Candidimonas bauzanensis sp. nov., isolated from soil, and emended description of the genus *Candidimonas* Vaz-Moreira *et al.* 2011

De-Chao Zhang,¹ Hans-Jürgen Busse,² Cornelia Wieser,² Hong-Can Liu,³ Yu-Guang Zhou,³ Franz Schinner¹ and Rosa Margesin¹

Correspondence

Rosa Margesin

Rosa.Margesin@uibk.ac.at

¹Institute of Microbiology, University of Innsbruck, Technikerstrasse 25, A-6020 Innsbruck, Austria

²Institute of Bacteriology, Mycology and Hygiene, University of Veterinary Medicine Vienna, Veterinärplatz 1, A-1210 Vienna, Austria

³China General Microbiological Culture Collection Center and State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, PR China

A Gram-negative, facultatively anaerobic, psychrophilic, motile rod, designated BZ59^T, was isolated from hydrocarbon-contaminated soil. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain BZ59^T belonged to the genus *Candidimonas* and had highest 16S rRNA gene sequence similarity with *Candidimonas nitroreducens* SC-089^T (97.7 %) and *Candidimonas humi* SC-092^T (97.6 %). The ubiquinone was Q-8 and the major fatty acids were C_{16:0}, C_{17:0} cyclo and summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH). The polar lipid profile contained the major compounds phosphatidylethanolamine, phosphatidylglycerol, phosphatidylmonomethylethanolamine and diphosphatidylglycerol. The major polyamines were putrescine and spermidine; a minor amount of 2-hydroxyputrescine was present. The DNA G + C content of strain BZ59^T was 61.6 mol%. Combined data from phenotypic, phylogenetic and DNA–DNA relatedness studies demonstrated that strain BZ59^T represents a novel species of the genus *Candidimonas*, for which the name *Candidimonas bauzanensis* sp. nov. is proposed. The type strain is BZ59^T (=DSM 22805^T=LMG 26046^T=CGMCC 1.10190^T). The description of the genus *Candidimonas* is emended.

The genus *Candidimonas* was proposed by Vaz-Moreira *et al.* (2011) to accommodate Gram-negative rods that contain Q-8 as the respiratory quinone and summed feature 2 (C_{14:0} 3-OH and/or iso-C_{16:1} I), C_{16:0} and C_{18:1}ω7c as the major fatty acids. At the time of writing, the genus *Candidimonas* includes two species: *Candidimonas nitroreducens* and *Candidimonas humi*. The type strains of these species were isolated from sewage sludge compost. In this study, we describe the characterization of a novel bacterium of the genus *Candidimonas*.

Strain BZ59^T was isolated from soil of an industrial site containing high amounts of heavy crude oil and heavy metals in Bozen, South Tyrol, Italy, as previously described (Zhang *et al.*, 2010). Briefly, soil was shaken with sodium

pyrophosphate solution and appropriate dilutions were plated on R2A agar (containing 0.05 % yeast extract, 0.05 % peptone, 0.05 % Casamino acids, 0.05 % glucose, 0.05 % starch, 0.03 % sodium pyruvate, 0.03 % K₂HPO₄, 0.005 % MgSO₄, 1.5 % agar; pH 7; Reasoner & Geldreich, 1985). Strain BZ59^T formed white colonies. Strain BZ59^T was routinely cultured on R2A agar and stored as a suspension in skim milk (10 %, w/v) at –80 °C. *C. nitroreducens* SC-089^T, *C. humi* SC-092^T, *Parapusillimonas granuli* KCTC 12668^T and *Pusillimonas ginsengisoli* KCTC 22046^T were routinely grown on R2A agar at 25 °C and used as reference strains.

