Flavobacterium rivuli sp. nov., *Flavobacterium subsaxonicum* sp. nov., *Flavobacterium swingsii* sp. nov. and *Flavobacterium reichenbachii* sp. nov., isolated from a hard water rivulet

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Strains WB 3.3-2^T, WB 3.2-61^T, WB 4.1-42^T and WB 2.3-68^T were isolated from the Westerhöfer Bach hard water rivulet, North Germany. The strains were Gram-staining-negative and catalase-, aminopeptidase- and oxidase-positive. The novel strains lacked flagella and only strain WB3.2-61^T showed gliding motility. Isolates WB 3.2-61^T, WB 4.1-42^T and WB 2.3-68^T produced flexirubin pigments. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the novel strains showed <98.2% similarity to the type strains of all recognized species of the genus *Flavobacterium*. Strains WB 3.3-2^T and WB 4.1-42^T shared 96.3% sequence similarity and were only distantly related to the type strains of all of the members of the genus *Flavobacterium*. Strain WB 3.2-61^T branched adjacent to *Flavobacterium limicola* DSM 15094^T (98.0%), while strain WB 2.3-68^T was a neighbour of *Flavobacterium psychrophilum* DSM 3660^T (97.1%). On R2A medium, iso-C_{15:0} was the common major fatty acid; fatty acids C_{15:0}, C_{16:0}, iso-C_{15:0} 3-OH, iso-C_{17:1ω9c}, iso-C_{17:0} 3-OH and summed feature 3 (comprising C_{16:1ω7c} and/or iso-C_{15:0} 2-OH) occurred in all strains though sometimes in low amounts. Metabolic properties revealed clear differences between the four isolates as well as between the isolates and their nearest phylogenetic neighbours. The lack of close relatedness was confirmed by Riboprinter and MALDI-TOF analyses of cell extracts. On the basis of a high number of phenotypic differentiating properties and phylogenetic uniqueness, four novel *Flavobacterium* species are proposed with the following names: *Flavobacterium rivuli* (type strain WB 3.3-2^T=DSM 21788^T=CIP 109865^T), *Flavobacterium subsaxonicum* (type strain WB 4.1-42^T=DSM 21790^T=CIP 109867^T), *Flavobacterium swingsii* (type strain WB 2.3-68^T=DSM 21789^T=CIP 109868^T) and *Flavobacterium reichenbachii* (type strain WB 3.2-61^T=DSM 21791^T=CIP 109866^T).

A recent assessment of the cultured microbial diversity of a hard water rivulet on the Western slopes of Harz Mountain, Germany (Brambilla *et al.*, 2007; Cousin *et al.*, 2008), resulted in the isolation and description of several novel species of the genera *Flavobacterium* (Cousin *et al.*, 2007) and *Pedobacter* (Muurholm *et al.*, 2007) and of proteobacterial genera as well as some novel genera

(Stackebrandt *et al.*, 2007, 2008; Verburg *et al.*, 2008). In a previous publication (Cousin *et al.*, 2008), the phylogeny of 362 flavobacterial strains was analysed and the distinct position of four isolates was determined. In this communication, we characterize these strains and conclude that they represent novel species of the genus *Flavobacterium*.

The isolation of strains from Westerhöfer Bach hard water rivulet has been described previously by Brambilla *et al.* (2007). Following isolation on R2A medium, the strains were transferred and maintained on the same medium at 20 °C. The same medium and temperature were used to grow the phylogenetically related type strains of the genus *Flavobacterium* that were used as references, *Flavobacterium limicola* DSM 15094^T and *Flavobacterium psychrophilum* DSM 3660^T, and some other type strains, i.e.

Abbreviations: NJ, Neighbour-joining; MALDI-TOF, matrix assisted laser desorption ionisation time-of-flight; ML, maximum-likelihood.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences for strains WB 3.3-2^T, WB 4.1-42^T, WB 2.3-68^T and WB 3.2-61^T are AM934661, AM934666, AM934651 and AM177616, respectively.

A dendrogram based on MALDI-TOF is available as a supplementary figure with the online version of this paper.

Flavobacterium saliperosum DSM 21664^T, *Flavobacterium terrigena* DSM 17934^T and *Flavobacterium xinjiangense* DSM 19743^T.

