Note

# *Chryseobacterium defluvii* sp. nov., isolated from wastewater

Peter Kämpfer,[1] Ute Dreyer,[2] Alexander Neef,[1] Wolfgang Dott[3] and Hans-Jürgen Busse[4,5]

Correspondence
Peter Kämpfer
peter.kaempfer@agrar.uni-giessen.de

[1]Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität Giessen, Heinrich-Buff-Ring 26–32, D-35390 Giessen, Germany

[2]Fachgebiet Hygiene und Umweltmikrobiologie, Technische Universität Berlin, D-13353 Berlin, Germany

[3]Institut für Hygiene und Umweltmedizin, Rheinisch-Westfälische Technische Hochschule, D-52057 Aachen, Germany

[4]Institut für Bakteriologie, Mykologie und Hygiene, Veterinärmedizinische Universität, A-1210 Vienna, Austria

[5]Institut für Mikrobiologie und Genetik, Universität Wien, A-1030 Vienna, Austria

A Gram-negative, rod-shaped, non-spore-forming, yellow-pigmented bacterium (strain B2$^T$) isolated from wastewater of a sequence batch reactor showing enhanced phosphorus removal was investigated to determine its taxonomic status. Complete 16S rRNA gene sequence analysis indicated that the organism should be placed in the genus *Chryseobacterium*. The strain contained a polyamine pattern with *sym*-homospermidine as the major compound, menaquinone MK-6 as the predominant menaquinone and ai-$C_{15:0}$, i-$C_{15:0}$ and $C_{16:1}$ as the major fatty acids. Phosphatidylethanolamine and several unidentified lipids were detected in the polar lipid profile. Phylogenetically, strain B2$^T$ was most closely related to *Chryseobacterium indoltheticum* and *Chryseobacterium gleum* (96·2 and 95·9 % 16S rRNA gene sequence similarity, respectively). The phylogenetic distance from any validly described species within the genus *Chryseobacterium*, as indicated from 16S rRNA gene sequence similarities, and its phenotypic properties demonstrate that strain B2$^T$ represents a novel species, for which the name *Chryseobacterium defluvii* sp. nov. is proposed; the type strain is B2$^T$ (=DSM 14219$^T$ =CIP 107207$^T$).

The recognition of the *Cytophaga–Flavobacterium–Bacteroides* group as a separate line of descent within the domain *Bacteria* has been clearly demonstrated by different rRNA-based studies (Bauwens & De Ley, 1981; Paster *et al.*, 1985; Segers *et al.*, 1993; Weisburg *et al.*, 1985; Woese *et al.*, 1990). Within the family *Flavobacteriaceae*, which was emended by Bernardet *et al.* (1996), the genera *Bergeyella*, *Chryseobacterium* and *Riemerella* form a separate branch on the basis of rRNA cistron similarity studies (Vandamme *et al.*, 1994) and phenotypic characteristics. At the time of writing, the genus *Chryseobacterium* contains the species *Chryseobacterium gleum*, *Chryseobacterium indologenes*, *Chryseobacterium balustinum*, *Chryseobacterium indoltheticum*, *Chryseobacterium meningosepticum*, *Chryseobacterium scophthalmum* and the recently described species '*Chryseobacterium proteolyticum*' (Yamaguchi & Yokoe, 2000). The genus *Riemerella* comprises the two species *Riemerella anatipestifer* (Segers *et al.*, 1993) and *Riemerella columbina* (Vancanneyt *et al.*, 1999) and the genus *Bergeyella* accommodates the single species *Bergeyella zoohelcum* (Vandamme *et al.*, 1994), which was originally described as *Weeksella zoohelcum* by Holmes *et al.* (1986).

During selective enrichment experiments of a phosphorus-removing defined mixed bacterial culture from activated sludge (Hollender *et al.*, 2002), four bacterial isolates were obtained, one of them showing yellow-pigmented colony morphology on nutrient agar. This strain (B2$^T$) was isolated from an activated sludge enrichment on R2A agar (Oxoid). The isolate was subcultivated on this medium at 25 °C for 48 h for further analyses. The Gram reaction was tested as described by Gerhardt *et al.* (1994). Cell morphologies were observed under a light microscope (1000× magnification) (Zeiss) with cells grown for 3 days at 30 °C on tryptone-soy agar (TSA; Oxoid). On nutrient agar (Oxoid), strain B2$^T$ formed visible colonies (diameter of about 2 mm) within 24 h at 30 °C. No growth was observed within 14 days at 4 °C or at temperatures above 45 °C. At 15 °C, very small colonies were visible after

The EMBL accession number for the 16S rRNA gene sequence of strain B2$^T$ is AJ309324.

