Flavobacterium aquidurense sp. nov. and *Flavobacterium hercynium* sp. nov., from a hard-water creek

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Ten new *Flavobacterium*-like strains were isolated from freshwater of the hard-water creek Westerhöfer Bach, northern Germany. These strains formed two phylogenetic groups: strains WB 1.1-56^T, WB 1.1-04, WB 1.1-14, WB 1.1-57 and WB 1.1-63; and strains WB 4.2-33^T, WB 4.1-86, WB 4.2-34, WB 4.2-32 and WB 4.2-78. Cells were Gram-negative, yellow-pigmented, chemoheterotrophic rods. Their major fatty acid profiles were similar, consisting of iso-C_{15:0}, iso-C_{15:0} 3-OH, iso-C_{17:0} 3-OH and summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH). DNA G + C contents for strains WB 1.1-56^T and WB 4.2-33^T were 33.5 and 37.5 mol%, respectively. Phylogenetic analysis based on almost complete 16S rRNA gene sequences indicated that strain WB 1.1-56^T was phylogenetically most closely related to *Flavobacterium frigidimaris* KUC-1^T, and that strain WB 4.2-33^T was related most closely to *F. frigidimaris* KUC-1^T and *Flavobacterium saccharophilum* DSM 1811^T. Levels of 16S rRNA gene sequence similarity between strains WB 1.1-56^T and WB 4.2-33^T and the type strains of recognized members of the genus *Flavobacterium* were below 98 %. DNA–DNA hybridization experiments confirmed the separate genomic status of strains WB 1.1-56^T and WB 4.2-33^T. Strains WB 1.1-56^T and WB 4.2-33^T and their respective relatives differed from phylogenetically related *Flavobacterium* species based on several phenotypic characteristics. On the basis of their phenotypic and phylogenetic distinctiveness, the two groups of strains are considered to represent two novel species, for which the names *Flavobacterium aquidurense* sp. nov. (type strain WB 1.1-56^T = DSM 18293^T = CIP 109242^T) and *Flavobacterium hercynium* sp. nov. (type strain WB 4.2-33^T = DSM 18292^T = CIP 109241^T) are proposed.

The genus *Flavobacterium* Bergey *et al.* 1923 belongs to the family *Flavobacteriaceae* in the phylum *Bacteroidetes*. With emendation of the description of the genus (Bernardet *et al.*, 1996), several species previously belonging to the genus were reclassified and placed in new or different genera. Recently, Bernardet & Bowman (2005) provided an excellent overview of the taxonomy and ecology of members of the genus *Flavobacterium*. Species of the genus have been isolated worldwide from habitats such as marine and freshwater environments, soil, sediment, sea-ice biofilms and diseased fish. In the past few years, many novel species have been added to the genus, isolated from freshwater sediments (Tamaki *et al.*, 2003; Wang *et al.*, 2006), glacier ice (Zhu

et al., 2003), soil (Yoon *et al.*, 2006; Kim *et al.*, 2006), Antarctic habitats (McCammon & Bowman, 2000; Humphry *et al.*, 2001; Van Trappen *et al.*, 2003, 2004, 2005; Yi *et al.*, 2005; Nogi *et al.*, 2005; Yi & Chun, 2006), the gut of an earthworm (Horn *et al.*, 2005) and bacterial aggregates of a wastewater treatment plant (Aslam *et al.*, 2005). A rich diversity of flavobacteria has also been reported from river epilithon (O'Sullivan *et al.*, 2006). In a recent study on the diversity of freshwater bacteria, hundreds of Gram-negative, rod-shaped *Flavobacterium*-like bacteria were isolated from water of a hard-water creek from the western slopes of the Harz Mountains, Germany. Following a pre-screening by MALDI-TOF mass spectrometry (Brambilla *et al.*, 2007) and partial 16S rRNA gene sequence analysis, several groups of isolates were found to cluster separately from the type strains of recognized members of the genus *Flavobacterium*. Two of these groups were subjected to a polyphasic taxonomic study.

Isolation of strains from the Westerhöfer Bach has been described by Brambilla *et al.* (2007). Strains WB 1.1-56^T,

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of WB 1.1-56^T, WB 4.2-33^T and WB 4.2-78 are AM177392, AM265623 and AM177627, respectively.