DNA of strain BZ59^T was extracted and purified as described by Sambrook *et al.* (1989). The 16S rRNA gene was amplified by PCR with the universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1541R (5'-AAGGAGGTGATCCAGCCGCA-3'). The amplification products were cloned using the pGEM-T Easy vector system (Promega). Sequencing reactions were carried out by Eurofins MWG Operon (Ebersberg, Germany) using the ABI Big Dye Terminator kit (v3.1) and an automated DNA sequencer (model ABI 3730 XL). The sequence was

Abbreviations: AL, Unidentified aminolipid; APL, aminophospholipid; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PME, phosphatidylmonomethylethanolamine.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BZ59^T is GQ246953.

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

aligned with sequences retrieved from GenBank and EMBL databases using CLUSTAL_X version 1.8 (Thompson *et al.*, 1997). Neighbour-joining phylogenetic analysis was carried out using MEGA version 4.0 (Tamura *et al.*, 2007). In addition, a maximum-likelihood tree was generated using PHYLIP version 3.69 (Felsenstein, 2009). The neighbour-joining tree (Fig. 1) showed that strain BZ59^T clustered with the genus *Candidimonas* and formed a distinct cluster with *C. nitroreducens* SC-089^T and *C. humi* SC-092^T, with which it exhibited 97.7% and 97.6% 16S rRNA gene sequence similarity, respectively. A similar topology was found in the maximum-likelihood tree (Fig. 1).

Morphology of cells grown on R2A agar at 25 °C was examined using phase-contrast microscopy ($\times 1000$; Dia-plan, Leitz) and transmission electron microscopy (Libra 120 EFTEM; Zeiss). Motility was examined using microscopy ($\times 1000$) and the API M system (bioMérieux). Gram-reaction was tested by Gram-staining and confirmed by KOH lysis. Catalase activity was determined by bubble production in 3% (v/v) H₂O₂ and cytochrome *c* oxidase activity was determined using 1% (w/v) *N,N,N',N'*-tetramethyl-*p*-phenylenediamine. Physiological and biochemical characteristics and enzyme activities were determined using the API 20 NE, API 20 E and API ZYM systems (bioMérieux) at 25 °C. Amylase, lipase and protease

Fig. 1. Neighbour-joining tree, based on 16S rRNA gene sequences, showing the phylogenetic position of strain BZ59^T and representatives of other taxa in the family *Alcaligenaceae*. Bootstrap values (>50%) based on 1000 replicates are shown at branch nodes. Asterisks indicate that the corresponding nodes were also recovered in the maximum-likelihood tree. Bar, 2% sequence divergence.

activities were also tested on R2A agar supplemented with the appropriate substrates (Margesin *et al.*, 2003). Growth under anaerobic conditions was determined after 7 days at 25 °C in an anaerobic jar containing Anaerocult A (Merck) to produce anaerobic conditions on nutrient agar (NA; 0.5 % peptone, 0.3 % meat extract, 1.5 % agar; pH 7) and R2A agar supplemented with 10 mM KNO₃. Growth at 1–45 °C was assessed on R2A agar and in R2A broth with shaking at 150 r.p.m. Growth at pH 5–10 (using buffered medium) and with 0–10 % (w/v) NaCl was determined on R2A agar. Growth with trypticase soy agar (TSA; 1.5 % casein peptone, 0.5 % soy peptone, 0.5 % sodium chloride, 1.5 % agar; pH 7) was also assessed. All tests were carried out simultaneously with strain BZ59^T and the four reference strains. The morphological, physiological and biochemical characteristics of strain BZ59^T are given in the species description and the features that differentiate strain BZ59^T from the reference strains are given in Table 1.