7 days incubation. The colonies were yellowish, translucent and shiny with entire edges. After prolonged incubation, colonies were not detectable as single entities because of the production of extracellular slimy substances. Oxidase activity was tested using Bactident-Oxidase test strips (Merck) according to the manufacturer's instructions. Strain B2$^T$ was oxidase-positive. Cells were non-motile, non-spore-forming rods (about 2 μm in length) and stained Gram-negative. Strain B2$^T$ was able to grow on various nutrient-rich media, including TSA (Oxoid), but was not able to grow on MacConkey agar. Physiological characterization was done as described previously (Kämpfer *et al.*, 1991), with the modification that all media were diluted threefold. Additional tests were performed using the Micronaut-E gallery (Merlin, formerly known as TTE-AS; Kämpfer, 1990).

Strain B2$^T$ produced acid from D-glucose, D-maltose, trehalose and D-cellobiose (weak). No acid was produced from several other sugars and related compounds. In addition, only a few carbon sources were utilized. Most of the tested *p*-nitrophenyl derivatives were hydrolysed (see species description).

Fatty acid methyl esters were extracted and prepared by the standard protocol of the Microbial Identification System (MIDI; Microbial ID). Extracts were analysed using a Hewlett Packard model HP6890A GC equipped with an FID, an automatic sampler, an integrator and a computer, as described previously (Kämpfer & Kroppenstedt, 1996). In strain B2$^T$, the fatty acids 15:0 iso (58·5 %), 17:0 iso 3-OH (14·1 %) and summed feature 4 (16:1ω7*c*/*t* and/or 15:0 iso 2-OH, 8·4 %) were predominant. The detailed fatty acid composition is shown in Table 1. Because the results shown in this table are based on fatty acid analyses under identical conditions, these results can be compared directly. It is obvious that isolate B2$^T$ had larger relative amounts of 15:0 iso and 13:0 iso than all other *Chryseobacterium* species. Although representatives of the genera *Riemerella* and *Bergeyella* showed similar fatty acid profiles, differentiation on the basis of amounts of several other fatty acids is possible (e.g. iso 17:1ω9*c* and 17:0 iso 3-OH).

Analysis of the respiratory quinones of strain B2$^T$ by HPLC (Tindall, 1990) gave only one characteristic peak, which corresponded to menaquinone MK-6. Polyamine analysis (Busse & Auling, 1988; Busse *et al.*, 1997) revealed a pattern for strain B2$^T$ that is characteristic for *Chryseobacterium* (Hamana & Matsuzaki, 1990, 1991). Strain B2$^T$ contained *sym*-homospermidine [35·6 μmol (g dry wt)$^{-1}$] as the major polyamine and minor amounts of spermine [2·1 μmol (g dry wt)$^{-1}$] and spermidine [0·4 μmol (g dry wt)$^{-1}$].

**Table 1.** Fatty acid composition of strain B2$^T$ in comparison with other related organisms

Strains: 1, *C. defluvii* sp. nov. B2$^T$; 2, *C. gleum* LMG 8334$^T$; 3, *C. indologenes* LMG 8337$^T$; 4, *C. balustinum* LMG 8329$^T$; 5, *C. indoltheticum* LMG 4025$^T$; 6, *C. meningosepticum* LMG 12279$^T$; 7, *C. scophthalmum*; 8, *R. anatipestifer*; 9, *R. columbina*; 10, *B. zoohelcum* LMG 8351$^T$. Data for reference species were taken from Segers *et al.* (1993) (other *Chryseobacterium* species except *C. scophthalmum* and *Bergeyella* species), Mudarris *et al.* (1994) (*C. scophthalmum*) and Vancanneyt *et al.* (1999) (*Riemerella*). Fatty acids that account for less than 1 % of the total fatty acids in all strains studied are not shown. Therefore, the percentages do not add up to 100 %. For *R. anatipestifer*, *R. columbina* and *C. scophthalmum*, the means and standard deviations for strains containing the fatty acids are shown. tr, Trace (less than 1 %); ND, not detected; ECL, equivalent chain length (i.e. the identity of the fatty acids is unknown).

