Flavobacterium indicum sp. nov., isolated from warm spring water in Assam, India

P. Saha and T. Chakrabarti

Correspondence

T. Chakrabarti

tapan@imtech.res.in

Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Sector 39A, Chandigarh 160 036, India

A polyphasic taxonomic approach was employed to characterize a strain designated GPTSA100-9^T, which was isolated from water sampled from a warm spring. The micro-organism, comprising Gram-negative, strictly aerobic rods, could not grow on nutritionally rich media such as tryptic soy broth. Analysis of the 16S rRNA gene sequence (1396 nt) of strain GPTSA100-9^T revealed that it is a member of the genus *Flavobacterium*, sharing 99.8 % sequence similarity with the CFB group bacterium strain A0653 (AF236016), 93.4 % with '[*Flexibacter*] *aurantiacus* subsp. *excathedrus*' and 93.2–92.0 % with *Flavobacterium saliperosum*, *Flavobacterium soli*, *Flavobacterium aquatile* and *Flavobacterium columnare*. The G+C content of the genomic DNA was 31.0 mol%. The major fatty acids of the strain grown on modified R2A agar were iso-C_{15:0} (18.5 %), iso-C_{15:1} G (18.0 %), summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1} ω7c, 16.6 %) and iso-C_{17:0} 3-OH (9.0 %). On the basis of phenotypic and genotypic characteristics, strain GPTSA100-9^T represents a novel species of the genus *Flavobacterium*, for which the name *Flavobacterium indicum* sp. nov. is proposed. The type strain is GPTSA100-9^T (=MTCC 6936^T=DSM 17447^T).

The description of the genus *Flavobacterium*, created by Frankland in 1889 (Bergey *et al.*, 1923), has been emended twice during the last 10 years (Bernardet *et al.*, 1996, 2002). Members of the family *Flavobacteriaceae* have been reported from diverse aquatic habitats (Bernardet *et al.*, 1996), where they may be significantly involved in the uptake and degradation of the high-molecular-mass fraction of dissolved organic matter (Kirchman, 2002), in the degradation of several biopolymers (Glöckner *et al.*, 1999; Bernardet *et al.*, 2002) and in the bacterioplankton biomass (Brettar *et al.*, 2004). Here we report the taxonomic characterization of strain GPTSA100-9^T, isolated from warm spring water in Assam, India. The spring was located in a forest reserve not disturbed by human activities but frequented by wild animals, especially elephants, which was evident from the surrounding dung. The spring water was clear and its temperature was 37–38 °C.

Strain GPTSA100-9^T was isolated by plating serial dilutions on a nutritionally poor medium, TSBA100 [tryptic soy broth (Difco), 100 × diluted with distilled water and solidified with 1.5 % agarose (SRL)]. Various phenotypic tests were performed according to standard methods (Cowan & Steel, 1965; Murray *et al.*, 1994; Smibert & Krieg, 1994; Powers, 1995). Growth at different temperatures (10, 15, 20, 25, 30, 37, 42 and 55 °C), pH values (5, 6, 7, 8, 9, 10, 11 and 12) and

NaCl concentrations (1, 2, 5, 7 and 10 %, w/v) was determined using TSBA100 as a basal medium. Temperature and pH tolerances were also assessed in liquid MR2A medium. Since the strain had been isolated on a nutritionally poor medium, we also assessed its ability to grow on the following media: MR2A agar (Saha & Chakrabarti, 2006), R3A agar [essentially according to Reasoner & Geldreich (1985) except that proteose peptone no. 2 and 0.0048 % MgSO₄·7H₂O (w/v) were used instead of proteose peptone no. 3 and 0.01 % MgSO₄·7H₂O, respectively], nutrient agar (Hi-Media), MacConkey agar (Hi-Media), LY agar (Reichenbach, 1989), cytophaga agar (Anacker & Ordal, 1959, as mentioned in Reichenbach, 1989), undiluted tryptic soy broth agar (TBSA), Luria–Bertani agar (Difco) and ZoBell marine agar (Hi-Media). The presence of gliding motility was assessed on MR2A agar as described by Bowman (2000) and in liquid MR2A and TSB100 media. For the detection of flexirubin-type pigments, bacterial cell mass was exposed to a 20 % (w/v) KOH solution, as described by Reichenbach (1989); this was then examined for an eventual colour shift.