For fatty acid methyl ester analysis, strain BZ59^T and the reference strains were grown on TSA at 25 °C for 3 days

after quadrant streak inoculation. All strains agreed in their growing behaviour and cells of comparable physiological age were harvested from the third sectors of the quadrant streaks. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the Sherlock Microbial Identification System (version 6.1; MIDI; Sasser, 1990), using an Agilent 6890N gas chromatograph and were identified using the TSBA database (version 4.10). The fatty acids were determined by the Identification Service of the DSMZ (Braunschweig, Germany). Strain BZ59^T contained the following cellular fatty acids (>3 %): C_{16:0} (29.6 %), C_{17:0} cyclo (24.8 %), summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; 20.4 %), C_{18:1}ω7c (8.9 %), C_{12:0} (3.8 %), C_{12:0} 2-OH (2.3 %), C_{12:0} 3-OH (1.8 %) and summed feature 2 (C_{14:0} 3-OH and/or iso-C_{16:1} I; 4.6 %) (Table S1, available in IJSEM Online). The fatty acid composition of strain BZ59^T resembled those of the members of the genus *Candidimonas* (Vaz-Moreira *et al.*, 2011), with the exception that strain BZ59^T contained higher amounts of C_{12:0} and lower amounts of summed feature 2 and C_{18:0} than *C. humi* and *C. nitroreducens*.

Table 1. Phenotypic characteristics that differentiate strain BZ59^T from its closest phylogenetic neighbours

Strains: 1, BZ59^T; 2, *C. humi* SC-092^T; 3, *C. nitroreducens* SC-089^T; 4, *Parapusillimonas granuli* KCTC 12668^T; 5, *Pusillimonas ginsengisoli* KCTC 22046^T. Data were taken from this study unless otherwise stated. All strains are positive for catalase, cytochrome oxidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase and assimilation of adipic acid. All strains are negative for amylase, protease, lipase (C14), trypsin, α- and β-galactosidases, α- and β-glucosidases, β-glucuronidase, α-mannosidase, α-fucosidase, N-acetyl-β-glucosaminidase, arginine dihydrolase and assimilation of D-mannitol, N-acetylglucosamine, D-mannose, maltose and capric acid. +, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3	4	5
Isolation source*	Hydrocarbon-contaminated soil	Sewage sludge compost	Sewage sludge compost	Wastewater-treatment bioreactor	Soil of a ginseng field
DNA G + C content (mol%)*†	61.6	65.0	64.0	67.9 ± 0.1	57.3
Anaerobic growth	+	w	w	+	–
Motility	+	–	–	+	–
Growth at:					
1–5 °C	+	–	–	–	–
10 °C	+	–	–	+	+
pH 5	–	+	+	–	–
End product of nitrate reduction	N ₂	–	NO ₃	N ₂	NO ₃
Enzyme activities					
Urease	+	–	–	–	–
Alkaline phosphatase	–	+	+	–	–
Assimilation (API 20 NE)					
D-Glucose	+	–	–	–	–
L-Arabinose	w	–	–	–	–
Potassium gluconate	+	–	–	–	–
Malic acid	+	–	w	–	+
Trisodium citrate	w	w	–	–	+
Phenylacetic acid	–	+	+	+	+

*Data for columns 2 and 3 were obtained from Vaz-Moreira *et al.* (2011), for column 4 from Kim *et al.* (2010) and for column 5 from Srinivasan *et al.* (2010).

†Data for column 1 were obtained by the liquid renaturation method and for columns 2–5 by HPLC.

