Transfer of *Chryseobacterium meningosepticum* and *Chryseobacterium miricola* to *Elizabethkingia* gen. nov. as *Elizabethkingia meningoseptica* comb. nov. and *Elizabethkingia miricola* comb. nov.

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The taxonomic positions of six strains (including the type strain) of *Chryseobacterium meningosepticum* (King 1959) Vandamme *et al.* 1994 and the type strain of *Chryseobacterium miricola* Li *et al.* 2004 were re-evaluated by using a polyphasic taxonomic approach. Phylogenetic analysis, based on 16S rRNA gene sequencing, showed that the strains represent a separate lineage from the type strains of the *Chryseobacterium*–*Bergeyella*–*Riemerella* branch within the family *Flavobacteriaceae* (90.7–93.9 % similarities), which was supported by phenotypic differences. Combined phylogenetic and phenotypic data showed that *C. meningosepticum* and *C. miricola* should be transferred to a new genus, *Elizabethkingia* gen. nov., with the names *Elizabethkingia meningoseptica* comb. nov. (type strain, ATCC 13253^T = NCTC 10016^T = LMG 12279^T = CCUG 214^T) and *Elizabethkingia miricola* comb. nov. (type strain, DSM 14571^T = JCM 11413^T = GTC 862^T) proposed.

The genus *Chryseobacterium* was first described during reclassification of members of the genus *Flavobacterium* on the basis of rRNA cistron similarity studies (Vandamme *et al.*, 1994; Bernardet *et al.*, 1996). At that time the genera *Chryseobacterium* (including six species previously considered *Flavobacterium* species, *Chryseobacterium balustinum*, *Chryseobacterium gleum*, *Chryseobacterium indologenes*, *Chryseobacterium indoltheticum*, *Chryseobacterium meningosepticum* and *Chryseobacterium scophthalmum*), *Bergeyella* (including a single species, *Bergeyella zoohelcum*, previously considered a *Weeksella* species) and *Riemerella* (including a single species, *Riemerella anatipestifer*, long considered a *Moraxella* species) composed a separate rRNA branch of the family *Flavobacteriaceae* in rRNA superfamily V. Although '*Chryseobacterium proteolyticum*' (Yamaguchi & Yokoe, 2000), *Chryseobacterium defluvii* (Kämpfer *et al.*, 2003), *Chryseobacterium joostei* (Hugo *et al.*, 2003), *Chryseobacterium miricola* (Li *et al.*, 2003) and *Riemerella columbina* (Vancanneyt *et al.*, 1999) have been described since then, the

Chryseobacterium–*Bergeyella*–*Riemerella* (CBR) branch retains its original taxonomic position.

However, recent 16S rRNA gene sequence similarity studies have revealed that the genus *Chryseobacterium* is genetically heterogeneous, and that *C. meningosepticum* and *C. miricola* can be readily differentiated from other *Chryseobacterium* species.

The aim of this study was to clarify the taxonomic positions of six strains of *C. meningosepticum* (King 1959) Vandamme *et al.* 1994 and *C. miricola* Li *et al.* 2004 within the family *Flavobacteriaceae* by using a polyphasic approach.

Strains used in this study are listed in Table 1. All were cultivated on nutrient agar (Difco) at 28 °C except the two *Riemerella* strains, which were cultivated on trypticase soy agar (TSA; BBL) at 37 °C microaerobically. For analysis of fatty acids, all strains were cultivated on TSA for 24 h for direct comparison.

The Gram reaction was performed as described by Gerhardt *et al.* (1994). Cell morphology was observed under a phase-contrast microscope (1000 × magnification; Nikon), with cells grown for 3 days on nutrient agar. Flexirubin-type pigment was detected with 20 % KOH according to the method of Fautz & Reichenbach (1980). Oxidase activity was tested using Bactident-Oxidase strips (Merck) and catalase activity was tested using 3 % H₂O₂. Growth was

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Abbreviation: CBR, *Chryseobacterium*–*Bergeyella*–*Riemerella*.

The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequences of *Elizabethkingia meningoseptica* ATCC 13253^T and *Elizabethkingia miricola* GTC 862^T are AJ704540 and AB071953, respectively.