The colony morphology of the novel isolates and reference strains was observed using a stereomicroscope on R2A (Difco), TSA (Caso), NA (Difco) agars and on Cytophaga agar [CY, medium M67 according to the DSMZ catalogue of strains (DSMZ, 2001)] after 1–3 days incubation at 20 °C. Gram staining was performed using the amino-peptidase and KOH reactions. The presence of flagella was detected using Gray's method (Smibert & Krieg, 1994). Cells were tested for oxidase (tetramethyl-p-phenyldiamine dihydrochloride test) and catalase (H₂O₂ test) activities. Flexirubin pigments and gliding motility were assessed as described by Bernardet *et al.* (2002). The temperature range and optimum for growth were determined using a temperature gradient incubator (model TN-3; Toyo Kagaku Sangyo Co) from 0 to 40 °C with 1 °C increments. Strains WB 3.3-2^T, WB 3.2-61^T and WB 4.1-42^T were grown in R2A broth. As strain WB 2.3-68^T did not grow in R2A broth, growth tests were performed in CY broth. Results were recorded after 48 h incubation. The optimal pH for growth was determined in buffered R2A broth at between pH 5.8 and 8.4, at 0.2 pH unit intervals. Strain WB 2.3-68^T, cultured in buffered CY broth, grew only at pH 7.2. The salt tolerance range was observed in CY broth supplemented with 1–6 % (w/v) NaCl, at 1 % intervals. Microaerophily was tested by the candle jar method (Gerhardt *et al.*, 1981). Anaerobic growth was assessed in the Anaerocult A mini gas pack system (Merck). The results of these tests are presented in the species descriptions.

Tests to determine the degradation of pectin and chitin (Sigma-Aldrich) (Reichenbach & Dworkin, 1981b), alginate (Sigma-Aldrich) (West & Colwell, 1984) (recorded after three weeks), casein (Reichenbach & Dworkin, 1981a) and starch and L-tyrosine (Barrow & Feltham, 1993) (recorded after one week) followed previously described procedures. Cellulose hydrolysis was determined by following the maceration of a cellulose (Whatman No. 1) strip (1 × 3 cm) in carbohydrate-free R2A broth. Formation of H₂S from cysteine was tested according to Dye (1968). DNA hydrolysis was determined on DNA agar (Difco). Other physiological properties were investigated using the API ZYM and API 20NE systems (bioMérieux) and the GN MicroPlate system (Biolog), according to the manufacturers' instructions. API ZYM tests were recorded after 6 h incubation while API 20NE and GN MicroPlate tests were read after 48 h. All systems were incubated at 20 °C. The results of the reactions are recorded in Table 1.

The methods used for DNA extraction and sequencing of the almost complete 16S rRNA gene were as described by Cousin *et al.* (2008). For phylogenetic analysis, the *Flavobacterium* database of the 'All-species living tree' was used (<http://www.arb-silva.de/projects/living-tree/>). The sequences of the novel isolates and of *Flavobacterium*

lindanitolerans IP-10^T, *Flavobacterium anhuiense* KCTC 22128^T, *Flavobacterium flevense* DSM 1076^T and *Flavobacterium pectinovorum* DSM 6368^T, which were not included in this database, were added and aligned by using the remote SINA aligner. The complete tree of type strains and novel isolates was then recalculated for the isolates and a selection of neighbouring type strains. Neighbour-joining (NJ) and maximum-likelihood (ML) analyses, as well as bootstrap calculations (with 1000 resamplings) were performed using the respective files in the ARB program (<http://www.arb-home.de/>). All of the novel strains showed 98.2 % or less sequence similarity with type strains of members of the genus *Flavobacterium*. A sequence similarity value of 96.3 % was found between strains WB 3.3-2^T and WB 4.1-42^T and significantly lower similarity values were found with other members of the genus. Strains WB 3.2-61^T and WB 2.3-68^T were related to *F. limicola* DSM 15094^T and *F. psychrophilum* DSM 3660^T, respectively (both with 98 % sequence similarity). The isolated position of the four novel isolates was apparent in the dendrograms obtained using the NJ (Fig. 1) and the ML (identical branching patterns are indicated in Fig. 1) algorithms. Only a few branching points of the dendrogram were supported by high bootstrap values, which is consistent with published data (Brambilla *et al.*, 2007).