Respiratory quinones were extracted and purified according to Collins (1985) and analysed by HPLC (Wu *et al.*, 1989). The isoprenoid quinone was Q-8. For polyamine and polar lipid analysis, strain BZ59^T was grown in PYE medium (0.3 % yeast extract, 0.3 % peptone; pH 7) and harvested at the late exponential growth phase. Polyamines were extracted as described by Busse & Auling (1988) and analysed as described by Busse *et al.* (1997) using the HPLC apparatus described by Stolz *et al.* (2007). The polyamine pattern contained putrescine [46.4 µmol (g dry mass)⁻¹], spermidine [19.6 µmol (g dry mass)⁻¹], 2-hydroxyputrescine [8.5 µmol (g dry mass)⁻¹], spermine [3.3 µmol (g dry mass)⁻¹] and cadaverine [1.3 µmol (g dry mass)⁻¹]. This polyamine pattern is in agreement with that of other members of the class *Betaproteobacteria*, which includes the family *Alcaligenaceae* (Busse & Auling, 1988). Polar lipids were analysed according to Tindall (1990a, b). The major polar lipids (Fig. S1) were phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylmonomethylethanolamine (PME) and diphosphatidylglycerol (DPG). Furthermore, minor amounts of an unidentified amino-lipid (AL), an aminophospholipid (APL) and two polar lipids (L1 and L2) were detected. This polar lipid profile exhibited several similarities with those of the genus *Candidimonas* (Vaz-Moreira *et al.*, 2011). As well as PE, PG and DPG, which have also been detected in other taxa of the family *Alcaligenaceae*, strain BZ59^T shared the presence of PME (designated APL1 by Vaz-Moreira *et al.*, 2011) with *C. humi* and *C. nitroreducens*. Of the members of the family *Alcaligenaceae*, a polar lipid showing the same staining and chromatographic behaviour as PME has only been previously reported for *C. humi* and *C. nitroreducens* and *Parapusillimonas granuli* (Vaz-Moreira *et al.*, 2011). On the other hand, the presence of AL, L1 and L2 and the absence of additional aminophospholipids clearly distinguished strain BZ59^T from the two recognized members of the genus *Candidimonas*. Considering the close phylogenetic relatedness between the isolate and *C. humi* and *C. nitroreducens*, the differences in polar lipid profiles should be considered as indicative of strain BZ59^T representing a novel species of the genus *Candidimonas* rather than a novel genus.

The DNA G + C content was determined using the thermal denaturation method with *Escherichia coli* K-12 as the reference. DNA–DNA hybridization was performed by the liquid renaturation method in triplicate, according to De Ley *et al.* (1970) as modified by Huß *et al.* (1983). Both experiments were carried out using a model Lambda 35 UV/VIS spectrometer equipped with a temperature program controller (Perkin–Elmer). The DNA G + C content of strain BZ59^T was 61.6 mol%. The DNA–DNA relatedness values between strain BZ59^T and *C. nitroreducens* SC-089^T and *C. humi* SC-092^T were 23.7 ± 2.3 % and 21.5 ± 3.5 %, respectively. These values are much lower than the 70 % threshold value proposed for separation of strains at the species level (Wayne *et al.*, 1987).

Strain BZ59^T could be differentiated from *C. nitroreducens* SC-089^T and *C. humi* SC-092^T by a number of physiological

properties, such as motility, ability to grow at 1–10 °C, urease activity, ability to assimilate glucose, potassium gluconate and malic acid and inability to assimilate phenylacetic acid and to produce alkaline phosphatase. In addition, the isolate differed from *Parapusillimonas granuli* KCTC 12668^T by its ability to grow at 1–5 °C and urease activity and further from *Pusillimonas ginsengisoli* KCTC 22046^T by motility and ability to grow under anaerobic conditions. The ability to produce the highest cell yields at 1–5 °C in liquid culture demonstrated the psychrophilic character of strain BZ59^T. We use the term psychrophile as a general term that describes a micro-organism that grows in a cold environment (Margesin *et al.*, 2008), since the use of growth rates to define the optimum growth temperature, as described by Morita (1975), has been shown to be ambiguous and inappropriate (Cavicchioli, 2006; Margesin, 2009).

The polar lipid profile of strain BZ59^T showed some striking similarities with those of the two recognized members of the genus *Candidimonas*, which were phylogenetically more closely related to each other than to strain BZ59^T (Fig. 1) and which also exhibited higher 16S rRNA gene sequence similarity with each other (99.1 %) than with strain BZ59^T (97.6–97.7 %). Similar degrees of differences were also seen in the polar lipid profiles: those of the two recognized members of the genus *Candidimonas* were almost indistinguishable, whereas strain BZ59^T lacked two of the four APLs listed in the genus description (Vaz-Moreira *et al.*, 2011). However, from our point of view, these differences should be considered as species specific and not indicative of a novel genus. Hence, we propose that strain BZ59^T is assigned to the genus *Candidimonas* as representing a novel species, *Candidimonas bauzanensis* sp. nov., and that the genus description is emended.