Table 1. Strains investigated and their sources of isolation

Abbreviations: ATCC, American Type Culture Collection, Manassas, VA, USA; CCUG, Culture Collection of the University of Göteborg, Sweden; CIP, Collection of the Institute Pasteur, Paris, France; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; JCM, Japan Collection of Microorganisms, Institute of Physical and Chemical Research (RIKEN), Saitama, Japan; KCTC, Korean Collection for Type Cultures, Daejeon, Korea; LMG, Culture Collection, Laboratorium voor Microbiologie, Ghent University, Belgium; NCTC, National Collection of Type Cultures, London, UK.

Strain (former name)	Other designation(s)	Isolation
<i>E. meningoseptica</i> ATCC 13253 ^T (<i>C. meningosepticum</i>)	NCTC 10016 ^T , LMG 12279 ^T , CCUG 214 ^T	Spinal fluid, MA, USA
<i>E. meningoseptica</i> ATCC 13254 (<i>C. meningosepticum</i>)	NCTC 10585	Blood, FL, USA
<i>E. meningoseptica</i> ATCC 13255 (<i>C. meningosepticum</i>)	NCTC 10586	Spinal fluid and throat, South CA, USA
<i>E. meningoseptica</i> ATCC 49470 (<i>C. meningosepticum</i>)	AmMS 250	Clinical specimen
<i>E. meningoseptica</i> ATCC 51720 (<i>C. meningosepticum</i>)	LMG 12883, CCUG 26117	Blood, Göteborg, Sweden
<i>E. miricola</i> DSM 14571 ^T (<i>C. miricola</i>) <i>E. miricola</i> ATCC 33958 (<i>C. meningosepticum</i>)	JCM 11413 ^T , GTC 862 ^T	Condensation water Contaminated enzyme
<i>C. balustinum</i> KCTC 2903 ^T <i>C. defluvii</i> DSM 14219 ^T	NCTC 11212 ^T , ATCC 33487 ^T , LMG 8329 ^T CIP 107207 ^T	Blood of freshwater fish, France Activated sludge, Germany
<i>C. gleum</i> KCTC 2904 ^T <i>C. indologenes</i> KCTC 2905 ^T	NCTC 11432 ^T , ATCC 35910 ^T , LMG 8334 ^T NCTC 10796 ^T , ATCC 29897 ^T , LMG 8337 ^T	High vaginal swab, London, UK Trachea at autopsy, USA
<i>C. indoltheticum</i> KCTC 2920 ^T <i>C. joostei</i> LMG 18212 ^T	ATCC 27950 ^T , LMG 4025 ^T , CCUG 33445 ^T CCUG 46665 ^T	Marine mud Raw tanker milk, Ixopo II, Kwazulu-Natal, RSA
<i>C. scopthalmum</i> KCTC 2907 ^T <i>B. zoohelcum</i> KCTC 2910 ^T	ATCC 700039 ^T , LMG 13028 ^T , CCUG 33454 ^T NCTC 11660 ^T , ATCC 43767 ^T , LMG 8351 ^T	Gills of diseased turbot, Scotland, UK Sputum, NE, USA
<i>R. anatipestifer</i> KCTC 2911 ^T <i>R. columbina</i> LMG 11607 ^T	NCTC 11014 ^T , ATCC 11845 ^T , LMG 11054 ^T CIP 106288 ^T	Duck blood, USA Pigeon palatine cleft, Germany

investigated at different temperatures (5, 37 and 42 °C), and on MacConkey agar, casein agar and starch agar (Difco). Acid production tests from sugar were performed as described by Yamaguchi & Yokoe (2000). Additional tests were performed using API 20NE, API 20E and API ZYM galleries according to the manufacturer's instructions (bioMérieux).

Fatty acid methyl esters were prepared and analysed as described by Klatte *et al.* (1994) using the standard Microbial Identification System (MIDI) for automated gas chromatographic analyses (Sasser, 1990; Kämpfer & Kroppenstedt, 1996).

Isoprenoid quinones were extracted and purified as described by Tindall (1990); dried preparations were dissolved in 200 µl of 2-propanol and 1 to 10 µl was separated by HPLC without further purification. Menaquinones were separated by HPLC on a COSMOSIL 5C18-MS column (nacalai tesque) at 40 °C using acetonitrile/2-propanol (65:35, v/v) as solvent (Kroppenstedt, 1982, 1985).