The *rrn* operon heterogeneity of the isolates and reference strains was analysed by the Riboprinter microbial characterization system (Qualicon). Sample preparation and analysis were performed according to the manufacturer's instructions using the restriction enzyme *PvuII* as it is able to cut the *rrn* operon of *Flavobacterium* strains. *EcoRI*, conventionally used to cut DNA for RiboPrint analyses, did not result in discriminatory banding patterns. The band patterns were compared by using the BioNumerics software (Applied Maths). Clustering was performed by the unweighted pair group method with arithmetic means (UPGMA) based on the Pearson correlation coefficient, using an optimization coefficient of 1.2 %. Each ribopattern was obviously different, confirming that none of the new isolates was closely genomically related to any of the reference strains used (Fig. 2).

The mol% G+C content of DNA was determined according to Mesbah *et al.* (1989). The G+C contents of strains WB 2.3-68^T and WB 3.2-61^T were 33.3 and 34.3 mol%, respectively. These values are consistent with those of recognized members of the genus *Flavobacterium*, which range from 30 to 37 mol% (Bernardet *et al.*, 1996; Van Trappen *et al.*, 2003). The values for strains WB 3.3-2^T and WB 4.1-42^T, however, were 40.4 and 43.3 mol%, respectively, expanding the range reported for the genus by up to 5 %. As the latter two strains are obviously phylogenetic members of the genus *Flavobacterium*, the higher mol% G+C of the DNA should not be used as an argument to exclude them from the genus. It might, however, be necessary to emend the genus description once further strains with higher than the above-mentioned range of DNA G+C content are included in the genus.

Table 1. Differential biochemical properties of the four novel isolates and reference *Flavobacterium* type strains

Isolates WB 3.2-61^T and WB 2.3-68^T are arranged next to their closest phylogenetic relative. Strains: 1, WB 3.3-2^T; 2, WB 4.1-42^T; 3, WB 3.2-61^T; 4, *F. limicola* DSM 15094^T; 5, WB 2.3-68^T; 6, *F. psychrophilum* DSM 3660^T; 7, *F. saliperosum* DSM 21664^T; 8, *F. terrigena* DSM 17934^T; 9, *F. xinjiangense* DSM 19743^T. All data are from this study. All strains were positive for dextrin and L-glutamic acid utilization (GN MicroPlate). All strains were negative for hydrolysis of alginate, cellulose and pectin and L-tyrosine degradation and, according to API ZYM, for β -glucosidase, β -glucuronidase, lipase (C14), α -mannosidase and α -fucosidase activities; production of indole, fermentation of glucose, arginine dihydrolase and urease activities, and assimilation of mannitol, potassium gluconate, capric acid, adipic acid, malate, trisodium citrate, and phenylacetic acid (API 20NE); utilization of Tween 80, N-acetyl-D-galactosamine, adonitol, D-arabitol, i-erythritol, myo-inositol, raffinose, D-sorbitol, cis-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-gluconic acid, D-glucosaminic acid, α -hydroxybutyric acid, β -hydroxybutyric acid, p-hydroxyphenylacetic acid, itaconic acid, α -ketoglutaric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, L-alaninamide, D-alanine, L-histidine, L-phenylalanine, D-mannitol, xylitol, L-pyroglutamic acid, D-serine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL- α -glycerol phosphate, DL-carnitine, γ -aminobutyric acid, urocanic acid and inosine (GN MicroPlate). +, Positive; –, negative; w, weak positive; NG, no growth.