Emended description of the genus *Candidimonas* Vaz-Moreira *et al.* 2011

The description of the genus *Candidimonas* is as given by Vaz-Moreira *et al.* (2011) with the following modifications. Mesophilic or psychrophilic. Cells are motile or non-motile. Urease and alkaline phosphatase activity are species dependent. The predominant cellular fatty acids are C_{16:0}, C_{17:0} cyclo and summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH). The polar lipid profile contains phosphatidylethanolamine, phosphatidylglycerol, phosphatidylmonomethylethanolamine and diphosphatidylglycerol. Variable numbers of unidentified aminophospholipids are detectable. The type species is *Candidimonas nitroreducens*.

Description of *Candidimonas bauzanensis* sp. nov.

Candidimonas bauzanensis (bau.zan.en'sis. N.L. fem. adj. *bauzanensis* referring to *Bauzanum* medieval Latin name of Bozen/Bolzano, a city in South Tyrol, Italy, where the type strain was isolated).

Cells are Gram-negative, facultatively anaerobic rods (0.7–0.9 × 1.2–1.9 µm after 3 days at 25 °C on R2A agar) and motile by polar flagella (Fig. S2). Colonies on R2A agar are creamy white, smooth, round and convex with entire margins (after 3 and 5 days at 25 °C, 1–1.2 and 1.5 mm in diameter, respectively). Nitrate is reduced to nitrogen gas. Catalase- and cytochrome oxidase-positive. Grows well at 1–30 °C in R2A broth and on R2A agar, grows weakly at 37 °C and does not grow at 42 °C. In liquid culture, growth is fastest at 25–30 °C and cell density is highest at 1–5 °C. Grows at pH 6–8 and with 0–3 % (w/v) NaCl. Grows on NA and TSA. Negative for indole production from tryptophan, H₂S production, citrate utilization and aesculin and gelatin hydrolysis. Positive for urease, leucine arylamidase, esterase (C4) and naphthol-AS-BI-phosphohydrolase, weakly positive for esterase lipase (C8) and acid phosphatase, and negative for arginine dihydrolase, lysine dihydrolase, ornithine dihydrolase, tryptophan deaminase, alkaline phosphatase, amylase, protease (skimmed milk), trypsin, α -chymotrypsin, lipase (C14), *N*-acetyl- β -glucosaminidase, α - and β -galactosidases, β -glucuronidase, α - and β -glucosidases, α -mannosidase and α -fucosidase. Assimilates D-glucose, adipic acid, malic acid, potassium gluconate, trisodium citrate (weakly) and L-arabinose (weakly), but does not assimilate D-mannose, D-mannitol, maltose, *N*-acetylglucosamine, capric acid or phenylacetic acid. Does not ferment D-glucose, D-mannitol, sucrose, inositol, D-sorbitol, L-rhamnose, melibiose, amygdalin or L-arabinose. The polyamine pattern is composed of major amounts of putrescine and spermidine and minor amounts of 2-hydroxyputrescine, spermine and cadaverine. The isoprenoid quinone is Q-8. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, phosphatidylmonomethylethanolamine and diphosphatidylglycerol. Minor amounts of one unidentified aminolipid, one aminophospholipid and two polar lipids are also detected. The major fatty acids are C_{16:0}, C_{17:0} cyclo and summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH).

The type strain is BZ59^T (=DSM 22805^T=LMG 26046^T=CGMCC 1.10190^T), isolated from soil from an industrial site in Bozen, South Tyrol, Italy. The DNA G+C content of the type strain is 61.6 mol%.