Chromosomal DNA was extracted and purified by using the DNeasy Tissue Kit and Genomic-tip system 100/G (Qiagen).

In vitro amplification of extracted 16S rRNA genes was performed as described by Yoon *et al.* (1997) with some modifications. The 16S rRNA gene sequences were aligned with published sequences retrieved from EMBL by using CLUSTAL_X (Thompson *et al.*, 1997) and edited using BioEdit (Hall, 1999). A phylogenetic tree was constructed on the basis of the neighbour-joining method (Saitou & Nei, 1987); evolutionary distances were estimated by the method of Jukes & Cantor (1969) using MEGA version 2.1 (Kumar *et al.*, 2001).

DNA base composition (G+C content) was determined by HPLC after hydrolysis as described by Tamaoka & Komagata (1984) and non-methylated λ DNA (Sigma) was used as a reference standard. DNA-DNA hybridization to determine genomic relatedness was performed fluorometrically by the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and microdilution wells.

Six strains formerly classified as *C. meningosepticum* and the type strain of *C. miricola* formed visible colonies (diameter of 1.0–1.5 mm) on nutrient agar within 24 h. Good growth was observed on TSA and nutrient agar at 28–37 °C, but no growth was observed at 5 or 42 °C after

Table 2. Characteristics that differentiate *Elizabethkingia* gen. nov. from the type strains of the CBR branch

Species: 1, *E. meningoseptica* (n=5); 2, *E. miricola* (n=2); 3, *C. balustinum*; 4, *C. defluvii*; 5, *C. gleum*; 6, *C. indologenes*; 7, *C. indoltheticum*; 8, *C. joostei*; 9, *C. scophthalmum*; 10, *B. zoohelcum*; 11, *R. anatipestifer*; 12, *R. columbina*. +, Positive; v, variable; –, negative; n, number of strains tested.

Characteristics	1	2	3	4	5	6	7	8	9	10	11	12
G + C content (mol%)	37.2±0.6	35.3±0.3	34.7	38.8	37.0	37.6	33.8	36.7	34.1	35.2	35.0	36.0
Flexirubin-type pigment	–	–	+	+	+	+	+	+	+	–	–	–
Indole production	+	v ^a	+	+	+	+	+	+	–	–	–	–
H ₂ S production	–	–	–	–	–	–	+	–	–	–	–	–
Citrate utilization	v ^b	+	–	–	+	–	–	–	–	–	–	–
Malonate utilization	–	–	–	–	–	+	–	–	–	–	–	–
Nitrate reduction	–	–	+	–	–	–	–	–	–	–	–	–
Acid production from:†												
Arabinose	–	–	–	–	+	–	–	–	–	–	–	–
Cellobiose	–	–	–	+	–	–	–	–	–	–	–	–
Fructose	+	+	+	+	+	+	–	+	–	–	–	–
Glucose	+	+	+	+	+	+	+	+	–	–	+	+
Glycerol	+	v ^c	–	+	+	–	–	+	–	–	–	–
Lactose	+	+	–	–	–	–	–	–	–	–	–	–
Maltose	+	+	–	+	+	+	+	+	–	–	+	+
Mannitol	+	+	–	–	–	–	–	+	–	–	–	–
Trehalose	+	+	–	+	+	+	–	+	+	–	–	–
Growth at/on:												
5 °C	–	–	+	–	–	–	+	+	+	–	–	–
37 °C	+	+	–	+	+	+	+	–	–	+	+	+
42 °C	–	–	–	+	+	+	–	–	–	–	+	+
MacConkey agar	v ^d	+	+	–	+	–	+	+	–	–	–	–
Hydrolysis of:†												
Aesculin	+	+	+	+	+	+	+	+	+	–	–	+
Starch	–	–	–	+	+	+	–	+	–	–	–	–
Urea	–	+	+	–	+	+	–	+	+	+	+	+
PNPG‡	+	+	–	–	+	+	–	–	–	–	–	–

*Variable reactions are scored as: a, positive for ATCC 33958; b, positive for ATCC 13254 and ATCC 49470; c, positive for ATCC 33958; d, positive for ATCC 13253^T, ATCC 13255 and ATCC 51720.

