Amantichitinum ursilacus gen. nov., sp. nov., a chitin-degrading bacterium isolated from soil

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A bacterial strain named IGB-41^T was isolated from a soil sample from an ant hill near Stuttgart, Germany. The strain was Gram-negative, rod-shaped, motile and facultatively anaerobic. Phylogenetic analysis based on 16S rRNA grouped the strain IGB-41^T within the class *Betaproteobacteria* into the family *Neisseriaceae* together with *Silvimonas amylolytica* NBRC 103189^T, *Silvimonas iriomotensis* NBRC 103188^T and *Silvimonas terrae* KM-45^T as the closest relatives with sequence similarities of 96.7, 96.6 and 96.1 %, respectively. The G + C content of the genomic DNA was determined to be 61.5 mol% and quinone analysis revealed Q-8 as the only detectable quinone. Major cellular fatty acids were identified as C_{16:0}, summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c) and C_{18:1}ω7c. Strain IGB-41^T was unique in harbouring phosphoaminolipids, aminolipids and glycoaminolipids when compared with *Silvimonas amylolytica* NBRC 103189^T in polar lipid analysis. On the basis of the physiological, phenotypic and genotypic characteristics of strain IGB-41^T, we suggest that the novel strain should be assigned to a new genus *Amantichitinum* and novel species *Amantichitinum ursilacus*. The type species of the genus *Amantichitinum* is *Amantichitinum ursilacus* and the type strain is IGB-41^T (=DSM 23761^T=CIP 110167^T).

Chitin is the second most abundant polymer on earth, synthesized for use as structural scaffold by a variety of organisms including fungi, crustaceans and insects (Patil *et al.*, 2000). Due to its omnipresence, many organisms are able to degrade chitin and use it as a carbon and energy source in metabolic processes (Campbell & Williams, 1951). Habitats characterized by a natural enrichment of chitinous polymer, such as ant hills and other agglomerations of arthropoda, are more likely to harbour chitinolytic micro-organisms than other habitats. Therefore, screening for chitin-metabolizing micro-organisms in such habitats is highly promising. During a research project focused on the identification and characterization of new chitinolytic bacteria for use in industrial processes, strain IGB-41^T was isolated from a soil sample taken near Stuttgart, Germany.

A soil sample with a temperature of 18 °C was taken from an abandoned ant hill located near Lake Bärensee in

Stuttgart, Germany. Chitinolytic organisms were enriched by sequential use of different media with chitin of rising complexity. In a first and second step, acidic pretreated chitin was used as carbon source, which in the third step was supplemented with protein-free milled chitin. The latter was the sole carbon source for the fourth culture step. Three grams of the soil sample were transferred into 100 ml screening medium (pH 4.2) containing 0.05 M potassium phosphate, 5 mg Ca(NO₃)₂·4H₂O l⁻¹, 20 mg MgSO₄·7H₂O l⁻¹, 100 mg (NH₄)₂SO₄ l⁻¹, 1 mg Fe(III)NH₄-citrate l⁻¹, 100 µg ZnSO₄·7H₂O l⁻¹, 30 µg MnCl₂·4H₂O l⁻¹, 300 µg H₃BO₃ l⁻¹, 200 µg CoCl₂·6H₂O l⁻¹, 10 µg CuCl₂·2H₂O l⁻¹, 20 µg NiCl₂·6H₂O l⁻¹, 30 µg Na₂MoO₄·2H₂O l⁻¹, 25 mg tryptone l⁻¹, 12.5 mg yeast extract l⁻¹, 25 mg NaCl l⁻¹ and 0.5 g wet mass colloidal chitin l⁻¹. The suspension was incubated at 20 °C. After 10 days, a 10 ml aliquot of the suspension was added to 90 ml fresh culture medium. This procedure was repeated twice with tryptone, yeast extract and NaCl omitted and 1 g chitin (Senn Chemicals) added for the third step. In a fourth step, colloidal chitin was also omitted.

The micro-organism suspension was diluted 1:10⁶, spread on Luria–Bertani (LB)-agar pH 4.2 and incubated for 5 days at 20 °C. Morphologically distinguishable colonies

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Amantichitinum ursilacus* IGB-41^T is FN994890.

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

were purified by transfer onto new plates for repeated incubation under equivalent conditions.