Acknowledgements

We are grateful to Dr Célia M. Manaia and Dr Deok-Chun Yang for providing us with the type strains of *C. nitroreducens* and *C. humi*, and *Parapusillimonas granuli* and *Pusillimonas ginsengisoli*, respectively. This research work was supported by the Autonome Provinz Bozen, Südtirol. We are grateful to W. Salvenmoser (Institute of Zoology, University of Innsbruck) for performing transmission electron microscopy. We thank P. Thurnbichler and J. Mair for technical assistance.

References

- Busse, H.-J. & Auling, G. (1988). Polyamine pattern as a chemotaxonomic marker within the *Proteobacteria*. *Syst Appl Microbiol* **11**, 1–8.
- Busse, H.-J., Bunka, S., Hensel, A. & Lubitz, W. (1997). Discrimination of members of the family *Pasteurellaceae* based on polyamine patterns. *Int J Syst Bacteriol* **47**, 698–708.
- Cavicchioli, R. (2006). Cold-adapted archaea. *Nat Rev Microbiol* **4**, 331–343.
- Collins, M. D. (1985). Isoprenoid quinone analysis in classification and identification. In *Chemical Methods in Bacterial Systematics*, pp. 267–287. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Felsenstein, J. (2009). PHYLIP (phylogeny inference package), version 3.69. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- HuB, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Kim, Y.-J., Kim, M. K., Im, W.-T., Srinivasan, S. & Yang, D.-C. (2010). *Parapusillimonas granuli* gen. nov., sp. nov., isolated from granules from a wastewater-treatment bioreactor. *Int J Syst Evol Microbiol* **60**, 1401–1406.
- Margesin, R. (2009). Effect of temperature on growth parameters of psychrophilic bacteria and yeasts. *Extremophiles* **13**, 257–262.
- Margesin, R., Gander, S., Zacke, G., Gounot, A. M. & Schinner, F. (2003). Hydrocarbon degradation and enzyme activities of cold-adapted bacteria and yeasts. *Extremophiles* **7**, 451–458.
- Margesin, R., Schinner, F., Marx, J. C. & Gerday, C. (editors) (2008). *Psychrophiles: from Biodiversity to Biotechnology*. Berlin, Heidelberg: Springer.
- Morita, R. Y. (1975). Psychrophilic bacteria. *Bacteriol Rev* **39**, 144–167.
- Reasoner, D. J. & Geldreich, E. E. (1985). A new medium for the enumeration and subculture of bacteria from potable water. *Appl Environ Microbiol* **49**, 1–7.
- Sambrook, J., Frisch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a laboratory manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Srinivasan, S., Kim, M. K., Sathiyaraj, G., Kim, Y.-J. & Yang, D.-C. (2010). *Pusillimonas ginsengisoli* sp. nov., isolated from soil of a ginseng field. *Int J Syst Evol Microbiol* **60**, 1783–1787.
- Stolz, A., Busse, H.-J. & Kämpfer, P. (2007). *Pseudomonas knackmussii* sp. nov. *Int J Syst Evol Microbiol* **57**, 572–576.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Tindall, B. J. (1990a). A comparative study of the lipid composition of *Halobacterium saccharovororum* from various sources. *Syst Appl Microbiol* **13**, 128–130.
- Tindall, B. J. (1990b). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.
- Vaz-Moreira, I., Figueira, V., Lopes, A. R., De Brandt, E., Vandamme, P., Nunes, O. C. & Manaia, C. M. (2011). *Candidimonas nitroreducens* gen. nov., sp. nov. and *Candidimonas humi* sp. nov., isolated from sewage sludge compost. *Int J Syst Evol Microbiol* **61**, 2238–2246.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

Wu, C., Lu, X., Qin, M., Wang, Y. & Ruan, J. (1989). Analysis of menaquinone compound in microbial cells by HPLC. *Microbiology* [English translation of *Microbiology (Beijing)*] **16**, 176–178.

Zhang, D.-C., Schinner, F. & Margesin, R. (2010). *Pedobacter bauzanensis* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **60**, 2592–2595.