Characteristic	1	2	3	4	5	6	7	8	9
Casein	–	–	+	–	–	–	–	–	–
Chitin	–	–	–	–	–	–	–	–	+
DNA	–	–	w	NG	NG	NG	NG	NG	NG
H ₂ S production	w	w	w	w	NG	w	+	w	NG
Starch	+	+	+	–	–	–	–	w	+
Tween 40	+	+	+	NG	–	+	–	–	–
Tween 80	+	+	–	–	+	+	+	+	–
API ZYM*									
Acid phosphatase	5	3	5	4	5	3	1	0	3
Alkaline phosphatase	2	4	5	5	5	5	5	5	5
α -Chymotrypsin	0	0	2	0	5	0	0	0	0
Cystine arylamidase	0	1	2	2	2	1	3	1	1
Esterase (C4)	0	0	0	1	0	0	0	0	0
Esterase lipase (C8)	1	1	1	1	2	1	1	0	1
α -Galactosidase	3	4	0	3	0	0	0	0	0
β -Galactosidase	5	2	2	0	0	0	0	0	1
α -Glucosidase	0	2	5	5	0	0	0	0	4
Leucine arylamidase	3	5	5	5	3	5	4	3	4
N-Acetyl- β -glucosaminidase	0	0	3	5	0	0	5	0	5
Naphthol-AS-BI-phosphohydrolase	4	2	5	4	3	1	1	1	3
Trypsin	1	2	1	1	0	1	2	4	0
Valine arylamidase	1	3	5	5	5	1	5	5	5
API 20NE									
β -Galactosidase	+	+	+	–	–	–	–	–	w
Assimilation of:									
N-Acetylglucosamine	–	–	+	–	–	–	–	–	–
L-Arabinose	w	+	+	–	–	–	–	–	–
D-Glucose	w	+	+	+	–	–	–	–	–
Maltose	+	+	+	+	–	–	–	–	–
D-Mannose	w	+	+	w	–	–	–	–	–
GN MicroPlate (utilization of)									
Acetic acid	+	–	–	–	–	–	–	–	–
N-Acetyl-D-glucosamine	–	–	+	–	–	–	–	–	w
L-Alanine	–	+	–	–	–	–	–	–	–
L-Alanylglycine	+	+	w	+	–	–	+	–	+
L-Arabinose	+	+	+	–	–	–	–	–	–
L-Asparagine	–	–	+	+	–	–	–	–	–
L-Aspartic acid	–	–	+	+	–	–	–	–	–
Cellobiose	+	+	+	–	–	–	–	–	w
α -Cyclodextrin	+	+	+	+	–	–	–	–	+
D-Fructose	+	+	+	–	–	–	–	–	w
L-Fucose	–	+	w	–	–	–	–	–	–
D-Galactose	+	+	–	–	–	–	–	–	–
D-Galacturonic acid	–	–	+	–	–	–	–	–	–

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8	9
Gentiobiose	+	+	+	–	–	–	–	–	+
α -D-Glucose 1-phosphate	–	–	+	+	–	–	–	–	w
D-Glucose 6-phosphate	–	–	+	–	–	–	–	–	w
α -D-Glucose	+	+	+	+	–	–	–	–	+
D-Glucuronic acid	–	–	+	–	–	–	–	–	–
Glycyl-L-aspartic acid	–	+	+	+	–	–	–	–	+
Glycyl-L-glutamic acid	–	+	+	+	–	+	w	–	+
Hydroxy-L-proline	–	w	–	–	–	–	w	–	–
α -Ketobutyric acid	–	+	+	–	–	–	+	+	–
α -Ketovaleric acid	–	w	–	–	–	–	–	+	–
DL-Lactic acid	–	+	–	–	–	–	–	–	–
α -D-Lactose	+	–	+	–	–	–	–	–	–
Lactulose	+	w	–	–	–	–	–	–	–
L-Leucine	–	w	–	–	–	–	–	–	–
Maltose	+	+	+	+	–	–	–	–	+
D-Mannose	+	+	+	–	–	–	–	–	+
Melibiose	+	+	w	–	–	–	–	–	–
Methyl β -D-glucoside	–	+	–	–	–	–	–	–	w
Methylpyruvate	+	+	+	+	–	–	w	–	w
Monomethyl succinate	+	w	w	+	–	–	–	–	+
L-Ornithine	–	+	+	+	–	+	w	–	w
L-Proline	+	+	+	+	–	+	+	+	+
D-Psicose	+	+	–	–	–	–	–	–	–
L-Rhamnose	–	+	–	–	–	–	–	–	–
L-Serine	–	+	–	+	–	–	–	–	–
Sucrose	–	–	–	–	–	–	–	–	+
L-Threonine	+	+	+	+	–	–	+	+	+
Thymidine	–	–	–	–	–	–	+	–	–
Trehalose	+	–	–	–	–	–	–	–	+
Turanose	–	w	+	+	–	–	–	–	w
Tween 40	+	–	+	–	+	+	–	+	w
Uridine	–	–	+	–	–	–	–	–	w

*Results are expressed as API ZYM scores: 0, no activity; 1, lowest activity; 5, highest activity.