A table giving the fatty acid compositions of the novel strains and related members of the genus *Flavobacterium* and a dendrogram based on these results are available as supplementary material in IJSEM Online.

WB 1.1-04, WB 1.1-14, WB 1.1-57 and WB 1.1-63 were isolated from spring water, and strains WB 4.2-33^T, WB 4.1-86, WB 4.2-34, WB 4.2-32 and WB 4.2-78 were isolated from creek water about 320 m downstream from this spring (station 4). Following isolation on R2A medium (Difco) strains were transferred to and maintained on medium 67 (M67; DSMZ, 2001) at 25 °C for at least 24 h. The same medium was also used to cultivate the reference strains *Flavobacterium saccharophilum* DSM 1811^T, *Flavobacterium frigidimaris* DSM 15937^T, *Flavobacterium flevense* DSM 1076^T and *Flavobacterium pectinovorum* DSM 6368^T.

DNA extraction and PCR amplification of the 16S rRNA genes were carried out as described by Rainey *et al.* (1996). The PCR amplificate was purified by using the QIAquick PCR purification kit (Qiagen) according to the manufacturer's instructions. Sequencing of the PCR products, manual alignment of the sequences with those of recognized *Flavobacterium* species and determination of similarity coefficients were performed as described by Rainey *et al.* (1996). The algorithm of De Soete (1983) and the neighbour-joining algorithm (Felsenstein, 1993) were used to generate tree topologies. Bootstrap values were calculated according to Felsenstein (1985).

All strains were first subjected to partial 16S rRNA gene sequence analysis (436 bp, 5' terminus). The five strains isolated from the spring water showed 100 % sequence similarity to each other. The partial 16S rRNA gene sequences of the five strains isolated from station 4 showed between 98.6 and 100 % similarity to each other.

The almost complete sequences of the 16S rRNA gene were determined for strains WB 1.1-56^T, WB 4.2-33^T and WB 4.2-78. The sequences of the type strains of *F. saccharophilum* and *F. pectinovorum* were reanalysed because of sequence ambiguity in the data deposited in public databases. A sequence similarity value of 97.9 % was found between strains WB 1.1-56^T and WB 4.2-33^T. These two novel strains showed 16S rRNA gene sequence similarity values of less than 98 % to the type strains of recognized members of the genus *Flavobacterium*. Strain WB 1.1-56^T was related most closely to *F. frigidimaris* DSM 15937^T (97.9 %), and WB 4.2-33^T to *F. saccharophilum* DSM 1811^T (96.7 %). None of the other recognized type strains of the genus *Flavobacterium* clustered closely to strains WB 1.1-56^T or WB 4.2-33^T. A neighbour-joining tree showing the phylogenetic position of strains WB 1.1-56^T, WB 4.2-33^T and WB 4.2-78 among related members of the genus *Flavobacterium* is shown in Fig. 1. The topologies of the trees generated with the neighbour-joining method and the algorithm of De Soete (1983) were almost identical. Only a few branch points of the dendrogram were supported by high bootstrap values, which is consistent with published data (Brambilla *et al.*, 2007). The 16S rRNA gene sequences of strains WB 4.2-33^T and WB 4.2-78 were identical.

The G + C content of the DNA of strains WB 1.1-56^T and WB 4.2-33^T was determined from *T_m* values according to

Fig. 1. Relationship between strains WB 1.1-56^T, WB 4.2-33^T and WB 4.2-78 and the type strains of neighbouring *Flavobacterium* species. The dendrogram is based on 16S rRNA gene sequence comparisons using the neighbour-joining algorithm of Felsenstein (1993). Numbers at branch points are bootstrap values > 50 % (500 resamplings). Bar, 5 % sequence divergence, as determined by measuring the length of the horizontal lines connecting any two species. The sequences of less closely related *Flavobacterium* species served as a root.

the formula of Frank-Kamenetskii (1971). The G + C content of strains WB 1.1-56^T and WB 4.2-33^T was 33.5 and 37.5 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).

