; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PS, phosphatidylserine; APL, aminophospholipid; AL, aminolipid; PL1–3, three uncharacterized phospholipids; L1–3, three uncharacterized lipids.

Characteristic	1	2
pH range for growth	5.5–9.0	6.0–9.0*
Nitrate reduction	−	+
Utilization of:		
Citrate	−	+
Tween 40	−	+
Glycerol	−	+
Hydrolysis of:		
Tyrosine	−	+
Voges-Proskauer test	−	+
Enzyme activities (API ZYM)		
Acid phosphatase	+	−
Cefoxitin (30 µg)	R	S
Polar lipids	DPG, PG, PE, PS, APL, PL1, PL2, PL3, L1, L2, L3	DPG, PG, PE, PS, APL, AL, PL3, L2
DNA G+C content (mol%)	42.3	45.8

*Data from Terasaki (1979).

Table 2. Fatty acid composition (as a percentage of the total) of strain $B3^T$ and *T. pusilla* DSM 6293^T

Strains: 1, $B3^T$; 2, *T. pusilla* DSM 6293^T. Data from this study. Fatty acids that represented <1 % in both strains are not shown. –, Not detected. Major components (≥10 %) are highlighted in bold.

Fatty acid	1	2
Saturated		
C12 : 0	5.6	4.8
C14 : 0	4.5	2.0
C16 : 0	**14.5**	**12.5**
Unsaturated		
C16 : 1 ω5c	1.4	1.7
C18 : 1 ω7c	**28.8**	**35.5**
Hydroxy		
C18 : 1 2-OH	–	2.9
Summed feature 2*	9.6	10.2
Summed feature 3*	**33.4**	**26.7**

*Summed feature 2 contained iso-$C_{16:1}$ I and/or $C_{14:0}$ 3-OH. Summed feature 3 contained $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH.

Downloaded from www.microbiologyresearch.org by
IP: 183.157.162.61
On: Tue, 14 Jun 2016 13:39:23