The hydrolysis of aesculin, cellulose (filter paper), chitin, tyrosine, hypoxanthine, starch, casein (2.8 %, w/v), DNA and gelatin was assessed according to standard protocols (Cowan & Steel, 1965; Smibert & Krieg, 1994), except that MMR2A agar (MR2A medium without glucose, starch and sodium pyruvate) was used as the basal medium. The hydrolysis of carboxymethylcellulose (Sigma) was assessed as reported previously (Saha & Chakrabarti, 2006). The

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GPTSA100-9^T is AY904351.

hydrolysis of alginate and pectin was investigated according to the methods described by Nakamura (1987) and Stanley *et al.* (1989), respectively. Acid production from various carbohydrates (0.5 %, w/v) and the hydrolysis of urea (2 %, w/v) were tested in basal MMR2A broth, using phenol red as an indicator. The hydrolysis of ONPG was assessed using an ONPG disc (Hi-Media). To determine the utilization of sole carbon sources, basal medium [containing (w/v) 0.2 % (NH₄)₂SO₄; 0.024 % K₂HPO₄; 0.024 % MgSO₄·7H₂O; 0.01 % KCl; 0.01 % yeast extract and 1.5 % agarose] was supplemented with the respective carbon sources (1 %). The production of H₂S from cysteine was assessed by placing a lead acetate paper strip above a culture in MMR2A broth supplemented with 0.05 % cysteine. The reaction with egg yolk was investigated using MMR2A agar supplemented with 1 % egg yolk. All biochemical tests were incubated at 37 °C.

Antibiotic susceptibility was investigated on MR2A agar, using discs (Hi-Media) containing antibiotics at the following concentrations: 10 µg ampicillin; 8 U bacitracin; 30 µg chloramphenicol; 15 µg erythromycin; 10 µg gentamicin; 30 µg kanamycin; 2 µg lincomycin; 30 µg neomycin; 30 µg novobiocin; 10 µg norfloxacin; 10 U penicillin G; 300 U polymyxin B; 2 µg rifampicin; 10 µg streptomycin; 300 µg sulfasomidine and 3 µg tetracycline. On MR2A agar, 5–7-day-old colonies showed a thin film of translucent growth with finger-like projections emanating from their irregular margins, suggesting that the strain could exhibit gliding motility. However, the presence of gliding motility could not be confirmed using phase-contrast microscopy on bacterial cells grown in liquid media. The major phenotypic properties of the strain are given in the species description and in Table 1.

For cellular fatty acid analysis, the strain was grown on MR2A agar at 30 °C for 24 h. Extraction and analysis of cellular fatty acids were performed according to the procedures of the Sherlock Microbial Identification system (MIDI), as described previously (Pandey *et al.*, 2002). The major fatty acids were iso-C_{15:0} (18.5 %), iso-C_{15:1} G (18.0 %), summed feature 3 (comprising iso-C_{15:0} 2-OH and/or C_{16:1ω7}C_{16:6} %), iso-C_{17:0} 3-OH (9.0 %), iso-C_{16:0} (5.1 %), iso-C_{15:0} 3-OH (5.0 %) and iso-C_{16:0} 3-OH (4.5 %).

Isolation of genomic DNA and determination of the G + C content (mol%) were carried out according to published methods (Saha *et al.*, 2005). Amplification of the 16S rRNA gene was performed using primers 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-TACGGYTACCTTGTTACGACTT-3'). The amplification reaction and the purification of the amplicon were performed as described previously (Pandey *et al.*, 2002). Sequencing reactions were performed using primers 27f, 357f, 685r, 926f, 1100r (Johnson, 1994) and 1492r. The almost-complete (1396 nt) 16S rRNA gene sequence of the strain was used to search for homologous sequences in the GenBank database. Sequence analyses were also

Table 1. Differential characteristics of strain GPTSA100-9^T and related members of the genus *Flavobacterium*