Genomic relatedness between strains WB 1.1-56^T and WB 4.2-33^T and their most closely related phylogenetic neighbours, i.e. the type strains of *F. frigidimaris* and *F. saccharophilum*, was determined according to the spectrophotometric DNA–DNA reassociation method. DNA was isolated using a French pressure cell (Thermo Spectronic) and was purified by hydroxyapatite chromatography as described by Cashion *et al.* (1977). DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970), with the modifications of Huß *et al.* (1983) and Escara & Hutton (1980), by using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with *in-situ* temperature probe (Varian). Hybridization values were determined at least twice for any given strain pair. Strain WB 1.1-56^T was moderately related to *F. saccharophilum* DSM 1811^T (60.5 %), while reassociation values with *F. frigidimaris* DSM 15937^T (26.2 %) and with WB 4.2-33^T and WB 4.2-78 (5.2 and 8.1 %, respectively) were significantly lower. Strain WB 4.2-33^T shared 100 % DNA–DNA relatedness with strains WB 4.2-78 and WB 4.1-86, i.e. two other members of the same 16S rRNA gene cluster. Reassociation values for strains WB 4.2-33^T and WB 4.2-78 with *F. frigidimaris* DSM 15937^T and *F. saccharophilum* DSM 1811^T were low, ranging between 31.4–41.8 and 44.0 %. These

values indicate that the two new strain groups represent two distinct novel *Flavobacterium* genospecies.

The ten novel strains were subjected to fatty acid methyl ester analysis in order to determine their chemotaxonomic coherence. Also included were their closest phylogenetic neighbours, *F. saccharophilum* DSM 1811^T and *F. frigidimar* DSM 15937^T, as well as the type strains of *Flavobacterium denitrificans*, *F. pectinovorum*, *F. flevense* and *Flavobacterium hibernum*. Fatty acids were extracted and analysed (Miller, 1982) according to the standard protocol of the Microbial Identification System (MIDI Microbial ID Inc.) by using the TSBA40 method. Extracts were analysed by using a Hewlett Packard model HP6890A gas chromatograph equipped with a flame-ionization detector as described by Kämpfer & Kroppenstedt (1996). The major compounds identified in the novel strains were iso-C_{15:0}, iso-C_{15:0} 3-OH, iso-C_{17:0} 3-OH and summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) (Table 1; the complete data set is given in Supplementary Table S1 in IJSEM Online). These fatty acids are generally present in recognized members of the genus *Flavobacterium*. WB 1.1-56^T and related strains contained larger amounts of C_{15:0} 3-OH, C_{15:0}, C_{15:1}ω6c, iso-C_{17:1}ω9c and C_{17:1}ω6c fatty acids than strain WB 4.2-33^T and its relatives. The latter strain group possessed larger amounts of C_{16:0} and C_{16:0} 3-OH, a feature also found for *F. frigidimar* DSM 15937^T. The main fatty acid differences between the novel strains and their closest described relatives consist of variations in the relative amounts of less abundant and minor components. Supplementary Fig. S1 (see IJSEM Online) provides a dendrogram of fatty acid similarities in which the Euclidean distances reveal the coherence of the two new strain clusters (<6%). On the basis of fatty acid similarities, *F. denitrificans* DSM 15936^T and *F. pectinovorum* DSM 6368^T group with *F. saccharophilum* DSM 1811^T, while *F. flevense* DSM 1076^T and *F. hibernum* DSM 12611^T group with *F. frigidimar* DSM 15937^T. None of these type strains cluster closely with the novel strains.

In order to test whether the novel genospecies could also be defined in terms of their phenotypic properties, they were subjected to cultural, biochemical and morphological analyses. The results are given in Table 2 and in the species descriptions below. Growth was measured by reading the OD at 560 nm.

The temperature optimum for growth was tested using a temperature gradient incubator model TN-3 (Toyo Kagaku Sangyo Co.) in 1 °C steps from 4 to 36 °C. Strains were grown in R2A medium for 12 h.

The optimal pH for growth was tested in buffered M67 at 25 °C between pH 5.8 and 8.4, with steps of 0.2 pH units. Salt tolerance was tested on R2A medium supplemented with 1–6% NaCl, in 1% step increases. Reading was after 1 day of incubation.