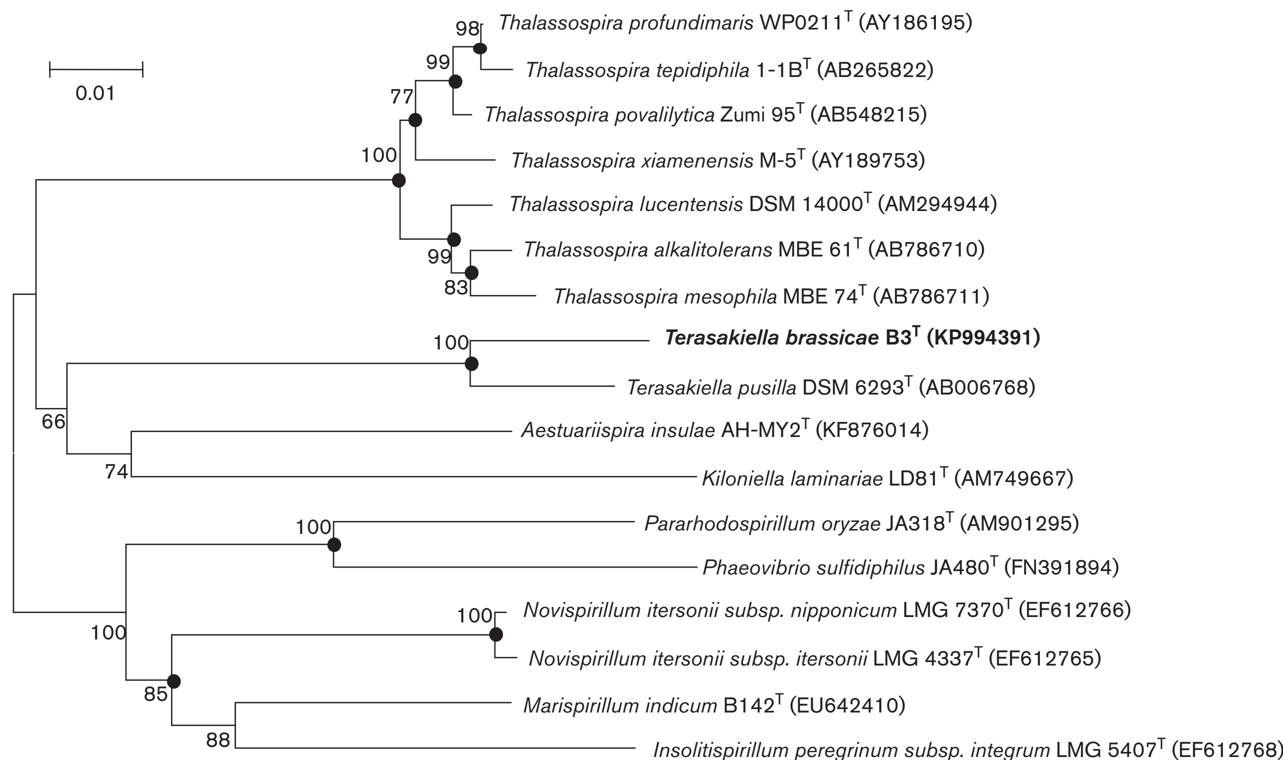


Fig. 2. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strain B3^T and related species. Bootstrap values based on 1000 replicates are listed as percentages at branching points. Only bootstrap values above 50 % are shown. Filled circles indicate that the corresponding nodes were also recovered in both maximum-likelihood and maximum-parsimony trees. Bar, 0.01 substitutions per nucleotide position.

acids ($C_{16:0}$, $C_{18:1}\omega 7c$ and $C_{16:1}\omega 7c$ and/or iso-$C_{15:0}$ 2-OH), major polar lipids and respiratory quinone (Q-10), along with the other characteristics presented in this study classify strain B3^T in the genus *Terasakiella*. However, there are some differential characteristics distinguishing strain B3^T from the reference strain, *T. pusilla* DSM 6293^T. Firstly, the fatty acids of *T. pusilla* DSM 6293^T contained $C_{18:1}$ 2-OH, but it was not detected in strain B3^T. Secondly, in this study, the polar lipid profiles of the two strains were different. AL, which was presented in *T. pusilla* DSM 6293^T, was not detected in strain B3^T and L3 was detected at a similar position. Moreover, B3^T contained three uncharacterized phospholipids, which is two more than in *T. pusilla* DSM 6293^T (PL1, PL2). Thirdly, some biochemical characteristics, such as susceptibility to antimicrobial compounds, the activity of acid phosphatase, nitrate reduction, the Voges–Proskauer reaction, the hydrolysis of tyrosine and carbon source untilization patterns differentiated strain B3^T from *T. pusilla* DSM 6293^T. Fourthly, the DNA G+C content of strain B3^T was 3.5 % lower than that of *T. pusilla*. Finally, the low DNA–DNA relatedness value (23.9 %) between strain B3^T and *T. pusilla* DSM 6293^T clearly indicated that strain B3^T should represent a novel species of the genus *Terasakiella*.

Based on of the polyphasic taxonomic characterization presented in this study, strain B3^T is proposed to represent a novel species of genus *Terasakiella*, with the name *Terasakiella brassicae* sp. nov.

Emended description of *Terasakiella pusilla* Terasaki (1973), emend. Satomi *et al.* (2002)

The major fatty acids include $C_{16:0}$, $C_{18:1}\omega 7c$, iso-$C_{16:1}$ I and/or $C_{14:0}$ 3-OH and $C_{16:1}\omega 7c$ and/or iso-$C_{15:0}$2-OH. The major polar lipids include diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylserine, aminophospholipid and aminolipid. The genomic DNA G+C content is 45.8 mol% (determined by HPLC). Other properties (Terasaki, 1973) are unchanged.