Taxa: 1, strain GPTSA100-9^T; 2, *Flavobacterium saliperosum*; 3, *Flavobacterium aquatile*; 4, *Flavobacterium columnare*; 5, *Flavobacterium soli*. Data are from Holmes *et al.* (1984), Bernardet & Grimont (1989), Bernardet *et al.* (1996, 2002), Wang *et al.* (2005) and Yoon *et al.* (2005). All of the strains are positive for growth without NaCl and for catalase, oxidase (except *F. saliperosum*) and gelatinase activities. All of the strains are negative for hydrolysis of agar, carboxymethylcellulose and chitin (except *F. soli*, for which no data are available). +, Positive; –, negative; W, weakly positive; ND, not determined; NDT, not detected; NG, no growth on medium tested.

Characteristic	1	2	3	4	5
Colony colour*	YO	Y	CY	GY	CY
Flexirubin-type pigments	–	+	–	+	–
Growth on:					
Nutrient agar	W	+	–	–	ND
Tryptic soy agar	–	+	+	–	+
Growth at 37 and 42 °C	+	–	–	–	–
Tolerance of 2 % NaCl	+	–	–	–	+
H ₂ S production	–	–	W	+	ND
Nitrate reduction	–	–	+	+	+
Hydrolysis of:					
Aesculin	NG	–	+	–	+
DNA	–	ND	–	+	–
Starch	+	–	+	–	–
Tween 20	–	ND	+	+	+
Tyrosine	–	+	+	–	–
Acid production from:					
Fructose	+	–	–	–	N/A†
Lactose	–	–	+	–	N/A†
Sucrose	–	–	+	–	N/A†
Sensitivity to:					
Gentamicin	+	+	ND	–	–
Kanamycin	+	+	ND	–	–
Neomycin	+	ND	ND	–	–
DNA G + C content (mol%)	31	41	33	32	36.9
C _{15:0} content (%)‡	NDT	3.9	12.7	4.4	8.2

*CY, cream-yellow; Y, yellow; YO, yellowish orange; GY, greenish yellow.

†Not applicable (does not produce acid from carbohydrates).

‡The fatty acid composition was determined using bacteria grown under different conditions.

performed with various online analysis tools available in the Ribosomal Database Project II database (release 9; <http://rdp.cme.msu.edu/>).

The sequence analysis revealed that strain GPTSA100-9^T belongs to the genus *Flavobacterium*. Its closest relative was found to be CFB group bacterium strain A0653 (GenBank accession number AF236016; 99.8 % sequence similarity), followed by the uncultured bacterial clones HP1A39

(AF502205; 94.4 %) from activated sludge (McMahon *et al.*, 2002) and 169ds20 (AY212620; 94.0 %) from equine manure (Simpson *et al.*, 2004). Among named bacteria, strain GPTSA100-9^T showed closest similarity to '[*Flexibacter*] *aurantiacus* subsp. *excathedrus*' IFO 16024 (93.4 %), *Flavobacterium saliperosum* S13^T (93.2 %), *Flavobacterium soli* DS-6^T (92.9 %), *Flavobacterium aquatile* ATCC 11947^T (92.1 %) and *Flavobacterium columnare* NCIMB 2248^T (90.0 %). Sequence similarities with other members of the family *Flavobacteriaceae* were below 90.0 %. Previous studies have shown that '[*Flexibacter*] *aurantiacus* subsp. *excathedrus*' is generically misclassified and should be considered as a species of the genus *Flavobacterium* pending further investigations (Bernardet *et al.*, 1996; Nakagawa *et al.*, 2002).