| Fatty acid | 1* | 2 | 3 | 4 | 5* | 6* | 7 | 8 | 9* | 10† |
|---|---|---|---|---|---|---|---|---|---|---|
| 13:0 iso | 2·8 | tr | tr | tr | tr | 1·9 | tr | 15·1±3·8 | 10·1±1·8 | 3·6 |
| ECL 13·566 | tr | 2·1 | 2·2 | 2·6 | tr | 1·4 | 3·3±0·5 | 1·4±0·4 | tr | 1·5 |
| 15:0 iso | 58·5 | 35·5 | 34·8 | 31·1 | 34·1 | 42·1 | 34·9±0·5 | 52·4±4·6 | 45·3±3·3 | 61·9 |
| 15:0 anteiso | 3·2 | ND | tr | 1·1 | 5·3 | 1·9 | tr | 5·4±1·1 | 22·1±3·3 | ND |
| Summed feature 4‡ | 8·4 | 13·3 | 11·6 | 8·9 | 10·2 | 17·3 | 11·8±0·6 | ND | 2·9±1·3 | 6·4 |
| 16:0 | 1·3 | 1·0 | 1·2 | 2·7 | 1·3 | tr | 1·1±0·1 | tr | tr | ND |
| 15:0 iso 3-OH | 2·6 | 3·0 | 2·8 | 2·6 | 2·0 | 3·7 | 2·9±0·1 | 8·2±248 | 3·9±0·9 | 4·1 |
| iso 17:1ω9*c* | 4·8 | 19·1 | 19·8 | 26·3 | 21·7 | 4·6 | 21·3±0·4 | ND | ND | 9·1 |
| ECL 16·580 | tr | 1·9 | 1·6 | 1·4 | 1·4 | 1·7 | 1·7±0·2 | tr | ND | ND |
| 17:0 iso | 2·0 | 1·3 | 1·1 | 1·7 | 1·0 | tr | 1·1±0·1 | ND | ND | tr |
| 16:0 iso 3-OH | tr | tr | tr | tr | tr | 1·1 | tr | tr | ND | ND |
| 16:0 3-OH | tr | 1·1 | 1·2 | 1·5 | 1·1 | 2·3 | 1·3±0·1 | ND | ND | ND |
| 17:0 iso 3-OH | 14·1 | 20·6 | 20·5 | 15·1 | 15·4 | 17·6 | 17·1±0·2 | 13·6±3·4 | 7·0±1·5 | 9·9 |

**C. defluvii* B2$^T$, *C. indoltheticum* LMG 4025$^T$, some strains of *C. meningosepticum* and strains of *R. columbina* also contain 17:0 2-OH in respective amounts of 0·3, 2·7 and 1·0±0·1 %.

†*B. zoohelcum* LMG 8351$^T$ also contains 1·5 % summed feature 5 (17:1 iso I and/or 17:1 anteiso B).

‡Summed feature 4 contains 15:0 iso 2-OH and/or 16:1ω7*c*/*t*.

Only trace amounts of 1,3-diaminopropane, cadaverine and putrescine were detected.

Polar lipids were determined by two-dimensional TLC as described previously (Ventosa *et al.*, 1993). Phosphatidylethanolamine was detected as the major lipid in B2$^T$. In addition, several unknown polar lipids were detected (Fig. 1).

The 16S rRNA gene was amplified by PCR using universal primers 27f and 1492r (Lane, 1991) and sequenced as described previously (Wieser *et al.*, 1999). Phylogenetic analysis was performed using the ARB software package (Strunk *et al.*, 1999). Distance matrix, maximum-parsimony and maximum-likelihood methods, as implemented in the ARB software package, were applied for tree construction (Strunk *et al.*, 1999) (Fig. 2).