†Acid production from raffinose, salicin, sucrose and xylose negative for all strains tested; hydrolysis of casein and gelatin positive for all strains tested.

‡PNPG, *p*-nitrophenyl-β-D-galactopyranoside.

2 weeks. Most strains could grow on MacConkey agar. Colonies were white–yellow, translucent and shiny with entire edges, becoming mucoid after 3 days incubation. Flexirubin-type pigment was not detected and acid was produced from lactose, in contrast to other *Chryseobacterium* species. Nitrate was not reduced as an electron acceptor and malonate was not utilized as a carbon source. As determined with the API ZYM system, a wide spectrum of substrates could be hydrolysed. Physiological and biochemical characteristics that differentiate these strains from the type strains of the CBR branch are summarized in Table 2 and Table 3.

The major quinone of the seven strains, as well as the type strains of the CBR branch, was menaquinone MK-6. The fatty acid profiles of the seven strains showed significant

differences from those of the type strains of the CBR branch (Table 4). The fatty acids 15:0 iso, 17:0 iso 3-OH and summed feature 4 (15:0 iso 2-OH and/or 16:1ω7*clt*) were predominant and only the *C. defluvii* type strain showed similar profiles within the CBR branch.

The almost-complete 16S rRNA gene sequences of the seven strains were compared with those of representatives within the family *Flavobacteriaceae*. They showed 90.7–93.9% similarity to the type strains of the CBR branch, and had quite high similarity (97.8–100%) with each other. In the phylogenetic tree (Fig. 1) they formed a clade that was distinct from related genera within the family *Flavobacteriaceae* and that could be divided into two different groups: a cluster of five strains (ATCC 13253^T, ATCC 13254, ATCC 13255, ATCC 49470 and ATCC 51720), including the

Table 3. API ZYM profiles of seven strains of *Elizabethkingia* gen. nov. and type strains of the CBR branch

Species: 1, *E. meningoseptica* ATCC 13253^T; 2, *E. meningoseptica* ATCC 13254; 3, *E. meningoseptica* ATCC 13255; 4, *E. meningoseptica* ATCC 49470; 5, *E. meningoseptica* ATCC 51720; 6, *E. miricola* DSM 14571^T; 7, *E. miricola* ATCC 33958; 8, *C. balustinum*; 9, *C. defluvii*; 10, *C. gleum*; 11, *C. indologenes*; 12, *C. indoltheticum*; 13, *C. joostei*; 14, *C. scophthalmum*; 15, *B. zoohelcum*; 16, *R. anatispestifer*; 17, *R. columbina*.

Substrate*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
2-Naphthyl butyrate	−†	−	−	−	−	+	+	−	−	−	−	−	−	+	−	+	+
L-Cystyl-2-naphthylamide	+	+	+	+	−	+	+	−	−	−	+	+	+	+	−	+	+
N-Benzoyl-DL-arginine-2-naphthylamide	+	+	+	+	+	+	+	−	−	−	−	−	+	+	−	−	−
N-Glutaryl-phenylalanine-2-naphthylamide	+	−	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−
6-Bromo-2-naphthyl- α -D-galactopyranoside	+	−	+	+	+	−	+	−	−	−	−	−	−	−	−	−	−
2-Naphthyl- β -D-galactopyranoside	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−
2-Naphthyl- α -D-glucopyranoside	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
6-Bromo-2-naphthyl- β -D-glucopyranoside	−	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−
1-Naphthyl-N-acetyl- β -D-glucosaminide	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−
2-Naphthyl- α -L-fucopyranoside	+	+	−	+	−	+	+	−	−	−	−	−	−	−	−	−	−

*All the organisms showed positive reactions for 2-naphthyl phosphate (pH 8.5), 2-naphthyl caprylate, L-leucyl-2-naphthylamide, L-valyl-2-naphthylamide, 2-naphthyl phosphate (pH 5.4) and naphthol-AS-BI-phosphate, and showed negative reactions for 2-naphthyl myristate, naphthol-AS-BI- β -D-glucuronide and 6-bromo-2-naphthyl- α -D-mannopyranoside.

†Colour intensity was measured on a scale from 0 to 5, and interpreted as negative for values between 0 and 1, and positive for values between 2 and 5 (Mudarris *et al.*, 1994).