The 16S rRNA gene sequences of CFB group bacterium A0653, the uncultured bacterial clones HP1A39 and 169ds20, the type strains of 28 members of the genus *Flavobacterium*, '[*Flexibacter*] *aurantiacus* subsp. *excathedrus*' and seven closely related genera in the family *Flavobacteriaceae* were compared using the CLUSTAL_X program (Thompson *et al.*, 1997) and edited manually. Aligned sequences were analysed by using the TREECON software package (Van de Peer & De Wachter,

1997). A neighbour-joining analysis (Saitou & Nei, 1987) was carried out using the correction of Jukes & Cantor (1969). The stability of the tree was estimated by bootstrap analysis of 1000 replications. The resulting phylogenetic tree (Fig. 1) confirmed that strain GPTSA100-9^T belongs to the genus *Flavobacterium*. Within the monophyletic *Flavobacterium* lineage, strain GPTSA100-9^T, the uncultured bacterial clone HP1A39 and CFB group bacterium A0653 together form a subcluster within the *Flavobacterium aquatile*–*Flavobacterium saliperosum* cluster. Parsimony analysis (DNAPARS with 100 replicates) using PHYLIP, version 3.5c (Felsenstein, 1993), produced a tree with similar overall topology. The lack of information on CFB group strain A0653 and the low levels of sequence similarity between the novel strain and cultured relatives warrant the categorization of strain GPTSA100-9^T as a novel species in the genus *Flavobacterium*.

Strain GPTSA100-9^T could be distinguished from its close phylogenetic relatives on the basis of phenotypic characteristics, DNA G + C content (mol%) and chemotaxonomic parameters (Table 1). Thus, on the basis of this polyphasic study, we conclude that GPTSA100-9^T represents a novel

Fig. 1. Neighbour-joining phylogenetic tree, based on the comparison of 16S rRNA gene sequences, showing the relationships between strain GPTSA100-9^T and the type strains of relatives within the family *Flavobacteriaceae*. Bootstrap values shown at the nodes are expressed as percentages of 1000 replications (if greater than 50 %). The sequence of *Myroides odoratus* ATCC 4651^T was used as an outgroup. Bar, 0.05 substitutions per site.

species of the genus *Flavobacterium*, for which the name *Flavobacterium indicum* sp. nov. is proposed.

Description of *Flavobacterium indicum* sp. nov.

Flavobacterium indicum (in'di.cum. L. neut. adj. *indicum* pertaining to India, where the bacterial strain was isolated).

Cells are Gram-negative, strictly aerobic, non-motile and occur mostly as single slender rods, but sometimes in pairs; tend to form filaments in old cultures. Cells are 1–3 µm long and 0.1–0.2 µm wide. Colonies on MR2A agar are yellowish orange, round, convex to slightly umbonate and have irregular margins; finger-like projections appear at the margins of 5–7-day-old colonies. Colonies on TSBA100 medium are much smaller, pale yellowish in colour and more adherent to the agar than those on MR2A agar. Good growth occurs on R2A agar, R3A agar, cytophaga agar, full- and quarter-strength ZoBell marine agar, yeast extract agar and nutrient agar devoid of NaCl. Weak growth occurs on nutrient agar, Luria–Bertani agar and LY agar. No growth occurs on tryptic soy broth, Methyl Red–Voges–Proskauer broth, Simmons' citrate agar, spirit blue agar, MacConkey agar or Kligler's iron agar. Flexirubin-type pigments are absent. Tests are positive for oxidase and very weakly positive for catalase. Grows at temperatures between 15 and 42 °C (optimum, 37 °C), at pH 5.0–11.0 (optimum, pH 7.4–8.0) and tolerates up to 2 % NaCl. Casein, gelatine and Tweens 40 and 60 are hydrolysed; starch is weakly hydrolysed. ONPG, tyrosine, urea, xylan, agar, aesculin, alginate, cellulose (filter paper and carboxymethylcellulose), chitin, DNA, hypoxanthine, pectin, xanthine and Tweens 20 and 80 are not hydrolysed. Negative in tests for H₂S production from cysteine. Gives a proteolytic reaction, but no precipitate, on egg-yolk agar. D-Fructose and (to a lesser extent) inulin are utilized. L-Arabinose, D-amylgdalin, D-galactose, D-glucose, glycerol, glycogen, D-mannose, L-rhamnose, D-sorbitol and L-xylose are very weakly utilized. Adonitol, D-arabinose, L-arabitol, arbutin, D-cellobiose, dulcitol, *myo*-inositol, D-lactose, D-mannitol, D-maltose, D-melibiose, D-melezitose, D-raffinose, D-ribose, L-sorbose, sucrose, trehalose, xylitol and D-xylose are not utilized. Produces acid from D-fructose and (very weakly) from D-glucose, glycogen, inulin, L-xylose and D-mannose. Acid is not produced from the other carbohydrates tested. Major fatty acids are iso-C_{15:0}, iso-C_{15:1} G, summed feature 3, iso-C_{17:0} 3-OH, iso-C_{16:0}, iso-C_{15:0} 3-OH and iso-C_{16:0} 3-OH; other fatty acids present are iso-C_{14:0}, C_{15:1}ω6c, iso-C_{13:0}, C_{15:0} 2-OH, iso-C_{17:1}ω9c, C_{17:1}ω6c, iso-C_{16:1} H, anteiso-C_{15:0} and an unknown fatty acid with an equivalent chain length of 13.565. The DNA G+C content is 31.0 mol%. Sensitive to ampicillin, bacitracin, chloramphenicol, erythromycin, gentamicin, kanamycin, lincomycin, neomycin, novobiocin, norfloxacin, penicillin G, polymyxin B, rifampicin, streptomycin, sulfasomidine and tetracycline.