The preparation of cell extracts and handling of samples for matrix assisted laser desorption ionisation time-of-flight (MALDI-TOF) analyses were conducted as described by Cousin *et al.* (2008). Samples were analysed on a Microflex L20 MS (Bruker Daltonics) and BioTyper software, Version 1.0 (Bruker Daltonics) was used for processing the raw spectra and comparing spectra in order to classify the strains (Cousin *et al.*, 2008). Spectra recorded with 16 replicates per strain were smoothed, their baselines were corrected and peak lists were generated automatically according to the parameter settings. The main spectra were created by extraction of the specific peak information from the individual spectra of a particular strain. Based on similarity scores of the main spectra, dendrograms were constructed showing the clustering of strains by their mass spectra. As the correlation between dendrograms based on MALDI-TOF protein patterns and 16S rRNA gene sequence similarities is high only at the intra-specific level (Cousin *et al.*, 2008), lack of correlation between the

relevant patterns of type strains was not surprising. Only the clustering of *F. psychrophilum* DSM 3660^T and strain WB 2.3-68^T was confirmed (see Supplementary Fig. S1 available in IJSEM online) while the other isolates were distinctly apart from all the *Flavobacterium* type strains.

Some of the reactions in the API ZYM system were positive for all strains investigated though with varying degrees of activity (Table 1). Of the new isolates, strain WB 2.3-68^T appeared less active and this restricted activity was shared by its closest relatives, *F. psychrophilum* DSM 3660^T, *F. saliperosum* DSM 21664^T and *F. terrigena* DSM 17934^T. In addition, according to the API 20NE and GN MicroPlate systems, isolates WB 3.3-2^T, WB 4.1-42^T and WB 3.2-61^T were metabolically more versatile than strain WB 2.3-68^T and the reference strains, except for *F. xinjiangense* DSM 19743^T. The phylogenetic neighbours strains WB 3.3-2^T and WB 4.1-42^T shared many positive reactions, mainly for carbohydrate assimilation, but they differed in several other reactions. Likewise, strains WB 3.2-61^T and *F. limicola*

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship of strains WB 3.2-61^T, WB 2.3-68^T, WB 4.1-42^T and WB 3.3-2^T and neighbouring *Flavobacterium* type strains. Branching points which are identical with those of the maximum-likelihood tree are indicated by filled circles. Numbers at branching points are bootstrap values >70% (1000 resamplings). Bar, 2% sequence divergence. *Terrimonas ferruginea* DSM 30193^T (GenBank accession no. AM230484) served as a root (not shown).

DSM 15094^T shared several common reactions, notably for the assimilation of amino acids, which were often different from those of the above-mentioned strain pair. All four new isolates could be unambiguously differentiated from each other and from the reference type strains.

Fatty acid analysis was performed according to Miller (1982) on the isolates and reference strains grown on R2A agar at 20 °C for 3 days. R2A agar was selected as strains WB 2.3-68^T, *F. psychrophilum* DSM 3660^T and *F. terrigena* DSM 17934^T did not grow within 24–72 h on trypticase soy agar

(TSA; Difco), the medium recommended by the Microbial Identification System (MIDI; Microbial ID). Fatty acid analysis was performed following the standard protocol of the MIDI system. Extracts were analysed in a GC (model HP6890A; Hewlett Packard) equipped with a flame-ionization detector (Kämpfer & Kroppenstedt, 1996). In all strains studied, the major constituents, though in varying quantities, included iso-C_{15:1}, iso-C_{15:0}, iso-C_{15:0} 3-OH, iso-C_{17:0} 3-OH and summed feature 3 (comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) (Table 2). However, strains WB 3.2-61^T and WB 2.3-68^T contained significant amounts of iso-C_{17:1}ω9c,

Fig. 2. *PvuII* restriction pattern ribotypes of the four novel isolates and type strains of reference *Flavobacterium* taxa. Analysis was performed with the Riboprinter microbial characterization system (Qualicon).