Colony morphology was determined on M67, R2A, nutrient agar (NA) and trypticase soy agar (TSA) (both Difco),

Table 1. Fatty acid composition of strains WB 1.1-565^T and WB 4.2-33^T and related strains and reference type strains of phylogenetically related members of the genus *Flavobacterium*

Strains: 1, WB 1.1-565^T and relatives (*n*=5); 2, WB 4.2-33^T and relatives (*n*=5) (values in columns 1 and 2 are means ± SD); 3, *F. denitrificans* DSM 15936^T; 4, *F. saccharophilum* DSM 1811^T; 5, *F. pectinovorum* DSM 6368^T; 6, *F. frigidimar* DSM 15937^T; 7, *F. flevense* DSM 1076^T; 8, *F. hibernum* DSM 12611^T. Fatty acids accounting for <1% are omitted. All data are percentages of total fatty acids and were obtained in this study (TSBA40 method). A more detailed analysis is provided in Supplementary Table S1 in IJSEM Online. ND, Not detected; tr, trace (<1% of total).

Fatty acid	1	2	3	4	5	6	7	8
C _{14:0}	tr	1.5 ± 0.1	tr	tr	tr	tr	ND	tr
iso-C _{15:1} G	6.0 ± 0.2	6.1 ± 0.2	5.4	5.4	5.6	3.0	5.0	3.6
iso-C _{15:0}	15.1 ± 1.1	23.0 ± 0.1	21.8	20.1	25.1	14.8	13.7	17.4
anteiso-C _{15:0}	2.8 ± 0.3	3.1 ± 0.4	2.1	3.1	3.5	3.5	6.2	1.8
C _{15:1} ω6c	6.7 ± 0.5	1.4 ± 0.3	3.8	3.5	1.2	2.4	2.3	3.3
C _{15:0}	7.1 ± 0.7	1.6 ± 0.2	7.1	5.4	1.9	3.3	5.6	5.8
C _{16:0}	tr	3.4 ± 0.3	1.4	1.6	1.8	3.6	3.8	4.7
iso-C _{15:0} 3-OH	8.3 ± 0.5	7.1 ± 0.2	6.8	9.4	11.5	7.4	6.0	8.4
iso-C _{17:1} ω9c	8.2 ± 0.3	3.4 ± 0.1	13.5	8.5	9.0	4.0	2.44	4.5
C _{15:0} 3-OH	2.8 ± 0.1	ND	ND	ND	ND	ND	1.3	ND
C _{17:1} ω6c	6.4 ± 0.7	tr	4.2	2.9	tr	2.6	2.6	2.1
iso-C _{16:0} 3-OH	2.3 ± 0.1	1.1 ± 0.1	1.1	3.0	1.4	2.5	1.8	3.1
C _{16:0} 3-OH	1.7 ± 0.1	6.7 ± 0.3	tr	1.8	2.1	7.1	5.8	4.9
iso-C _{17:0} 3-OH	11.6 ± 0.7	7.5 ± 0.3	11.3	14.8	14.9	9.1	8.17	10.4
Summed feature 3*	11.8 ± 0.9	26.1 ± 0.6	10.9	10.5	13.2	25.4	27.7	19.9
Summed feature 4*	ND	1.19 ± 0.09	1.9	2.3	1.6	1.9	ND	1.4

*Summed feature 3 comprises C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; summed feature 4 comprises iso-C_{17:1} I and/or anteiso-C_{17:1} B.

MacConkey and DNA agar (Merck) and Anacker & Ordal agar (AOA) (Anacker & Ordal, 1955) after 5 days. Cells were tested for flexirubin pigments (Bernardet *et al.*, 2002), Gram staining using the aminopeptidase and KOH reactions and for oxidase and catalase (H₂O₂ test) activities. Results are given in the species descriptions below and in Table 2. As determined by electron microscopy, cells of strains WB 1.1-56^T and WB 4.2-33^T lacked flagella (not shown). Physiological properties were determined by using the commercial API ZYM and API 20NE systems (bioMérieux), as well as the GN MicroPlate system (Biolog), according to the manufacturers' instructions. API ZYM tests were read after 6 h incubation while API 20NE and GN MicroPlate tests were read after 48 h. Incubation was at 25 °C. Also investigated were gliding motility (hanging drop technique;

Table 2. Biochemical characteristics that differentiate members of the two novel genospecies, *F. saccharophilum* DSM 1811^T and *F. frigidimaris* DSM 15937^T as generated by the API and Biolog substrate panels