The type strain, GPTSA100-9^T (=MTCC 6936^T=DSM 17447^T), was isolated from water sampled from a warm spring in Assam, India.

Acknowledgements

We are grateful to Dr K. Ganesan for his help with DNA sequencing. The help of Dr T. C. Bora (Biotechnology Division, Regional Research Laboratory, Jorhat, India) with sample collection is gratefully acknowledged. This work was supported by grants from DBT, Government of India and CSIR. We thank Dr A. K. Mondal, Dr G. S. Prasad and Mr S. Mayilraj for useful discussions. We also thank the Editor and the referees for making useful suggestions. P.S. is the recipient of a CSIR fellowship. This is IMTECH communication number 62/2005.

References

- Bergey, D. H., Harrison, F. C., Breeds, R. S., Hammer, B. W. & Huntoon, F. M. (1923). Genus II. *Flavobacterium* gen. nov. In *Bergey's Manual of Determinative Bacteriology*, pp. 97–117. Baltimore: Williams & Wilkins.
- Bernardet, J.-F. & Grimont, P. A. D. (1989). Deoxyribonucleic acid relatedness and phenotypic characterization of *Flexibacter columnaris* sp. nov., nom. rev., *Flexibacter psychrophilus* sp. nov., nom. rev., and *Flexibacter maritimus* Wakabayashi, Hikida, and Masumura 1986. *Int J Syst Bacteriol* **39**, 346–354.
- Bernardet, J.-F., Seger, P., Vancanneyt, M., Berthe, F., Kersters, K. & Vandamme, P. (1996). Cutting a Gordian knot: emended classification and description of the genus *Flavobacterium*, emended description of the family *Flavobacteriaceae*, and proposal of *Flavobacterium hydatis* nom. nov. (Basonym, *Cytophaga aquatilis* Strohl and Tait 1778). *Int J Syst Bacteriol* **46**, 128–148.
- Bernardet, J.-F., Nakagawa, Y. & Holmes, B. (2002). Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *Int J Syst Evol Microbiol* **52**, 1049–1070.
- Bowman, J. P. (2000). Description of *Cellulophaga algicola* sp. nov., isolated from the surfaces of Antarctic algae, and reclassification of *Cytophaga uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Cellulophaga uliginosa* comb. nov. *Int J Syst Evol Microbiol* **50**, 1861–1868.
- Brettar, I., Christen, R. & Höfle, M. G. (2004). *Belliella baltica* gen. nov., sp. nov., a novel marine bacterium of the *Cytophaga–Flavobacterium–Bacteroides* group isolated from surface water of the central Baltic Sea. *Int J Syst Evol Microbiol* **54**, 65–70.
- Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.
- Felsenstein, J. (1993). PHYLIP (Phylogeny inference package), version 3.5c. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Glöckner, F. O., Fuch, B. M. & Amann, R. (1999). Bacterioplankton composition of lakes and oceans: a first comparison based on fluorescence in situ hybridization. *Appl Environ Microbiol* **65**, 3721–3726.
- Holmes, B. R., Owen, R. J. & McMeekin, T. A. (1984). Genus *Flavobacterium* Bergey, Harrison, Breed, Hammer and Huntoon 1923, 97^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 353–361. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.
- Johnson, J. L. (1994). Similarity analysis of rRNAs. In *Methods for General and Molecular Bacteriology*, pp. 683–700. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