Table 2. Fatty acid profile (%) of the four novel isolates and type strains of reference *Flavobacterium* species grown on R2A agar

Isolates WB 3.2-61^T and WB 2.3-68^T are arranged next to their closest phylogenetic relative. Strains: 1, WB 3.3-2^T; 2, WB 4.1-42^T; 3, WB 3.2-61^T; 4, *F. limicola* DSM 15094^T; 5, WB 2.3-68^T; 6, *F. psychrophilum* DSM 3660^T; 7, *F. saliperosum* DSM 21664^T; 8, *F. terrigena* DSM 17934^T; 9, *F. xinjiangense* DSM 19743^T. All data are from this study. Fatty acids amounting to ≤ 1 % of the total fatty acids in all strains studied are omitted. ND, Not detected; tr, trace (<1 % of total).

Fatty acid	1	2	3	4	5	6	7	8	9
iso-C _{14:0}	ND	ND	tr	4.2	tr	tr	tr	tr	3.9
iso-C _{15:1}	4.3	2.9	7.0	3.5	11.8	13.9	8.2	12.4	2.4
iso-C _{15:0}	11.2	23.4	19.9	3.6	20.2	22.5	21.7	22.3	10.4
anteiso-C _{15:0}	5.6	8.5	4.3	5.4	tr	2.4	5.1	2.2	3.3
C _{15:1} ω6c	tr	tr	6.5	8.5	7.0	11.0	2.5	3.1	11.5
C _{15:0}	4.3	3.1	12.4	6.9	23.0	10.3	13.5	12.3	12.1
iso-C _{16:1}	ND	ND	1.2	8.4	tr	1.2	2.2	1.6	2.8
iso-C _{16:0}	1.3	1.5	1.7	9.7	1.3	1.0	8.7	6.6	6.9
C _{16:0}	8.8	9.4	1.9	1.7	tr	tr	tr	1.5	2.1
iso-C _{15:0} 3-OH	3.9	3.7	8.8	4.1	9.2	7.7	4.0	5.1	4.3
iso-C _{17:1} ω9c	1.4	tr	3.8	3.0	7.8	10.0	17.0	tr	2.2
C _{15:0} 3-OH	ND	ND	2.2	tr	1.5	ND	tr	tr	tr
C _{17:1} ω8c	ND	ND	tr	tr	2.0	tr	1.5	tr	tr
C _{17:1} ω6c	tr	ND	4.9	6.9	3.7	3.2	tr	1.4	6.4
iso-C _{16:0} 3-OH	3.3	3.6	3.5	13.7	1.0	1.5	1.6	2.6	tr
C _{16:0} 3-OH	7.0	7.3	1.7	tr	tr	ND	tr	tr	1.9
C _{18:1} ω5c	ND	ND	ND	tr	1.2	1.6	ND	ND	ND
iso-C _{17:0} 3-OH	5.2	7.8	9.8	4.5	11.8	8.6	7.3	10.1	5.0
C _{17:0} 2-OH	1.7	2.5	1.0	tr	ND	ND	1.6	tr	tr
Summed feature 3*	35.1	20.7	8.1	11.1	2.2	1.6	1.8	4.1	14.2

*Summed features are groups of two or three fatty acids that cannot be separated by GLC using the MIDI System. Summed feature 3 comprised C_{16:1}ω7c and/or iso-C_{15:0} 2-OH.

as was also the case for *F. limicola* DSM 15094^T, *F. psychrophilum* DSM 3660^T and *F. saliperosum* DSM 21664^T. Strains WB 3.2-61^T and WB 2.3-68^T also contained a higher percentage of C_{15:0} and iso-C_{15:0} 3-OH in comparison with all of the five *Flavobacterium* type strains tested. Strains WB 3.3-2^T and WB 4.1-42^T showed the highest percentage of C_{16:0} and summed feature 3 compared with all other strains. The fatty acid patterns of the six strains that were able to grow on TSA differed from those of the same strains grown on R2A due to the absence of C_{15:0}; the other main components were present, though in different proportions (data not shown). The fatty acid compositions reported in the original descriptions of some of the reference strains studied essentially agreed with those obtained in this study, although the growth medium was either not specified (Tamaki *et al.*, 2003) or different (Zhu *et al.*, 2003; Wang *et al.*, 2006). For example, *F. saliperosum* reportedly lacked summed feature 3, iso-C_{17:0} 3-OH, iso-C_{16:0} and iso-C_{15:0} 3-OH (Wang *et al.*, 2006).