The 16S rDNA sequence of strain B2$^T$ consisting of a continuous stretch of 1415 bp (positions 28–1443, according to *Escherichia coli* numbering) was used to search GenBank, EMBL and the Ribosomal Database Project. Sequence searches showed that strain B2$^T$ was phylogenetically most closely related to representatives of the family *Flavobacteriaceae*. The results of the sequence similarity calculations indicated that the nearest relatives of strain B2$^T$ are *C. indoltheticum* (96·2 % sequence similarity), *C. gleum* (95·9 %) and *C. indologenes* (95·9 %). A sequence similarity of 95·0 % was observed to '*C. proteolyticum*'. Only slightly lower sequence similarities (92·0–95·4 %) were found to species from the genera *Riemerella* and *Bergeyella*. From these results, it is evident that strain B2$^T$ belongs to the genus *Chryseobacterium*. The results of maximum-parsimony analysis showed a separate position for strain B2$^T$, most closely related to '*C. proteolyticum*', but also closely related to *C. gleum* and *C. indoltheticum*. The branching patterns of the trees were not stable; however, strain B2$^T$ was always clearly separate from all other species.

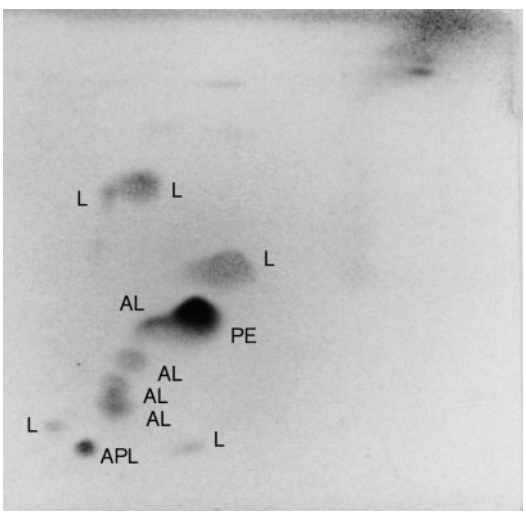


**Fig. 1.** Two-dimensional TLC of polar lipids of strain B2$^T$. PE, Phosphatidylethanolamine; AL, aminolipids; APL, unidentified aminophospholipids; L, unknown lipids.

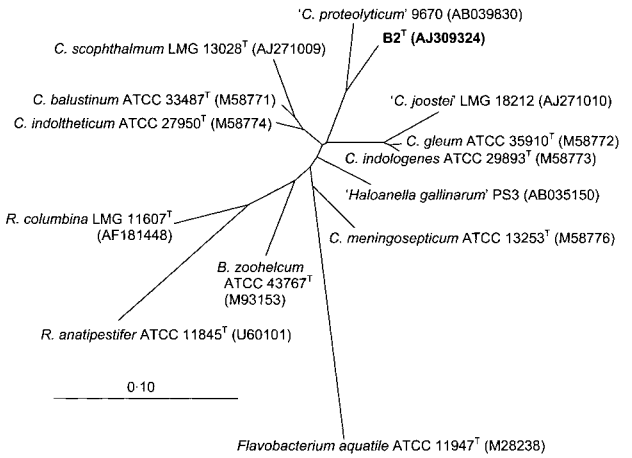


**Fig. 2.** Unrooted tree indicating the estimated phylogenetic relationship between strain B2$^T$ (AJ309324) and other members of the genera *Chryseobacterium*, *Riemerella* and *Bergeyella* based on a comparison of 16S rDNA sequences. The tree was constructed using the maximum-parsimony method. Sequence data for other strains were obtained from the EMBL database. The tree also includes the 16S rRNA sequence of the novel species '*Chryseobacterium joostei*', which has not yet been published (Hugo *et al.*, 2003).

The detection of menaquinone MK-6 as the only quinone, *sym*-homospermidine in the polyamine pattern, the large amounts of 15 : 0 iso and the 16S rRNA sequence clearly placed strain B2$^T$ in the genus *Chryseobacterium*. However, strain B2$^T$ differs from all other previously described species belonging to this genus.

The main reason for creation of a novel species for strain B2$^T$ was the rather high degree of sequence divergence of the 16S rRNA gene in comparison with the corresponding sequences from different species of the genus *Chryseobacterium*. It was shown clearly by Yamaguchi & Yokoe (2000) that *Chryseobacterium* species that share 16S rRNA gene sequence similarities of 94·9–96·0 % are distinct species on the basis of DNA–DNA pairing studies, with only 3·0–31·0 % DNA–DNA similarity. In addition, differences in physiological features were found in comparison with described *Chryseobacterium* species.