Emended description of the genus *Terasakiella* Satomi *et al.* (2002)

Nitrate is reduced to nitrite or not. The major fatty acids include $C_{16:0}$, $C_{18:1}\omega 7c$, $C_{16:1}\omega 7c$ and/or iso-$C1_{5:0}$ 2-OH. The major polar lipids include diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylserine and aminophospholipid. The G+C content of the genomic DNA varies between 42 and 46 mol%. The rest of

Downloaded from www.microbiologyresearch.org by
IP: 183.157.162.61
On: Tue, 14 Jun 2016 13:39:23

the description is identical to that given by Satomi *et al.* (2002). The type species is *Terasakiella pusilla.*

Description of *Terasakiella brassicae* sp. nov.

Terasakiella brassicae (bras'si.cae. L. gen. n. *brassicae* of cabbage, referring to the pickle ingredients, which the type strain was isolated from).

Cells are Gram-stain-negative, polyhydroxybutyrate-accumulating, S-shaped and helically counter-clockwise 1.5 are the predominant cell forms. Cells are approximately 0.3–0.5 µm wide and 1.5–4 µm long, and motile by means of single bipolar flagellum. After incubation for 24 h on marine agar 2216 plates, colonies are 0.5–1 mm in diameter, slightly convex, non-pigmented, smooth and circular. Growth occurs at pH 5.5–9.0, optimum 6.5–7.5. The optimal growth temperature is 32–37 °C and no growth is detected at 4 or 40 °C. The NaCl concentration range for growth is 0.5–8 % (w/v) and optimal growth occurs at 1–2 % (w/v). No growth is observed in anaerobic conditions by anaerobic respiration with $S_2O_3^{2-}$, SO_3^{2-}, SO_4^{2-}, NO_2^- or NO_3^- as electron acceptors. Negative for the catalase, methyl red and Voges–Proskauer reactions, nitrate or nitrite reduction, indole production and arginine dihydrolase. No acid is produced from the carbohydrates tested. Positive for oxidase and H_2S production (from thiosulfate or L-cysteine). No hydrolysis of aesculin, urea, casein, gelatin, tyrosine, starch or CM-cellulose. The following compounds are utilized as sole carbon sources: acetate, propionate, lactate, butyrate, succinate, malate and pyruvate. The following substrates are not utilized as sole carbon sources: formate, oxalate, tartrate, alginate, citrate, gluconate, glucose, arabinose, mannose, galactose, lactose, maltose, sucrose, xylose, fructose, rhamnose, cellobiose, trehalose, Tweens 40, Tweens 80 and glycerol. Alkaline and acid phosphatase, butyrate esterase, caprylate esterase lipase and naphthol-AS-BI-phosphohydrolase are positive in the API ZYM system. Susceptible to erythromycin, neomycin, amoxicillin, chloramphenicol, novobiocin, carbenicillin, penicillin G, ampicillin, kanamycin, gentamicin, norfloxacin, streptomycin, but resistant to bacitracin, tetracycline, polymyxin B and cefoxitin. The polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylserine, aminophospholipid, three uncharacterized phospholipids and three uncharacterized lipids. The respiratory quinone is ubiquinone Q-10. Major fatty acids are $C_{16:0}$, $C_{18:1}\omega7c$ and $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$2-OH.

The type strain, $B3^T$ (=CGMCC 1.15254^T=KCTC 42652^T), was isolated from wastewater collected from a pickle-processing factory in Zhejiang province, PR China. The DNA G+C content of the type strain is 42.3 mol% (determined by HPLC).

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (No. 31470005) and China Ocean Mineral Resources R & D Association (COMRA) Special Foundation (DY125-14-E-02).

References

Cui, H.-L., Gao, X., Yang, X. & Xu, X.-W. (2011). *Halolamina pelagica* gen. nov., sp. nov., a new member of the family *Halobacteriaceae. Int J Syst Evol Microbiol* **61**, 1617–1621.