The 16S rRNA gene sequence similarities of ≤ 98 % among the four novel isolates and between them and all *Flavobacterium* type strains demonstrated that they belong to unique genospecies (Stackebrandt & Ebers, 2006). It was therefore not necessary to determine the level of DNA–DNA hybridization. The genomic distinctiveness is corro-

borated by distinct *rrn* operon patterns and MALDI-TOF patterns of whole-cell extracts to which ribosomal proteins contribute significantly. Chemotaxonomically, the four novel isolates show a subset of fatty acids that confirm their affiliation to the genus *Flavobacterium*; the other fatty acids, however, show sufficient qualitative and quantitative differences to allow their identification. As the cultural and metabolic properties support the uniqueness of the new isolates, it is suggested that the isolates represent four novel species of the genus *Flavobacterium* for which the following names are proposed: *Flavobacterium rivuli* for strain WB 3.3-2^T, *Flavobacterium subsaxonicum* for strain WB 4.1-42^T, *Flavobacterium swingsii* for strain WB 2.3-68^T and *Flavobacterium reichenbachii* for strain WB 3.2-61^T.

Description of *Flavobacterium rivuli* sp. nov.

Flavobacterium rivuli (ri'vu.li. L. gen. n. *rivuli* of/from a small river).

Gram-staining-negative (KOH and aminopeptidase reactions positive), aerobic and microaerobic rods (0.4–0.6 × 1.5–2.5 µm). Does not grow anaerobically. Growth occurs between 4 and 29 °C with an optimal temperature between 16 and 24 °C. Growth between 4 and 8 °C is sparse; no growth occurs below 4 °C or above 29 °C. Growth occurs

between pH 6.4 and 7.8 with an optimal pH of 7.0. Growth occurs at NaCl concentrations between 0 and 2 % (w/v) with optimal growth at 1 % (w/v) NaCl. Gliding motility and flagella are absent. Colonies on R2A, NA, TSA and CY agars are circular with entire margins, convex, smooth, translucent, soft and butyrous. Colonies are pearl white on R2A and CY agars and yellow on TSA and NA agars. Colonies are 2–2.5 mm in diameter on R2A agar and 0.3–0.8 mm on NA, TSA and CY agars. Flexirubin pigments are absent. Catalase- and oxidase-positive. Nitrate reduction is negative. Aesculin and glycogen hydrolysis are positive; cellulose (Whatman No. 1), chitin and gelatin hydrolysis are negative. Other physiological reactions are given in Table 1. The major fatty acids (>7.5 % of total) are iso-C_{15:0}, C_{16:0} and summed feature 3 (comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH).

The type strain, WB 3.3-2^T (=DSM 21788^T=CIP 109865^T), was isolated from a spring of Westerhöfer Bach, Westerhof, 40 km north of Göttingen, Germany. The DNA G + C content of the type strain is 40.4 mol%.

Description of *Flavobacterium subsaxonicum* sp. nov.

Flavobacterium subsaxonicum (sub.sa.xo'ni.cum. L. prep. sub low, below, under; L. adj. *saxonicus* pertaining to Saxony; N.L. neut. adj. *subsaxonicum* pertaining to Lower Saxony).

Gram-staining-negative (KOH and aminopeptidase reactions positive), aerobic and microaerobic rods (1.5 × 3.5–4.5 µm). Does not grow anaerobically. Growth occurs between 2 and 38 °C with an optimal temperature between 24 and 28 °C. Growth between 2 and 9 °C is sparse; no growth occurs below 2 °C or above 38 °C. Growth occurs between pH 7.0 and 7.6 with optimal pH of 7.2. Growth occurs at NaCl concentrations between 0 and 2 % (w/v) with optimal growth at 0 % (w/v) NaCl. Gliding motility and flagella are absent. Colonies on R2A, NA, TSA and CY agars are circular with entire margins, convex, smooth, opaque, soft and butyrous. Colonies are light yellow on R2A, NA and TSA agars and pearl white on CY agar. Colonies are 0.8–1 mm in diameter on R2A agar and 0.2–0.3 mm on CY agar. Flexirubin pigments are present. Catalase- and oxidase-positive. Nitrate reduction is negative. Aesculin, gelatin and glycogen hydrolysis are positive; cellulose (Whatman No. 1) and chitin hydrolysis are negative. Other physiological reactions are given in Table 1. The major fatty acids (>7.5 % of total) are iso-C_{15:0}, anteiso-C_{15:0}, C_{16:0}, iso-C_{17:0} 3-OH and summed feature 3 (comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH).