The basic medium for growth determination was modified LB (LB_{mod}) containing 10 g tryptone l⁻¹ and 5 g yeast extract l⁻¹; 2 % agar, buffer or NaCl was added, if necessary. Growth at different temperatures was analysed on LB_{mod}-agar plates at pH 6.85 at temperatures between 4 and 40 °C (4, 10, 15, 20, 25, 30, 35, 37 and 40 °C). For the determination of pH-dependent growth, LB_{mod}-agar plates were supplemented with either 0.05 M citric acid/NaOH or ammonium chloride/ammonia buffer to yield pH values between 4 and 10 (pH 4.14, 4.85, 5.73, 6.61, 6.98, 8.19 and 9.2). The final pH was determined prior to sterilization. Salt tolerance was determined by growth at NaCl concentrations between 0 and 4 % (0, 1, 2, 3 and 4 %). Growth was determined after 3, 5 and 10 days and evaluated by colony size. Isolate IGB-41^T was maintained as glycerol stock (50%, by vol.) at -80 °C.

Gram reaction was conducted as described by Bast (2001) with *Lactobacillus plantarum* and *Escherichia coli* as controls. Cell morphology was examined by light microscopy and scanning electron microscopy after fixation with glutaraldehyde and dehydration by sequential washing with rising ethanol concentrations.

Growth under anaerobic conditions, lysis by KOH, aminopeptidase-, oxidase- and catalase activities as well as use or hydrolysis of different compounds such as adipate, citrate, malate, phenylacetate, gelatin, DNA, Tween 80 and starch were tested by the Deutsche Stammsammlung für Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) according to standard procedures (Reddy *et al.*, 2007).

For the determination of G + C content, genomic DNA was purified according to the procedure of Cashion *et al.* (1977). DNA was hydrolysed and prepared for measurement according to the protocol of Mesbah *et al.* (1989). Analysis of G + C content was done by HPLC according to the methods of Tamaoka & Komagata (1984) and Mesbah *et al.* (1989). The reaction was controlled with genomic DNA of *Bacillus subtilis* DSM 402 (43.5 mol% G + C), *Xanthomonas campestris* DSM 3586^T (65.1 mol% G + C) and *Streptomyces violaceoruber* DSM 40783 (72.1 mol% G + C). The determination of G + C content was performed in commission by the DSMZ.

DNA-DNA hybridization experiments were performed with isolate IGB-41^T, *Silvimonas amylolytica* NBRC 103189^T, *Silvimonas iriomotensis* NBRC 103188^T and *Silvimonas terrae* DSM 18233^T by the DSMZ. DNA was isolated using the procedure of Cashion *et al.* (1977). DNA-DNA hybridization was carried out as previously described (Huß *et al.*, 1983; De Ley *et al.*, 1970) using a Cary 100 Bio UV-VIS-spectrophotometer equipped with a Peltier-thermostat-regulated 6 × 6 multicell changer and a temperature controller with an *in situ* temperature probe (Varian). Results were obtained in 2 × SSC, 5 % formamide at 70 °C.

Genomic DNA was isolated after mechanical cell disruption according to Ausubel *et al.* (1987). Nearly full-length 16S rRNA (1492 bp) of isolate IGB-41^T was amplified using standard PCR primers (for-16S: AGAGTTTGATC-MTGGCTCAG; rev-16S: TACGGYTACCTGTTACGAC-TT). PCR was performed under the following cycling conditions: initial denaturation at 94 °C for 3 min, 94 °C for 30 s, annealing for 45 s at 56 °C and elongation at 72 °C for 2 min. After 30 cycles, final elongation was allowed for 10 min at 72 °C. Phylogenetic trees were reconstructed based on 16S rRNA using the ARB software package (Ludwig *et al.*, 2004). 16S rRNA gene sequence quality of isolate IGB-41^T was checked for sequence anomalies as described by Ashelford *et al.* (2006). 16S rRNA sequences were aligned locally using CLUSTAL_X v2.1 (Larkin *et al.*, 2007). The resulting alignment was used for tree reconstruction and is available as a supplementary FASTA file in IJSEM Online. The neighbour-joining algorithm (Saitou & Nei, 1987) with Jukes-Cantor correction was applied to reconstruct a phylogenetic tree with 1000-fold resampling. Tree topology was evaluated using Randomized Accelerated Maximum-likelihood RAXML v7.0.3 implemented into ARB (Stamatakis, 2006).