C. meningosepticum type strain, showing 98.2–100 % 16S rRNA gene sequence similarity with each other; and a cluster of two strains (DSM 14571^T and ATCC 33958), including

the *C. miricola* type strain, showing 99.5 % similarity with each other. Five strains of *C. meningosepticum* showed 97.8–98.4 % similarities to two strains of *C. miricola*.

Table 4. Cellular fatty acids of *Elizabethkingia* gen. nov. and type strains of the CBR branch

Species: 1, *E. meningoseptica* ($n=5$); 2, *E. miricola* ($n=2$); 3, *C. balustinum*; 4, *C. defluvii*; 5, *C. gleum*; 6, *C. indologenes*; 7, *C. indoltheticum*; 8, *C. joostei*; 9, *C. scophthalmum*; 10, *B. zoohelcum*; 11, *R. anatispestifer*; 12, *R. columbina*. n , Number of strains tested. Fatty acids are listed using standard abbreviations (no. of carbon atoms: no. of double bonds). Fatty acids that account for less than 1.0 % of the total in all strains studied are not shown. Therefore, the percentages do not total 100 %. Means $\pm$ SD are given where appropriate. tr, Trace (less than 1.0 %); ND, not detected; ECL, equivalent chain-length (i.e. the identity of the fatty acid is unknown).

Composition	1	2	3	4	5	6	7	8	9	10	11	12
13:0 iso	1.3 $\pm$ 0.3	2.0 $\pm$ 0.5	1.2	3.0	ND	ND	ND	1.5	1.0	4.5	13.3	11.7
Unknown ECL 13:566	1.9 $\pm$ 0.3	1.5 $\pm$ 0.2	1.4	tr	1.5	3.3	1.4	1.3	2.8	1.4	1.7	2.1
15:0 iso	43.9 $\pm$ 2.0	46.4 $\pm$ 2.2	36.8	56.3	35.6	35.1	34.6	36.8	37.1	60.8	53.9	47.8
15:0 iso 3-OH	2.8 $\pm$ 0.3	3.0 $\pm$ 0.6	2.7	2.6	2.5	2.7	2.2	2.9	3	3.7	8.4	3.6
15:0 anteiso	1.1 $\pm$ 0.8	1.0 $\pm$ 0.6	1.1	2.5	ND	ND	6.3	ND	tr	ND	6.2	21.7
16:0	tr	1.2 $\pm$ 0.1	1.4	1.3	1.3	1.1	1.2	tr	1.0	tr	tr	tr
16:0 3-OH	2.6 $\pm$ 0.4	3.0 $\pm$ 0.6	1.2	tr	1.2	1.1	1.0	tr	1.1	ND	ND	ND
16:0 iso 3-OH	tr	tr	ND	ND	ND	ND	tr	ND	ND	ND	tr	tr
Unknown ECL 16:580	1.6 $\pm$ 0.1	1.3 $\pm$ 0.6	1.0	tr	1.4	1.1	tr	tr	tr	tr	tr	ND
17:0 2-OH	ND	ND	ND	ND	ND	ND	2.2	ND	ND	ND	ND	1.1
17:0 iso	tr	tr	1.0	2.1	1.5	tr	tr	tr	tr	ND	ND	ND
17:0 iso 3-OH	14.6 $\pm$ 1.0	15.3 $\pm$ 0.2	16.3	15.9	20.8	18.2	13.2	18.7	17.1	9.0	14.1	7.4
17:1 iso ω 9c	7.8 $\pm$ 1.3	6.6 $\pm$ 0.2	27.5	4.8	20.2	22.9	24.1	22.2	24.5	10.2	ND	ND
18:1 ω 5c	tr	tr	ND	ND	ND	tr	tr	tr	ND	tr	ND	ND
Summed feature 4*	19.6 $\pm$ 1.0	17.0 $\pm$ 1.3	8.4	9.4	14.0	12.4	10.9	12.8	10.3	6.5	ND	3.1
Summed feature 5*	tr	ND	ND	ND	ND	Tr	ND	ND	ND	1.6	ND	ND

*Summed feature 4 contains 15:0 iso 2-OH and/or 16:1 ω 7c/t. Summed feature 5 contains 17:1 iso I and/or 17:1 anteiso B.