The type strain, WB 4.1-42^T (=DSM 21790^T=CIP 109867^T), was isolated from a spring of Westerhöfer Bach, Westerhof, 40 km north of Göttingen, Germany. The DNA G + C content of the type strain is 43.3 mol%.

Description of *Flavobacterium swingsii* sp. nov.

Flavobacterium swingsii (swing'si.i. N.L. masc. gen. n. *swingsii* of Swings, named after Jean Swings, a famous

Belgian microbiologist and former President of the World Federation of Culture Collections).

Gram-staining-negative (KOH and aminopeptidase reactions positive), aerobic and microaerobic rods (0.3 × 4.5–6.0 µm). Does not grow anaerobically. Growth occurs between 3 and 26 °C with an optimal temperature between 24 and 26 °C. Growth between 3 and 8 °C is sparse; no growth occurs below 3 °C or above 26 °C. Growth occurs at pH 7.0 (tested on R2A agar). Growth occurs with 0 % (w/v) NaCl; no growth occurs with 1 % (w/v) NaCl. Gliding motility and flagella are absent. Colonies on R2A and CY agars are yellow and 0.4–0.5 mm in diameter, circular with entire margins, convex, smooth, translucent, sticky and butyrous. Growth on NA agar is poor and no growth occurs on TSA agar or in R2A broth. Flexirubin pigments are present. Catalase- and oxidase-positive. Nitrate reduction is positive. Chitin hydrolysis is positive; cellulose (Whatman No. 1) aesculin, glycogen and gelatin hydrolysis are negative. Other physiological reactions are given in Table 1. The major fatty acids (>7.5 % of total) are iso-C_{15:1}, iso-C_{15:0}, C_{15:0}, iso-C_{15:0} 3-OH, iso-C_{17:1}ω9c, and iso-C_{17:0} 3-OH.

The type strain, WB 2.3-68^T (=DSM 21789^T=CIP 109868^T), was isolated from a spring of Westerhöfer Bach, Westerhof, 40 km north of Göttingen, Germany. The DNA G + C content of the type strain is 33.3 mol%.

Description of *Flavobacterium reichenbachii* sp. nov.

Flavobacterium reichenbachii (rei.chen.ba'chi.i. N.L. masc. gen. n. *reichenbachii* of Reichenbach, named after Hans Reichenbach, a famous German microbiologist, who pioneered the study of gliding bacteria).

Gram-staining-negative (KOH and aminopeptidase reactions positive), aerobic and microaerobic rods (1.0 × 5.5–8.0 µm). Does not grow anaerobically. Growth occurs between 6 and 30 °C with an optimal temperature between 20 and 26 °C. Growth between 6 and 9 °C is sparse; no growth occurs below 6 °C or above 30 °C. Growth occurs between pH 5.8 and 8.0 with an optimal pH of 6.2. Growth occurs at NaCl concentrations between 0 and 2 % (w/v), with optimal growth at 0 % (w/v) NaCl. Gliding motility is present; flagella are absent. Colonies are golden yellow on R2A and CY agars, and orange–yellow on NA and TSA agars. Colonies are irregular with spreading and undulate margins, flat, smooth, translucent, shiny and butyrous. Colonies are 2.0–5.0 mm in diameter on R2A and NA agars and 1–2 mm in diameter on TSA and CY agars. Flexirubin pigments are present. Catalase- and oxidase-positive. Nitrate reduction is negative. Chitin, aesculin and gelatin hydrolysis is positive; cellulose (Whatman No. 1) and glycogen hydrolysis are negative. Other physiological reactions are given in Table 1. The major fatty acids (>7.5 % of total) are C_{15:0}, iso-C_{15:0}, iso-C_{15:0} 3-OH, iso-C_{17:0} 3-OH and summed feature 3 (comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH).

The type strain, WB 3.2-61^T (=DSM 21791^T=CIP 109866^T), was isolated from a spring of Westerhöfer Bach, Westerhof, 40 km north of Göttingen, Germany. The DNA G+C content of the type strain is 34.3 mol%.

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