Fatty acid methyl esters were obtained from 40 mg cell mass, grown on trypticase soy broth agar (TSA) for two days at 28 °C. Cells were scraped from Petri dishes followed by saponification, methylation and extraction using minor modifications of the method described by Miller (1982) and Kuykendall *et al.* (1988). The fatty acid methyl ester mixtures were separated by the DSMZ using the Sherlock Microbial identification System (MIS) (MIDI, Microbial ID).

Polar lipid analysis and analysis of respiratory quinones was performed by the Identification Service of the DSMZ and Dr B. J. Tindall, DSMZ. Cells were grown in LB_{mod} without NaCl at 25 °C prior to analysis. Lipid extraction was done from 100 mg of freeze-dried cells using the two stage method described by Tindall (1990a, b). Respiratory quinones were extracted using methanol:hexane (2 : 1, by vol.) followed by phase separation into hexane (Tindall, 1990a, b). Separation of lipoquinones into their different classes was conducted by TLC on silica gel (Macherey-Nagel) using hexane:tert-butylmethylether [9:1 (v/v)] as the solvent. UV-absorbing bands corresponding to different quinone classes were further analysed by HPLC. This step was carried out on a LDC Analytical (Thermo Separation Products) HPLC fitted with a reverse phase column (Macherey-Nagel, 2 mm × 125 mm, 3 µm, RP18) using methanol:heptane [9:1 (v/v)] as the eluent. Respiratory lipoquinones were detected at 269 nm.

Polar lipids were extracted by adjusting the remaining methanol:0.3 % aqueous NaCl phase to give a chloroform:methanol:0.3 % aqueous NaCl mixture 1 : 2 : 0.8 (by volume). The extraction solvent was stirred overnight and cell debris was pelleted by centrifugation. Polar lipids were recovered into the chloroform phase by adjusting the

chloroform:methanol:0.3 % aqueous NaCl mixture to a ratio of 1:1:0.9 (by volume) and separated by two dimensional silica gel TLC (Macherey–Nagel). The first direction was developed in chloroform:methanol:water (65:25:4, by volume) and the second in chloroform:methanol:acetic acid:water (80:12:15:4, by volume). Total lipid material and specific functional groups were detected using dodecamolybdophosphoric acid (total lipids), Zinzadze reagent (phosphate), ninhydrin (free amino groups), periodate-Schiff (α -glycols), Dragendorff (quaternary nitrogen) and α -naphthol-sulphuric acid (glycolipids) (Tindall *et al.*, 2007).

Isolate IGB-41^T was found to be a representative of Gram-negative, rod-shaped (see Fig. S1a, b), motile and facultatively anaerobic bacteria with Q-8 found as the only detectable isoprenoid quinone.

Phylogenetic analysis revealed that isolate IGB-41^T belonged to the class *Betaproteobacteria*. Clustering was always observed within the family *Neisseriaceae* together with *Silvimonas amylolytica* NBRC 103189^T, *Silvimonas iriomotensis* NBRC 103188^T and *Silvimonas terrae* KM-45^T as the phylogenetically closest relatives with 96.7, 96.6 and 96.1 % sequence similarity, respectively. The divergent branching pattern between isolate IGB-41^T and the inner cluster of species of the genus *Silvimonas* was highly reproducible with solid bootstrap recovery using the neighbour-joining algorithm. This topology was further confirmed using RAXML (Fig. 1). *Jeongeupia naejangsensis* BIO-TAS4-2^T, *Andreprevotia lacus* GFC-1^T and *Andreprevotia chitinilytica* JS11-7^T formed an intrinsically stable sibling cluster to the cluster including isolate IGB-41^T and exhibited sequence similarities of only 93.3, 93.2 and 93.0 %, respectively, to strain IGB-41^T. The level of sequence similarity observed between IGB-41^T and its phylogenetically closest neighbour *Silvimonas amylolytica* NBRC 103189^T was significantly below the interspecies level of sequence similarity of recognized species of the genus

Silvimonas which is in a range of 98.8–99.2 %. This suggests that strain IGB-41^T forms an independent phylum in proximity to species of the genus *Silvimonas*.

The level of DNA–DNA relatedness as determined by DNA–DNA hybridization between isolate IGB-41^T and the type strains of *Silvimonas amylolytica*, *Silvimonas iriomotensis* and *Silvimonas terrae* was found with values of 18.4, 13.1 and 9.9 %, respectively. This is far below the proposed threshold for species delineation of 70 % (Wayne *et al.*, 1987), which indicates that strain IGB-41^T is a representative of a new genus and not a member of the genus *Silvimonas*.