All data are from this study. The following reactions gave the same result for all strains. Positive: API ZYM – production of alkaline phosphatase, leucine arylamidase, valine arylamidase and acid phosphatase; API 20NE – production of β -galactosidase, assimilation of glucose and *N*-acetylglucosamine; Biolog – utilization of dextrin, *N*-acetyl-D-glucosamine, gentiobiose, α -D-glucose, maltose, D-mannose, D-galacturonic acid, L-glutamic acid and glycyl L-glutamic acid. Positive or weakly positive: API ZYM – production of naphthol-AS-BI-phosphohydrolase and α -glucosidase. Negative: API ZYM – production of esterase, lipase, trypsin, α -chymotrypsin, α -galactosidase, β -glucuronidase, α -mannosidase and α -fucosidase; API 20NE – production of indole and assimilation of D-glucose, L-arginine, potassium gluconate, capric acid, adipic acid, malic acid, phenylacetic acid and potassium gluconate; Biolog – utilization of adonitol, i-erythritol, *myo*-inositol, lactulose, D-psicose, L-rhamnose, D-sorbitol, xylitol, acetic acid, *cis*-aconitic acid, formic acid, L-fucose, D-galactonic acid lactone, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α -hydroxybutyric acid, β -hydroxybutyric acid, γ -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α -ketoglutaric acid, DL-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, D-alanine, L-histidine, hydroxy-L-proline, L-leucine, L-phenylalanine, L-proline, L-pyrroglutamic acid, D-serine, L-serine, DL-carnitine, γ -aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL- α -glycerol phosphate, glucose 1-phosphate and glucose 6-phosphate.

Characteristic	<i>F. aquidurens</i> sp. nov.		<i>F. saccharophilum</i> DSM 1811 ^T	<i>F. hercynium</i> sp. nov.		<i>F. frigidimaris</i> DSM 15937 ^T
	WB 1.1-56 ^T	WB 1.1-04, WB 1.1-14, WB 1.1-57, WB 1.1-63		WB 4.2-33 ^T	WB 4.1-86, WB 4.2-32, WB 4.2-34, WB 4.2-78	
API ZYM						
Production of:						
Esterase lipase	—	W*, —	—	W	W, —	W
Cystine arylamidase	W	W	—	—	—	—
β-Galactosidase	W	W, —	+	—	—	W
β-Glucosidase	—	—	—	+	+	+
N-Acetyl-β-glucosaminidase	+	+	+	—	—	+
API 20NE						
Nitrate reduction	—	+, —	—	+	+	—
Urease production	—	—	+	—	—	—
Aesculin hydrolysis	+	+	—	+	+	+
Gelatin hydrolysis	—	—	+	+	+, —	+
Assimilation of:						
L-Arabinose	+	+	+	—	–, +	+
D-Mannose	+	+	—	+	+	+
D-Mannitol	—	—	+	+	+	+
D-Maltose	+	+	—	+	+	+
Trisodium citrate	—	—	—	+	+, —	—
Biolog						
Utilization of:						
α-Cyclodextrin	+	+, W	—	+	+	+
Glycogen	—	—	W	+	+	+
Tween 20	—	–, —	—	—	–, W	—
Tween 80	—	W, —	—	—	—	—
N-Acetyl-D-galactosamine	—	–, +	—	W	–, W	W
L-Arabinose	—	–, +	+	—	+, —	—
D-Arabitol	—	—	—	+	+	—
D-Cellobiose	—	W, +, —	—	+	+	W
D-Fructose	+	+, —	+	+	+	+
D-Galactose	—	–, +	+	+	+	W
α-D-Lactose	—	–, +	—	—	—	—
D-Melibiose	—	–, +	—	—	—	+
Methyl β-D-glucoside	—	–, W	—	+	+, W	+
D-Raffinose	—	—	—	—	—	+
Sucrose	—	–, +	+	—	—	+

Table 2. cont.