On the basis of these results, strain B2$^T$ is proposed as a representative of a novel species of the genus *Chryseobacterium*, *Chryseobacterium defluvii* sp. nov.

## Description of *Chryseobacterium defluvii* sp. nov.

*Chryseobacterium defluvii* (de.flu′vi.i. L. gen. n. *defluvii* of sewage).

**Table 2.** Characteristics that differentiate strain B2$^T$ from other *Chryseobacterium*, *Riemerella* and *Bergeyella* species

Species: 1, *C. defluvii* sp. nov. B2$^T$; 2, *C. proteolyticum* (*n*=2); 3, *C. gleum* (*n*=12); 4, *C. indologenes* (*n*=13); 5, *C. balustinum* (*n*=1); 6, *C. indoltheticum* (*n*=1); 7, *C. meningosepticum* (*n*=49); 8, *C. scophthalmum* (*n*=7); 9, *R. anatipestifer* (*n*=16); 10, *R. columbina* (*n*=13); 11, *B. zoohelcum* (*n*=30). Data for reference species were taken from Yamaguchi & Yokoe (2000), Holmes *et al.* (1984, 1986), Yabuuchi *et al.* (1983), Segers *et al.* (1993), Mudarris *et al.* (1994) and Vancanneyt *et al.* (1999). *n*, Number of strains tested. +, All strains tested positive; (+), weakly positive; −, all strains tested negative; V, variable, but the number of positive strains is not available; NA, not available. Two figures separated by a solidus (/) refer to the number of positive strains/number of strains tested.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Acid production from:* | | | | | | | | | | | |
| Glucose | + | + | + | + | + | + | + | − | V | + | − |
| Sucrose | − | + | − | − | − | − | − | − | − | − | − |
| Mannitol | − | + | − | 4/13 | − | − | 31/49 | − | − | − | − |
| Arabinose | − | + | 10/12 | − | − | − | 1/49 | − | NA | − | − |
| Cellobiose | (+) | − | − | − | − | − | 4/49 | − | NA | NA | − |
| Fructose | NA | NA | + | + | + | − | 28/49 | − | NA | + | − |
| Lactose | NA | − | − | − | − | − | 27/49 | − | NA | − | − |
| Maltose | + | + | + | + | − | + | 46/49 | − | V | + | − |
| Raffinose | − | − | − | − | − | − | − | − | NA | NA | − |
| Salicin | − | − | − | − | − | − | − | − | NA | − | − |
| Trehalose | + | + | + | + | − | − | 42/49 | − | NA | (+) | − |
| Xylose | − | NA | 4/12 | − | − | − | 3/49 | − | NA | − | − |
| Casein digestion | NA | + | + | NA | + | + | + | − | NA | NA | NA |
| Aesculin hydrolysis | + | + | + | + | − | + | 47/49 | − | − | + | − |
| Growth at 36–37 °C | + | + | + | + | − | + | + | − | + | + | + |
| Growth on MacConkey agar | − | − | + | V | + | + | + | − | − | − | − |
| Nitrate reduction | − | − | 7/12 | V | + | − | − | − | − | − | − |
| Urease activity | − | − | 7/12 | − | − | − | 18/49 | + | V | V | + |
| Indole production | + | + | + | + | + | + | 24/49 | − | V | − | + |
| Malonate utilization | − | − | NA | + | NA | NA | NA | NA | V | − | − |

*The methods for testing acid formation from different sugars are different and the original papers should be consulted for direct comparisons.