The predominant fatty acids of strain IGB-41^T were identified as C_{16:0} (26.1 %), summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c) (26.1 %) and C_{18:1}ω7c (22.4 %). Differences between the fatty acid composition of species of the genus *Silvimonas* and isolate IGB-41^T were evident, especially for short-chain and hydroxylated fatty acids. In this comparison, strain IGB-41^T was the only representative with the metabolic compounds C_{14:0} 2-OH (2.2 %), C_{15:0} (1.4 %) and C_{18:1} 2-OH (1.5 %) (Table S1), whereas the other fatty acids were found with similar patterns and concentration ranges. Therefore, to further discriminate between the organisms, we performed polar lipid analysis.

Two dimensional TLC revealed fundamental differences between the polar lipid cell membrane composition of strains IGB-41^T and *Silvimonas amylolytica* NBRC 103189^T. Glycolipids were found in *Silvimonas amylolytica* NBRC 103189^T, while they were absent in isolate IGB-41^T. Strain IGB-41^T was unique in harbouring phosphoaminolipids, aminolipids and glycoaminolipids. This underlines the phylogenetic divergence between strain IGB-41^T and *Silvimonas amylolytica* NBRC 103189^T. Both strains gave a positive result for diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and phospholipids. Quantitative consideration revealed higher contents of phosphatidylethanolamine for *Silvimonas amylolytica* NBRC 103189^T than for strain IGB-41^T as can be deduced

Fig. 1. Rooted neighbour-joining tree showing phylogenetically close neighbours of isolate IGB-41^T within the family *Neisseriaceae*. Filled circles indicate branching patterns also recovered using RAXML. Bootstrap values ≥ 50 % are shown. Bar, 1 nt substitution per 100 bases.

Table 1. Differential characteristics of strain IGB-41^T and the closest phylogenetic neighbours

Taxa: 1, *Amantichitinum ursilacus* IGB-41^T; 2, *Silvimonas amylolytica* NBRC 103189^T (Muramatsu *et al.*, 2010); 3, *Silvimonas iriomotensis* NBRC 103188^T (Muramatsu *et al.*, 2010); 4, *Silvimonas terrae* KM-45^T (Yang *et al.*, 2005; Muramatsu *et al.*, 2010); 5, *Jeongeupia naejangsensis* BIO-TAS4-2^T (Yoon *et al.*, 2010); 6, *Andreprevotia chitinilytica* JS11-7^T (Weon *et al.*, 2007); 7, *Andreprevotia lacus* GFC-1^T (Sheu *et al.*, 2009). (+), weakly positive; +, positive; –, negative; ND, not determined. All strains are rod-shaped.

Characteristic	1	2	3	4	5	6	7
Cell size (µm)	0.7–0.8 × 1.5–3.0	0.4–0.5 × 0.8–1.3	0.3–0.5 × 1.0–1.5	0.5–0.8 × 1.3–2.0	0.3–0.7 × 0.7–2.5	0.7–0.9 × 2.0–3.0	0.5 × 1.0–2.0
Anaerobic growth	+	+	+	+	+	–	–
pH range	6–9	4–9	4–9	6–8	≥ 5	5–8	7–9
pH optimum	7	4	4	7	7–8	6–7	7
Temp. range (°C)	10–35	5–40	5–40	15–40	10–50	5–35	15–37
Temp. optimum (°C)	20–25	30	30	25–30	30	28	30
NaCl tolerance (w/v, %)	0	0–1	0–3	0–2	0–3.0	0–2	0–1
Optimal NaCl Concentration (w/v, %)	0	0	0	ND	0–1.0	ND	0–0.5
DNA G+C content (mol%)	61.5	57.5	59.9	58	63.8	62	63.0
Major quinone	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8	ND
Assimilation of mannose	–	+	+	+	+	(+)	+
Hydrolysis of							
Chitin	+	–	–	– (+*)	ND	–	+
Gelatin	–	–	–	–	–	+	ND
Aesculin	+	+	+	–	–	–	–
DNA	–	–	–	+	ND	ND	ND

*According to original description.

from the TLC data images given in a format defined by Tindall *et al.* (2010) (Fig. S2).