Fig. 1. 16S rRNA gene sequence dendrogram obtained by distance matrix (neighbour-joining) analysis, showing the positions of the seven strains of *Elizabethkingia* gen. nov. Species of some genera within the family *Flavobacteriaceae* were used to define the root. Numbers at branching points refer to bootstrap values (1000 resamplings, only values above 50% are shown). Bar, 2 substitutions per 100 nucleotide positions. Abbreviations: *C.*, *Chryseobacterium*; *B.*, *Bergeyella*; *E.*, *Elizabethkingia*; *R.*, *Riemerella*.

The level of DNA–DNA hybridization between strain ATCC 33958 and the *C. miricola* type strain (84%) demonstrated that the former belongs to *C. miricola* and not to *C. meningosepticum* as assumed previously (Stackebrandt & Goebel, 1994) and in turn these two *C. miricola* strains showed levels of DNA–DNA hybridization of 23–54% to five strains of *C. meningosepticum*. By contrast, *C. meningosepticum* ATCC 13253^T showed a DNA–DNA hybridization level of only 31–35% to the remaining four strains of *C. meningosepticum*, levels among these latter four being 90–100%. This genetic heterogeneity using DNA–DNA hybridization was reported by Ursing & Bruun (1987). In their studies, [*Flavobacterium*] *meningosepticum* could be divided into two genomic groups; group I (including the type strain) showed about 40–55% DNA–DNA hybridization to group II. Subsequent studies investigating the phenotypic characterization and antimicrobial susceptibility of [*F.*] *meningosepticum* showed no characteristics differentiating the two genomic groups (Bruun & Ursing, 1987; Bruun, 1987). We detected no phenotypic or ecological differences among the five *C. meningosepticum* strains and therefore a proposal for a novel binomial name for the second genomovar within this species is not warranted.

Combined phylogenetic and phenotypic data show that *C. meningosepticum* and *C. miricola* should be transferred to a new genus, *Elizabethkingia* gen. nov., with the names *Elizabethkingia meningoseptica* comb. nov. and *Elizabethkingia miricola* comb. nov. proposed.

Description of *Elizabethkingia* gen. nov.

Elizabethkingia (E.liz.a.beth.kin'gi.a. N.L. fem. n. *Elizabethkingia* in honour of Elizabeth O. King, who first described bacteria associated with infant meningitis, notably [*Flavobacterium*] *meningosepticum* in 1959).

Cells are Gram-negative, non-motile, non-spore-forming rods (0.5 × 1.0–2.5 µm). Good growth is observed on TSA and nutrient agar at 28–37 °C, but no growth is observed at 5 or 42 °C. Colonies are white–yellow, non-pigmented, semi-translucent, circular and shiny with entire edges. Catalase, oxidase, phosphatase and β-galactosidase activities are positive. H₂S is not produced. Casein, aesculin and gelatin are hydrolysed, but starch is not. Malonate is not utilized. Nitrate is not reduced. Acid is produced from D-fructose, D-glucose, lactose, D-maltose, D-mannitol and trehalose, but not from L-arabinose, D-cellobiose, raffinose, sucrose, salicin or D-xylose. As determined with the API ZYM system, the following substrates are hydrolysed: 2-naphthyl phosphate (pH 8.5), 2-naphthyl caprylate, L-leucyl-2-naphthylamide, L-valyl-2-naphthylamide, N-benzoyl-DL-arginine-2-naphthylamide, 2-naphthyl phosphate (pH 5.4), naphthol-AS-BI-phosphate, 2-naphthyl α-D-glucopyranoside and 1-naphthyl-N-acetyl-β-D-glucosaminide, but the following substrates are not hydrolysed: 2-naphthyl myristate, naphthol-AS-BI-β-D-glucuronide, 6-bromo-2-naphthyl-β-D-glucopyranoside and 6-bromo-2-naphthyl-α-D-mannopyranoside. The fatty acid profile consists largely of 15:0 iso, 17:0 iso 3-OH and summed feature 4 (15:0 iso 2-OH and/or 16:1ω7*cl*). Menaquinone MK-6 is the predominant quinone. The G + C content of the DNA is 35.0–38.2 mol%.