Cells are non-motile, non-spore-forming rods (approx. 2 μm in length). Gram-negative, oxidase-positive, showing an oxidative metabolism. Good growth is observed on R2A agar, TSA and nutrient agar at 25–30 °C, but not on MacConkey agar. Colonies are yellowish, translucent and shiny with entire edges. Menaquinone MK-6 is the predominant quinone and *sym*-homospermidine is the major polyamine. Phosphatidylethanolamine is the major lipid; several unknown polar lipids are also present. The fatty acid profile is composed largely of 15 : 0 iso (58·5 %), 17 : 0 iso 3-OH (14·1 %) and summed feature 4 (16 : 1ω7*c*/*t* and/or iso 15 : 0 2-OH, 8·4 %). Produces acid from D-glucose, D-maltose, trehalose and D-cellobiose (weak). No acid produced from adonitol, L-arabinose, D-arabitol, dulcitol, erythritol, i-inositol, lactose, D-mannitol, D-melibiose, methyl α-D-glucoside, raffinose, L-rhamnose, salicin, D-sorbitol, sucrose or D-xylose. The following compounds are utilized as sole sources of carbon: D-glucose, D-maltose, D-mannose, D-trehalose, acetate (weak) and propionate (weak). The following carbon sources are not utilized as sole sources of carbon: *N*-acetylgalactosamine, *N*-acetylglucosamine, L-arabinose, L-arbutin, D-cellobiose, D-galactose, gluconate, glycerol, D-fructose, D-mannitol, maltitol, α-D-melibiose, L-rhamnose, D-ribose, D-sucrose, salicin, D-trehalose, D-xylose, adonitol, i-inositol, D-sorbitol, putrescine, *cis*-aconitate, *trans*-aconitate, 4-aminobutyrate, adipate, azelate, fumarate, glutarate, DL-3-hydroxybutyrate, itaconate, DL-lactate, 2-oxoglutarate, pyruvate, suberate, citrate, mesaconate, L-alanine, β-alanine, L-ornithine, L-phenylalanine, L-serine, L-aspartate, L-histidine, L-leucine, L-proline, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate. The chromogenic substrates *p*-nitrophenyl α-D-glucopyranoside, *p*-nitrophenyl β-D-glucopyranoside, bis-*p*-nitrophenyl phosphate, bis-*p*-nitrophenyl phenylphosphonate, bis-*p*-nitrophenyl phosphorylcholine, 2-deoxythymidine-2′-*p*-nitrophenyl phosphate, L-alanine *p*-nitroanilide, γ-L-glutamate *p*-nitroanilide and L-proline *p*-nitroanilide are hydrolysed. The following compounds are not hydrolysed: *p*-nitrophenyl β-D-galactopyranoside, *p*-nitrophenyl β-D-glucuronide and *p*-nitrophenyl β-D-xylopyranoside.

The type strain is strain B2$^T$ (=DSM 14219$^T$ =CIP 107207$^T$), isolated from sewage sludge (for details see Hollender *et al.*, 2002).

## References

**Bauwens, M. & De Ley, J. (1981).** Improvements in the taxonomy of *Flavobacterium* by DNA:rRNA hybridization. In *The Flavobacterium–Cytophaga Group*, pp. 27–31. GBF Monograph series no. 5. Edited by H. Reichenbach & O. B. Weeks. Weinheim, Germany: Verlag Chemie.

**Bernardet, J.-F., Segers, 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 1978). *Int J Syst Bacteriol* **46**, 128–148.

**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.

**Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (editors) (1994).** *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology.

**Hamana, K. & Matsuzaki, S. (1990).** Occurrence of homospermidine as a major polyamine in the authentic genus *Flavobacterium*. *Can J Microbiol* **36**, 228–231.

**Hamana, K. & Matsuzaki, S. (1991).** Polyamine distributions in the *Flavobacterium–Cytophaga–Sphingobacterium* complex. *Can J Microbiol* **37**, 885–888.

**Hollender, J., Dreyer, U., Kornberger, L., Kämpfer, P. & Dott, W. (2002).** Selective enrichment and characterization of a phosphorus-removing bacteria consortium from activated sludge. *Appl Microbiol Biotechnol* **58**, 106–111.

**Holmes, B., Owen, R. J., Steigerwalt, A. G. & Brenner, D. J. (1984).** *Flavobacterium gleum*, a new species found in human clinical specimens. *Int J Syst Bacteriol* **34**, 21–25.

**Holmes, B., Steigerwalt, A. G., Weaver, R. E. & Brenner, D. J. (1986).** *Weeksella zoohelcum* sp. nov. (formerly group IIj) from human clinical specimens. *Syst Appl Microbiol* **8**, 191–196.

**Hugo, C., Segers, P., Hoste, B., Vancanneyt, M. & Kersters, K. (2003).** *Chryseobacterium joostei* sp. nov., isolated from the dairy environment. *Int J Syst Evol Microbiol* **53** (in press).

**Kämpfer, P. (1990).** Evaluation of the Titertek-Enterobac-Automated System (TTE-AS) for identification of members of the family *Enterobacteriaceae*. *Zentbl Bakteriol* **273**, 164–172.