Further differences were observed in biochemical and physiological characteristics. Strain IGB-41^T was able to grow with pH values ranging from 6 to 9. However, growth occurred only in the absence of NaCl. A growth temperature range from 10 to 35 °C with an optimum at 20–25 °C was observed. In contrast, species of the genus *Silvimonas* show growth at up to 40 °C, are characterized by higher salt tolerance and are reported to be positive for mannose assimilation, while the latter was not found for isolate IGB-41^T. *Silvimonas terrae* KM-45^T has been reported to be positive for chitinolytic activity (Yang *et al.*, 2005). However, this was not confirmed in a more recent study (Muramatsu *et al.*, 2010) suggesting that none of the species of the genus *Silvimonas* known so far is positive for chitinolytic activity. Thus, strain IGB-41^T can be clearly distinguished from these species based on its chitinolytic and mannose-assimilating activities. The G+C content of strain IGB-41^T was found to be 61.5 mol% and this is outside the threshold of 57–60 mol% given in the emended genus definition of *Silvimonas* produced by Muramatsu *et al.* (2010). Further fundamental differences between the strains discussed are summarized in Table 1.

According to the physiological data observed, the highly reproducible clustering of strain IGB-41^T, which was never observed with species within the genus *Silvimonas*, and the assumptions on the assignment of new genera and species made in previous works (Stackebrandt & Goebel, 1994; Wayne *et al.*, 1987), we propose the assignment of isolate IGB-41^T to a new genus and a novel species. We suggest the name *Amantichitinum ursilacus* gen. nov., sp. nov. with isolate IGB-41^T as type strain.

Description of *Amantichitinum* gen. nov.

Amantichitinum [A.man.ti.chi'ti.num. L. part. adj. *amans* –antis loving; N.L. *chitinum* chitin; N.L. neut. n. *Amantichitinum* loving-chitin (bacterium)].

Cells of this genus are Gram-negative, motile, rod-shaped (1.5–3.0 µm × 0.7–0.8 µm) and facultatively anaerobic. They occur singly or in pairs (Fig. S1). Colonies on LB_{mod} are milky white to beige, circular, convex and about 1 mm in diameter. Representatives are catalase- and oxidase-positive. The only detectable quinone is Q-8. Major fatty acids (>10%) are C_{16:0}, summed feature 3 (comprising iso-C_{15:0} 2-OH and/or C_{16:1}ω7c) and C_{18:1}ω7c. Based on 16S rRNA sequence analysis, *Amantichitinum* belongs to the *Betaproteobacteria*. The type species is *Amantichitinum ursilacus*.

Description of *Amantichitinum ursilacus* sp. nov.

Amantichitinum ursilacus [ur.si.la'cus. L. n. *ursus* –i a bear; L. n. *lacus* a lake; N.L. gen. n. *ursilacus* of Bear Lake (Bärensee), a lake nearby Stuttgart, Germany, on the edge of which the strain was isolated].

Exhibits the following properties in addition to those given in the genus description. Growth is observed between 10 and 35 °C and at pH values of 6–9 (optimum pH 7). Growth occurs in the absence of NaCl. Gives a positive result in tests for nitrate reduction and acid formation from glucose. Hydrolyses aesculin, Tween 80, starch and chitin, but not gelatin or DNA. According to the API 50 CH panel, positive for fermentation of L-arabinose, D-ribose, DL-xylose, galactose, glucose, fructose, D-mannose, D-mannitol, N-acetylglucosamine, amygdalin, arbutin, aesculin, ferric citrate, salicin, D-cellobiose, D-maltose, starch, glycogen and D-arabitol. A weak reaction is observed for L-rhamnose, D-lactose (bovine), D-sucrose, gentiobiose and D-lyxose metabolism. The reactions for glycerol, erythritol, D-arabinose, D-adonitol, methyl β-D-xylopyranoside, L-sorbose, dulcitol, inositol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, D-melibiose, D-trehalose, inulin, D-melezitose, D-raffinose, xylitol, D-turanose, D-tagatose, D-fucose, L-fucose, L-arabitol, potassium gluconate, potassium-2-ketogluconate and potassium-5-ketogluconate are negative. Assimilation of adipate, citrate, malate and phenylacetate is negative. The major fatty acids (>5%) are C_{16:0} (26.1%), summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c) (26.1%), C_{18:1}ω7c (22.4%), C_{17:0}cyclo (6.0%) and C_{14:0} (5.5%).