The type species is *Elizabethkingia meningoseptica*.

Description of *Elizabethkingia meningoseptica* comb. nov.

Elizabethkingia meningoseptica (me.nin.go.sep'ti.ca. Gr. n. *meninx*, *meninges* meninges, membrane covering the brain; Gr. adj. *septikos* putrefactive; N.L. fem. adj. *meningoseptica* apparently referring to association of the bacterium with

both meningitis and septicaemia, but not septic meningitis as the name implies).

Basonym: *Flavobacterium meningosepticum* King 1959 (Approved Lists 1980).

Cells are Gram-negative, non-motile, non-spore-forming rods ($0.5 \times 1.0\text{--}2.0\ \mu\text{m}$). Growth on MacConkey agar is strain-dependent. Indole is produced. Urea is not hydrolysed. Acid is produced from D-fructose, ethanol, D-glucose, glycerol, lactose, D-maltose, D-mannitol and trehalose, but not from L-arabinose, D-cellobiose, raffinose, sucrose, salicin or D-xylose. As determined with the API ZYM system, the following substrates are hydrolysed: 2-naphthyl phosphate (pH 8.5), 2-naphthyl caprylate, L-leucyl-2-naphthylamide, L-valyl-2-naphthylamide, N-benzoyl-DL-arginine-2-naphthylamide, 2-naphthyl phosphate (pH 5.4), naphthol-AS-BI-phosphate, 2-naphthyl- α -D-glucopyranoside and 1-naphthyl-N-acetyl- β -D-glucosaminide, but the following substrates are not hydrolysed: 2-naphthyl butyrate, 2-naphthyl myristate, naphthol-AS-BI- β -D-glucuronide, 6-bromo-2-naphthyl- β -D-glucopyranoside and 6-bromo-2-naphthyl- α -D-mannopyranoside. The fatty acid profile consists largely of 15:0 iso ($43.9 \pm 2.0\%$), 17:0 iso 3-OH ($14.6 \pm 1.0\%$) and summed feature 4 (15:0 iso 2-OH and/or 16:1 ω 7c/t; $19.6 \pm 1.0\%$). The G + C content of the DNA is $37.2 \pm 0.6\%$ (37.1% for the type strain).

The type strain is ATCC 13253^T (=NCTC 10016^T = LMG 12279^T = CCUG 214^T).

Description of *Elizabethkingia miricola* comb. nov.

Elizabethkingia miricola [mi.ri'co.la. N.L. neut. n. *mirum* derived from *mir* (peace) (name of Russian space station); L. suff. -*cola* from L. masc. or fem. n. *incola* inhabitant; N.L. masc. or fem. n. *miricola* inhabitant of the Mir space station].

Basonym: *Chryseobacterium miricola* Li *et al.* 2004.

Cells are Gram-negative, non-motile, non-spore-forming rods ($0.5 \times 1.0\text{--}2.5\ \mu\text{m}$). Good growth is observed on MacConkey agar. Colonies are very sticky on solid medium. Indole is produced. Urea is hydrolysed. Acid is produced from D-fructose, D-glucose, lactose, D-maltose, D-mannitol and trehalose, but not from L-arabinose, D-cellobiose, raffinose, sucrose, salicin or D-xylose. As determined with the API ZYM system, the following substrates are hydrolysed: 2-naphthyl phosphate (pH 8.5), 2-naphthyl butyrate, 2-naphthyl caprylate, L-leucyl-2-naphthylamide, L-valyl-2-naphthylamide, L-cystyl-2-naphthylamide, N-benzoyl-DL-arginine-2-naphthylamide, 2-naphthyl phosphate (pH 5.4), naphthol-AS-BI-phosphate, 2-naphthyl- α -D-glucopyranoside, 1-naphthyl-N-acetyl- β -D-glucosaminide and 2-naphthyl- α -L-fucopyranoside, but the following substrates are not hydrolysed: 2-naphthyl myristate, N-glutaryl-phenylalanine-2-naphthylamide, naphthol-AS-BI- β -D-glucuronide, 6-bromo-2-naphthyl- β -D-glucopyranoside and 6-bromo-2-naphthyl- α -D-mannopyranoside. The fatty acid profile

consists largely of 15:0 iso ($46.4 \pm 2.2\%$), 17:0 iso 3-OH ($15.3 \pm 0.2\%$) and summed feature 4 (15:0 iso 2-OH and/or 16:1 ω 7c/t; $17.0 \pm 1.3\%$). The G + C content of the DNA is $35.3 \pm 0.3\%$ (35.0% for the type strain).