- Kirchman, D. L. (2002). The ecology of *Cytophaga*–*Flavobacteria* in aquatic environments. *FEMS Microbiol Ecol* **39**, 91–100.
- McMahon, K. D., Dojka, M. A., Pace, N. R., Jenkins, D. & Keasling, J. D. (2002). Polyphosphate kinase from activated sludge performing enhanced biological phosphorus removal. *Appl Environ Microbiol* **68**, 4971–4978.
- Murray, R. G. E., Doetsch, R. N. & Robinow, C. F. (1994). Determinative and cytological light microscopy. In *Methods for General and Molecular Bacteriology*, pp. 21–41. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Nakagawa, Y., Sakane, T., Suzuki, M. & Hatano, K. (2002). Phylogenetic structure of the genera *Flexibacter*, *Flexithrix* and *Microscilla* deduced from 16S rRNA sequence analysis. *J Gen Appl Microbiol* **48**, 156–165.
- Nakamura, L. K. (1987). *Bacillus alginolyticus* sp. nov. and *Bacillus chondroitinus* sp. nov., two alginate-degrading species. *Int J Syst Bacteriol* **37**, 284–286.
- Pandey, K. K., Mayilraj, S. & Chakrabarti, T. (2002). *Pseudomonas indica* sp. nov., a novel butane-utilizing species. *Int J Syst Evol Microbiol* **52**, 1559–1567.
- Powers, E. M. (1995). Efficacy of the Ryu nonstaining KOH technique for rapidly determining Gram reaction of food-borne and waterborne bacteria and yeasts. *Appl Environ Microbiol* **61**, 3756–3758.
- 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.
- Reichenbach, H. (1989). Genus I. *Cytophaga* Winogradsky 1929, 577^{AL} emend. Reichenbach, H. In *Bergey's Manual of Systematic Bacteriology*, vol. 3, pp. 2015–2050. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.
- Saha, P. & Chakrabarti, T. (2006). *Emticicia oligotrophica* gen. nov., sp. nov., a new member of the family 'Flexibacteraceae', phylum Bacteroidetes. *Int J Syst Evol Microbiol* **56**, 991–995.
- Saha, P., Krishnamurthi, S., Mayilraj, S., Prasad, G. S., Bora, T. C. & Chakrabarti, T. (2005). *Aquimonas voraii* gen. nov., sp. nov., a novel gammaproteobacterium isolated from a warm spring of Assam, India. *Int J Syst Evol Microbiol* **55**, 1491–1495.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Simpson, J. M., Santo Domingo, J. W. & Reasoner, D. J. (2004). Assessment of equine fecal contamination: the search for alternative bacterial source-tracking targets. *FEMS Microbiol Ecol* **47**, 65–75.
- 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.
- Stanley, T. W., Goodfellow, M. & Alderson, G. (1989). Genus *Streptomyces* Waksman and Henrici 1943, 339^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 4, pp. 2452–2492. Edited by S. T. Williams. Baltimore: Williams & Wilkins.
- 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 tool. *Nucleic Acids Res* **25**, 4876–4882.
- Van de Peer, Y. & De Wachter, R. (1997). Construction of evolutionary distance trees with TREECON for Windows: accounting for variation in nucleotide substitution rate among sites. *Comput Appl Biosci* **13**, 227–230.
- Wang, Z.-W., Liu, Y.-H., Dai, X., Wang, B.-J., Jiang, C.-Y. & Liu, S.-J. (2005). *Flavobacterium saliperosum* sp. nov., isolated from freshwater lake sediment. *Int J Syst Evol Microbiol* **56**, 439–442.
- Yoon, J.-H., Kang, S.-J. & Oh, T.-K. (2005). *Flavobacterium soli* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **56**, 997–1000.