The type strain, IGB-41^T (=DSM 23761^T=CIP 110167^T), was isolated from soil from an abandoned ant hill located near lake Bärensee in Stuttgart, Germany. The DNA G+C content of the type strain is 61.5 mol%.

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References

- Ashelford, K. E., Chuzhanova, N. A., Fry, J. C., Jones, A. J. & Weightman, A. J. (2006). New screening software shows that most recent large 16S rRNA gene clone libraries contain chimeras. *Appl Environ Microbiol* 72, 5734–5741.
- Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (editors) (1987). *Current Protocols in Molecular Biology*. New York: Green Publishing & Wiley Interscience.
- Bast, E. (2001). *Mikrobiologische Methoden: Eine Einführung in grundlegende Arbeitstechniken*. Heidelberg: Spektrum Akademischer Verlag GmbH.
- Campbell, L. L. & Williams, O. B. (1951). A study of chitin-decomposing micro-organisms of marine origin. *J Gen Microbiol* 5 (Suppl.), 894–905.

- Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Huß, 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.
- 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.
- Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A. & other authors (2007). CLUSTAL W and CLUSTAL_X version 2.0. *Bioinformatics* **23**, 2947–2948.
- Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S. & other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.
- 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.
- Miller, L. T. (1982). Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *J Clin Microbiol* **16**, 584–586.
- Muramatsu, Y., Suzuki, K. & Nakagawa, Y. (2010). *Silvimonas iriomotensis* sp. nov. and *Silvimonas amylolytica* sp. nov., new members of the class *Betaproteobacteria* isolated from the subtropical zone in Japan. *Int J Syst Evol Microbiol* **60**, 174–178.
- Patil, R. S., Ghormade, V. & Deshpande, M. V. (2000). Chitinolytic enzymes: an exploration. *Enzyme Microb Technol* **26**, 473–483.
- Reddy, C. A., Beveridge, T. J., Breznak, J. A., Snyder, L., Schmidt, T. M. & Marzluf, G. A. (2007). *Methods for General and Molecular Microbiology*. Washington, DC: American Society for Microbiology.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sheu, S. Y., Chiu, T. F., Chou, J. H., Sheu, D. S., Arun, A. B., Young, C. C., Chen, C. A., Wang, J. T. & Chen, W. M. (2009). *Andreprevotia lacus* sp. nov., isolated from a fish-culture pond. *Int J Syst Evol Microbiol* **59**, 2482–2485.
- 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.
- Stamatakis, A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**, 2688–2690.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Tindall, B. J. (1990a). A comparative study of the lipid composition of *Halobacterium sacharovorum* from various sources. *Syst Appl Microbiol* **13**, 128–130.
- Tindall, B. J. (1990b). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.
- Tindall, B. J., Sikorski, J., Smibert, R. M. & Kreig, N. R. (2007). Phenotypic characterization and the principles of comparative systematics. In *Methods for General and Molecular Microbiology*, 3rd edn. pp. 330–393. Edited by C. A. Reddy, T. J. Beveridge, J. A. Breznak, G. A. Marzluf, T. M. Schmidt & L. Snyder. Washington, DC, USA: American Society for Microbiology.
- Tindall, B. J., Rosselló-Móra, R., Busse, H. J., Ludwig, W. & Kämpfer, P. (2010). Notes on the characterization of prokaryote strains for taxonomic purposes. *Int J Syst Evol Microbiol* **60**, 249–266.
- 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.
- Weon, H.-Y., Kim, B.-Y., Yoo, S.-H., Joa, J.-H., Kwon, S.-W. & Kim, W.-G. (2007). *Andreprevotia chitinilytica* gen. nov., sp. nov., isolated from forest soil from Halla Mountain, Jeju Island, Korea. *Int J Syst Evol Microbiol* **57**, 1572–1575.
- Yang, H.-C., Im, W.-T., An, D.-S., Park, W. S., Kim, I. S. & Lee, S.-T. (2005). *Silvimonas terrae* gen. nov., sp. nov., a novel chitin-degrading facultative anaerobe belonging to the ‘*Betaproteobacteria*’. *Int J Syst Evol Microbiol* **55**, 2329–2332.
- Yoon, J. H., Choi, J. H., Kang, S. J., Choi, N. S., Lee, J. S. & Song, J. J. (2010). *Jeongeupia naejangsanensis* gen. nov., sp. nov., a cellulose-degrading bacterium isolated from forest soil from Naejang Mountain in Korea. *Int J Syst Evol Microbiol* **60**, 615–619.