The type strain is DSM 14571^T (=JCM 11413^T = GTC 862^T).

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References

- 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 hydati* nom. nov. (basonym, *Cytophaga aquatilis* Strohl and Tait 1978). *Int J Syst Bacteriol* **46**, 128–148.
- Bruun, B. (1987). Antimicrobial susceptibility of *Flavobacterium meningosepticum* strains identified by DNA-DNA hybridization. *Acta Pathol Microbiol Immunol Scand [B]* **95**, 95–101.
- Bruun, B. & Ursing, J. (1987). Phenotypic characterization of *Flavobacterium meningosepticum* strains identified by DNA-DNA hybridization. *Acta Pathol Microbiol Immunol Scand [B]* **95**, 41–47.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Fautz, E. & Reichenbach, H. (1980). A simple test for flexirubin-type pigments. *FEMS Microbiol Lett* **8**, 87–91.
- 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.
- Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.
- Hugo, C. J., Segers, P., Hoste, B., Vancanneyt, M. & Kersters, K. (2003). *Chryseobacterium joostei* sp. nov., isolated from the dairy environment. *Int J Syst Evol Microbiol* **53**, 771–777.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- 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., Dreyer, U., Neef, A., Dott, W. & Busse, H.-J. (2003). *Chryseobacterium defluvii* sp. nov., isolated from wastewater. *Int J Syst Evol Microbiol* **53**, 93–97.
- King, E. O. (1959). Studies on a group of previously unclassified bacteria associated with meningitis in infants. *Am J Clin Pathol* **31**, 241–247.
- Klatte, S., Rainey, F. A. & Kroppenstedt, R. M. (1994). Transfer of *Rhodococcus aichiensis* Tsukamura 1982 and *Nocardia amarae* Lechevalier and Lechevalier 1974 to the genus *Gordona* as *Gordona aichiensis* comb. nov. and *Gordona amarae* comb. nov. *Int J Syst Bacteriol* **44**, 769–773.

- Kroppenstedt, R. M. (1982).** Separation of bacterial menaquinones by HPLC using reverse phase (RP-18) and a silver loaded ion exchanger. *J Liquid Chromatogr* **5**, 2359–2367.
- Kroppenstedt, R. M. (1985).** Fatty acid and menaquinone analysis of actinomycetes and related organisms. In *Chemical Methods in Bacterial Systematics*, no. 20 SAB Technical Series, pp. 173–199. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- Kumar, S., Tamura, K., Jakobsen, I. B. & Nei, M. (2001).** MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics* **17**, 1244–1245.
- Li, Y., Kawamura, K., Fujiwara, N., Naka, T., Liu, H., Huang, X., Kobayashi, K. & Ezaki, T. (2003).** *Chryseobacterium miricola* sp. nov., a novel species isolated from condensation water of space station Mir. *Syst Appl Microbiol* **26**, 523–528.
- 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.
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990).** *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Stackebrandt, E. & Goebel, B. M. (1994).** Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Tamaoka, J. & Komagata, K. (1984).** Determination of DNA base composition by reverse-phased high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- 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 tools. *Nucleic Acids Res* **25**, 4876–4882.
- Tindall, B. J. (1990).** A comparative study of the lipid composition of *Halobacterium saccharovororum* from various sources. *Syst Appl Microbiol* **13**, 128–130.
- Ursing, J. & Bruun, B. (1987).** Genetic heterogeneity of *Flavobacterium meningosepticum* demonstrated by DNA-DNA hybridization. *Acta Pathol Microbiol Immunol Scand [B]* **95**, 33–39.
- 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.
- 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.
- Yoon, J.-H., Lee, S. T., Kim, S.-B., Kim, W. Y., Goodfellow, M. & Park, Y.-H. (1997).** Restriction fragment length polymorphism analysis of PCR-amplified 16S ribosomal DNA for rapid identification of *Saccharomonospora* strains. *Int J Syst Bacteriol* **47**, 111–114.