**Kämpfer, P. & Kroppenstedt, R. M. (1996).** Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.

**Kämpfer, P., Steiof, M. & Dott, W. (1991).** Microbiological characterization of a fuel oil contaminated site including numerical identification of heterotroph water and soil bacteria. *Microb Ecol* **21**, 227–251.

**Lane, D. J. (1991).** 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.

**Mudarris, M., Austin, B., Segers, P., Vancanneyt, M., Hoste, B. & Bernardet, J. F. (1994).** *Flavobacterium scophthalmum* sp. nov., a pathogen of turbot (*Scophthalmus maximus* L.). *Int J Syst Bacteriol* **44**, 447–453.

**Paster, B. J., Ludwig, W., Weisburg, W. G., Stackebrandt, E., Hespell, R. B., Hahn, C. M., Reichenbach, H., Stetter, K. O. & Woese, C. R. (1985).** A phylogenetic grouping of the bacteroides, cytophagas, and certain flavobacteria. *Syst Appl Microbiol* **6**, 34–42.

**Segers, P., Mannheim, W., Vancanneyt, M., De Brandt, K., Hinz, K.-H., Kersters, K. & Vandamme, P. (1993).** *Riemerella anatipestifer* gen. nov., comb. nov., the causative agent of septicemia anserum exsudativa, and its phylogenetic affiliation within the *Flavobacterium-Cytophaga* rRNA homology group. *Int J Syst Bacteriol* **43**, 768–776.

**Strunk, O., Gross, O., Reichel, B. & 11 other authors (1999).** ARB: a software environment for sequence data. Department of Microbiology, Technische Universität München, Munich, Germany. http://www.mikro.biologie.tu-muenchen.de

**Tindall, B. J. (1990).** A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* **13**, 128–130.

**Vancanneyt, M., Vandamme, P., Segers, P., Torck, U., Coopman, R., Kersters, K. & Hinz, K.-H. (1999).** *Riemerella columbina* sp. nov., a bacterium associated with respiratory disease in pigeons. *Int J Syst Bacteriol* **49**, 289–295.

**Vandamme, P., Bernardet, J.-F., Segers, P., Kersters, K. & Holmes, B. (1994).** New perspectives in the classification of the flavobacteria: description of *Chryseobacterium* gen. nov., *Bergeyella* gen. nov., and *Empedobacter* nom. rev. *Int J Syst Bacteriol* **44**, 827–831.

**Ventosa, A., Marquez, M. C., Kocur, M. & Tindall, B. J. (1993).** Comparative study of '*Micrococcus* sp.' strains CCM 168 and CCM 1405 and members of the genus *Salinicoccus*. *Int J Syst Bacteriol* **43**, 245–248.

**Weisburg, W. G., Oyaizu, Y., Oyaizu, H. & Woese, C. R. (1985).** Natural relationship between bacteroides and flavobacteria. *J Bacteriol* **164**, 230–236.

**Wieser, M., Schumann, P., Martin, K., Altenburger, P., Burghardt, J., Lubitz, W. & Busse, H.-J. (1999).** *Agrococcus citreus* sp. nov., isolated from a medieval wall painting of the chapel of Castle Herberstein (Austria). *Int J Syst Bacteriol* **49**, 1165–1170.

**Woese, C. R., Yang, D., Mandelco, L. & Stetter, K. O. (1990).** The *Flexibacter–Flavobacterium* connection. *Syst Appl Microbiol* **13**, 161–165.

**Yabuuchi, E., Kaneko, T., Yano, I., Moss, C. W. & Miyoshi, N. (1983).** *Sphingobacterium* gen. nov., *Sphingobacterium spiritivorum* comb. nov., *Sphingobacterium multivorum* comb. nov., *Sphingobacterium mizutae* sp. nov., and *Flavobacterium indologenes* sp. nov.: glucose-nonfermenting Gram-negative rods in CDC groups IIK-2 and IIb. *Int J Syst Bacteriol* **33**, 580–598.

**Yamaguchi, S. & Yokoe, M. (2000).** A novel protein-deamidating enzyme from *Chryseobacterium proteolyticum* sp. nov., a newly isolated bacterium from soil. *Appl Environ Microbiol* **66**, 3337–3343.