Characteristic	<i>F. aquidurens</i> sp. nov.		<i>F. saccharophilum</i> DSM 1811 ^T	<i>F. hercynium</i> sp. nov.		<i>F. frigidimaris</i> DSM 15937 ^T
	WB 1.1-56 ^T	WB 1.1-04, WB 1.1-14, WB 1.1-57, WB 1.1-63		WB 4.2-33 ^T	WB 4.1-86, WB 4.2-32, WB 4.2-34, WB 4.2-78	
D-Trehalose	—	—, +	—	+	+	+
Turanose	—	—, +	—	—	—	—
Methyl pyruvate	—	—, +	—	—	—	+
Monomethyl succinate	—	w, —	—	—	—	—
Citric acid	—	—	—	w	—, w	—
α-Ketobutyric acid	—	—, w	—	—	—	—
α-Ketovaleric acid	—	w, —	—	—	—	w
L-Alaninamide	—	w, +	—	—	—	—
L-Alanine	—	—, +, w	—	—	—	—
L-Alanyl glycine	—	w, —	—	—	—, w	—
L-Asparagine	+	+	—	w	w	—
L-Aspartic acid	+	+	+	+	+	—
Glycyl L-aspartic acid	+	+, —	+	w	w	w
L-Ornithine	w	+	w	w	w	w
L-Threonine	+	+, w	w	w	w	w

*Scoring according to API instructions was translated as follows: 5 and 4, + (positive); 3, w (weak); 2, 1 and 0, — (negative). In those cases where strains of a given cluster differ in their reaction, the dominant reaction is listed first. Reactions in which the new strain clusters differ from the phylogenetically neighbouring species are indicated in bold.

Bernardet *et al.*, 2002), formation of coccoid degenerative cells (spheroplasts; phase-contrast microscopy), degradation of casein (Reichenbach & Dworkin, 1981), DNA (by using DNA agar from Difco), cellulose (strip of Whatman paper No. 1 in M67 broth), starch and L-tyrosine (Barrow & Feltham, 1993) and production of diffusible pigments on L-tyrosine agar. Reactions were read after 3–10 days. Microaerophily was tested by the candle jar method (Gerhardt *et al.*, 1981).

The two novel strain clusters differed from each other in several physiological reactions, and both could clearly be distinguished from the type strains of *F. saccharophilum* and *F. frigidimaris* (Table 2). Identical reactions for the strains investigated are listed in the legend to Table 2. For the following properties, strains related to WB 4.2-33^T showed a larger number of positive reactions (at least 80 % of strains) in the substrate test panel supplied than those strains related to strain WB 1.1-56^T: β-glucosidase, glycogen, gelatin, trisodium citrate, D-arabitol, D-galactose, D-mannitol, methyl β-D-glycoside and D-trehalose. Positive reactions for at least 80 % of the strains related to WB 1.1-56^T and negative reactions for the strains related to WB 4.2-33^T were found for cystine arylamidase, N-acetyl-β-glucosaminidase, β-galactosidase and L-arabinose (Table 2).

In conclusion, the novel strains have been shown to be phylogenetically related to members of the genus *Flavobacterium* (Fig. 1; Brambilla *et al.*, 2007) and based

on similarities in Gram-negative staining behaviour, chemoheterotrophy, fatty acid profiles, the presence of flexirubin pigments and base composition of DNA. They also show the typical morphological and cultural characteristics of members of the genus *Flavobacterium* as given by Bernardet *et al.* (1996) in their emended description of the genus. Strains WB 1.1-56^T and WB 4.2-33^T show less than 98.0 % 16S rRNA gene sequence similarity to their closest phylogenetic neighbours and have DNA–DNA relatedness values distinctly lower than 70 %, the threshold value considered for species delineation (Wayne *et al.*, 1987). As the two genospecies show sufficient intra-cluster coherence with respect to phenotype, fatty acid profiles and 16S rRNA gene sequence similarities, while showing sufficient differences among each other and their closest recognized neighbours in phenotypic properties to allow their phenotypic identification, we propose the description of two novel species, *Flavobacterium aquidurens* sp. nov. and *Flavobacterium hercynium* sp. nov.

Description of *Flavobacterium aquidurens* sp. nov.

Flavobacterium aquidurens (a.qui.du.ren'se. L. fem. n. *aqua* water; L. fem. adj. *durus* hard; N.L. neut. adj. *aquidurens* pertaining to hard water).