Farmer, J. J., III & Hickman-Brenner, F. W. (2006). The genera *Vibrio* and *Photobacterium.* In *The Prokaryotes: a Handbook on the Biology of Bacteria,* 3rd edn, vol. 6, pp. 508–563. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer & E. Stackebrandt. New York: Springer.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

Huo, Y. Y., Xu, X. W., Cui, H. L. & Wu, M. (2010). *Gracilibacillus ureilyticus* sp. nov., a halotolerant bacterium from a saline-alkaline soil. *Int J Syst Evol Microbiol* **60**, 1383–1386.

Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon, Y. S., Lee, J. H. & other authors (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* **62**, 716–721.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

Kuykendall, L. D., Roy, M. A., O'Neill, J. J. & Devine, T. E. (1988). Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum. Int J Syst Bacteriol* **38**, 358–361.

Mata, J. A., Martínez-Cánovas, J., Quesada, E. & Béjar, V. (2002). A detailed phenotypic characterisation of the type strains of *Halomonas* species. *Syst Appl Microbiol* **25**, 360–375.

Mesbah, M. & Whitman, W. B. (1989). Measurement of deoxyguanosine/thymidine ratios in complex mixtures by high-performance liquid chromatography for determination of the mole percentage guanine + cytosine of DNA. *J Chromatogr A* **479**, 297–306.

Minnikin, D. E., Odonnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.

Nokhal, T. H. & Schlegel, H. G. (1983). Taxonomic study of *Paracoccus denitrificans. Int J Syst Bacteriol* **33**, 26–37.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Satomi, M., Kimura, B., Hamada, T., Harayama, S. & Fujii, T. (2002). Phylogenetic study of the genus *Oceanospirillum* based on 16S rRNA and gyrB genes: emended description of the genus *Oceanospirillum*, description of *Pseudospirillum* gen. nov., *Oceanobacter* gen. nov. and *Terasakiella* gen. nov. and transfer of *Oceanospirillum jannaschii* and *Pseudomonas stanieri* to *Marinobacterium* as *Marinobacterium jannaschii* comb. nov. and *Marinobacterium stanieri* comb. nov. *Int J Syst Evol Microbiol* **52**, 739–747.

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.

Terasaki, Y. (1973). Studies on the genus *Spirillum* Ehrenberg. II. Comments on type and reference strains of *Spirillum* and description of new species and subspecies. *Bull Suzugamine Women's Coll Nat Sci* **17**, 1–71.

Downloaded from www.microbiologyresearch.org by
IP: 183.157.162.61
On: Tue, 14 Jun 2016 13:39:23

Terasaki, Y. (1979). Transfer of five species and two subspecies of *Spirillum* to other genera (*Aquaspirillum* and *Oceanospirillum*), with emended descriptions of the species and subspecies. *Int J Syst Bacteriol* **29**, 130–144.

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.

Tindall, B. J. (1990). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.

Xu, X. W., Wu, Y. H., Zhou, Z., Wang, C. S., Zhou, Y. G., Zhang, H. B., Wang, Y. & Wu, M. (2007). *Halomonas saccharevitans* sp. nov., *Halomonas arcis* sp. nov. and *Halomonas subterranea* sp. nov., halophilic bacteria isolated from hypersaline environments of China. *Int J Syst Evol Microbiol* **57**, 1619–1624.

Zhang, X. Q., Ying, Y., Ye, Y., Xu, X. W., Zhu, X. F. & Wu, M. (2010). *Thermus arciformis* sp. nov., a thermophilic species from a geothermal area. *Int J Syst Evol Microbiol* **60**, 834–839.

Zhu, X.-F., Jia, X.-M., Zhang, X.-Q., Wu, Y.-H. & Chen, Z.-Y. (2011). *Modern Experimental Technique of Microbiology*. Hangzhou: Zhejiang University Press (English translation).

Downloaded from www.microbiologyresearch.org by
IP: 183.157.162.61
On: Tue, 14 Jun 2016 13:39:23