Gram-negative, aerobic and microaerobic rods (0.9 × 2.8 μm). Growth occurs between 13 and 30 °C with

an optimal temperature between 19 and 28 °C; no growth occurs below 8 °C or above 31.5 °C. Growth occurs between pH 6.0 and 6.6 with an optimal pH of 6.4. Growth occurs at NaCl concentrations between 0 and 1 %. No gliding motility; no flagella. Non-swarming; local spreading around colonies (maximum 6 mm) is observed on some growth media. Spheroplasts form after 3–4 days in R2A broth. Colonies formed on M67 agar after 5 days incubation are 3–8 mm in diameter, yellowish, spreading, irregular, flat with a slight rise at the centre but not umbonate, smooth, shiny, glistening, transparent and butyrous with filamentous margins. Colonies on AOA are zinc yellow, spreading, punctiform, flat with a central rise and with undulated margins fading to transparent. Colonies on R2A are 1–2 mm in diameter (mean 1.8 mm), zinc yellow, spreading, circular, convex, entire, smooth, translucent and butyrous with filamentous margins. Colonies on DNA agar are 0.9–1.5 mm in diameter (mean 1 mm), maize yellow, circular, non-spreading, convex-pyramidal, smooth and translucent to slightly opaque in the centre with undulated margins. Colonies on TSA are 0.7–1 mm in diameter, honey yellow, non-spreading, circular, convex, smooth, translucent and butyrous with undulate margins. Colonies on NA are 2.2 mm in diameter, maize yellow, have a central bump, are umbonate, smooth and translucent with undulate margins; irregular swarming is observed. No growth on MacConkey agar. Colonies do not adhere to the agar. Non-diffusible flexirubin pigments are present. Catalase- and oxidase-positive. Starch, L-tyrosine and casein are degraded. A brown pigment is produced on L-tyrosine agar. Cellulose, DNA and agar are not hydrolysed. Other physiological reactions are given in Table 1. Cells contain iso-C_{15:0}, iso-C_{17:0} 3-OH, iso-C_{15:0} 3-OH, iso-C_{17:1}ω9c, C_{15:0} and summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) as main fatty acids. The DNA G+C content of the type strain is 33.5 mol%.

The type strain, WB 1.1-56^T (=DSM 18293^T=CIP 109242^T), was isolated from the spring of the Westerhöfer Bach, Westerhof, 40 km north of Göttingen, Germany.

Description of *Flavobacterium hercynium* sp. nov.

Flavobacterium hercynium (her.cy'ni.um. L. neut. adj. *hercynium* pertaining to Hercynia, the Roman name for a mountain range, including the Harz Mountains, that forms the southern border of the North German plain).

Gram-negative, aerobic and microaerobic rods (0.7–0.8 × 5.8–6.3 µm). Growth occurs between 12 and 29 °C with an optimal growth temperature of 20–27 °C; no growth occurs below 7 °C or above 30.5 °C. Growth occurs between pH 6.4 and 8.0 with an optimum of pH 6.4–7.8. Growth occurs at NaCl concentrations between 0 and 1 %. Motile by gliding; no flagella are observed. Cells glide on M67, AOA, NA and R2A media but not on DNA agar or TSA. Spheroplasts form after 3–4 days in R2A broth. On M67 agar growth is brown beige on plates and orange-yellow on loops; colony surface is

effuse, irregular, glistening, spreading and with irregular lobate margins. Growth on AOA is brown beige; the surface is smooth, flat, translucent to transparent, and no margin is observed. Growth on R2A is dark orange to yellow; the surface is flat, glistening, translucent to transparent, with thinly lobate margins. Colonies on DNA agar are 4–8 mm in diameter, maize yellow, circular, non-spreading, umbonate, glistening, slightly irregular and translucent with undulate margins. Colonies on TSA are 4–7 mm in diameter, maize-yellow, non-spreading, circular, slightly umbonate, smooth, glistening and slightly irregular with undulate margins. Growth on NA is maize-yellow, glistening, irregular, flat and translucent. No growth is observed on MacConkey agar. Colonies do not adhere to the agar. Non-diffusible flexirubin pigments are present. Catalase- and oxidase-positive. Starch, L-tyrosine and casein are degraded. A brown pigment is produced on L-tyrosine agar. Cellulose, DNA and agar are not hydrolysed. Cells contain iso-C_{15:0}, iso-C_{17:0} 3-OH, iso-C_{15:0} 3-OH and summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) as main fatty acids. The DNA G+C content of the type strain is 37.5 mol%.

The type strain, WB 4.2-33^T (=DSM 18292^T=CIP 109241^T), was isolated from a site about 320 m downstream of the spring of the Westerhöfer Bach, Westerhof, 40 km north of Göttingen, Germany.

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