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Flavobacterium salmonis sp. nov. isolated from Atlantic salmon (*Salmo salar*) and formal proposal to reclassify *Flavobacterium spartansii* as a later heterotypic synonym of *Flavobacterium tructae*

Peter Kämpfer^{1,*}, Rute Irgang², Stefanie P. Glaeser¹, Hans-Jürgen Busse³, Alexis Criscuolo⁴, Dominique Clermont⁵ and Ruben Avendaño-Herrera^{2,6,7,*}

Abstract

A Gram-staining-negative non endospore-forming strain, T13(2019)^T was isolated from water samples from Atlantic salmon (*Salmo salar*) fry culture in Chile and studied in detail for its taxonomic position. The isolate shared highest 16S rRNA gene sequence similarities with the type strains of *Flavobacterium chungangense* (98.44%) followed by *Flavobacterium tructae* and *Flavobacterium spartansii* (both 98.22%). Menaquinone MK-6 was the predominant respiratory quinone in T13(2019)^T. Major polar lipids were phosphatidylethanolamine, an ornithine lipid and the unidentified polar lipids L1, L3 and L4 lacking a functional group. The major polyamine was *sym*-homospermidine. The fatty acid profile contained major amounts of iso-C_{15:0}, iso-C_{15:0} 3-OH, iso-C_{17:0} 3-OH, C_{15:0}, summed feature 3 (C_{16:1} ω7c and/or iso-C_{15:0} 2-OH) and various hydroxylated fatty acids in smaller amounts, among them iso-C_{16:0} 3-OH, and C_{15:0} 3-OH, which supported the grouping of the isolate into the genus *Flavobacterium*. Physiological/biochemical characterisation and ANI calculations with the type strains of the most closely related species allowed a clear phenotypic and genotypic differentiation. In addition it became obvious, that the type strains of *F. tructae* and *F. spartansii* showed 100% 16S rRNA gene sequence similarities and ANI values of 97.21%/ 97.59% and DDH values of 80.40% [77.5 and 83%]. These data indicate that *F. tructae* and *F. spartansii* belong to the same species and it is proposed that *F. spartansii* is a later heterotypic synonym of *F. tructae*. For strain T13(2019)^T (=CIP 111411^T=LMG 30298^T=CCM 8798^T) a new species with the name *Flavobacterium salmonis* sp. nov. is proposed.

The genus *Flavobacterium* comprises a very diverse group of short to long bacterial rods forming different tones of yellow colonies that have been isolated from various sources, occurring from warm to polar locations, algae, mammals, diseased fish, soil, fresh to marine waters [1] and at the time of writing 226 species with validly published names (<https://lpsn.dsmz.de/genus/flavobacterium>). Among the *Flavobacterium* species affecting fish, these have been isolated from fish culturing systems such as lakes, ponds and fish farms [2–5]. The most studied species of this genus have been first described three

decades ago such as *F. psychrophilum*, *F. columnare* and *F. brachiophilum* which cause severe diseases in a wide range of different fish species worldwide (see review of Bernardet and Bowman [6]). In this study, a new *Flavobacterium*-like bacterium was characterized by a polyphasic taxonomic approach and a novel species of the genus *Flavobacterium* is proposed.

Strain T13(2019)^T was isolated from water samples obtained from Atlantic salmon (*Salmo salar*) fry cultured in Chile in November 2013. The aim of the sampling was to identify

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Keywords: *Flavobacterium*; *salmonis*; taxonomy.

Abbreviations: ANI, Average Nucleotide Identity; *is*DDH, *in silico* DNA–DNA hybridization; oNP, ortho-nitrophenyl; pNP, para-nitrophenyl.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain T13(2019)^T is MN594468. Genome Shotgun project of strains T13(2019)^T and *Flavobacterium chungangense* CJ7^T (CIP110025^T) have been deposited at DDBJ/ENA/GenBank under the accessions CAIJDP00000000 and CAIJDP00000000.

One supplementary table and one supplementary figure are available with the online version of this article.

the dominant bacterial species in the water column of the Atlantic salmon fry culturing farm (S 35°45'0" W 71° 0'0") at the time. Serial dilutions of water samples were made on tryptone yeast extract salts (TYES) agar plates [7] and a total bacterial load of 4.1×10^4 colony forming units per ml was obtained. In the same sample, four dominant bacterial species were observed and identified by 16S rRNA sequencing and according to the closest similar as belonging to *Arthrobacter phenanthrenivorans* (99.42%), *Brevundimonas staleyi* (98.48%), *Planomicrobium chinense* (99.79%) and strain T13(2019)^T as *Flavobacterium chungangense* (98.44%). An isolated orange and translucent small (2–3 mm in diameter) colony designated T13(2019)^T was streaked onto fresh TYES plates, purified and stored at –80 °C for further studies. Several bacterial culture media were tested such as nutrient agar (Oxoid), tryptone soya agar (TSA, Difco), Columbia agar with 5% defibrillated sheep blood (bioMérieux), R2A (Oxoid) and Mueller-Hinton (Oxoid), Marine agar 2216 (Difco) and Thiosulfate-citrate-bile salts-sucrose (Oxoid) agars. Growth at different temperatures (4, 11, 20, 25, 30, 37 and 45 °C), different salinity ranges (0.5–8.0% NaCl, w/v) and pH ranges (4.5, 5.5, 6.5, 8.5, 9.5 and 10.5) were tested with TSA plates and TYES broth employed as basal medium. Strain T13(2019)^T was routinely grown on TSA and TYES media incubated at 25 °C for 48 h for these studies.

Gram-staining was performed using the modified Hucker and Conn method as described by Gerhardt *et al.* [8]. The cell morphology and gliding motility were recorded with a Zeiss light microscope at 1000-fold magnification using cells grown for 24 h at 25 °C TSA and in TYES broth, respectively. Presence of cell wall-associated flexirubin type pigments and absorption of Congo red was determined according to Bernardet *et al.* [9]. Catalase (3% H₂O₂ v/v, Merck) and oxidase activity (BD oxidase agent dropper) were also tested.

Physiological characterization of the strains and additional biochemical tests were performed as described previously [10]. The following additional biochemical tests were performed: the production of hydrogen sulphide, and triple-sugar-iron methods; indole reaction with Ehrlich's and Kovac's reagents; arginine and lysine dihydrolase and ornithine decarboxylase. Hydrolysis of DNase (Liofilchem), casein (1% w/v), gelatin (2% w/v), starch (0.4% w/v), Tween 80 (1% v/v) and tyrosine (0.4% w/v) [10] were tested by TYES plate assays incubated at 25 °C for a week. Also, the miniaturized galleries API 20NE and API ZYM (bioMérieux) were prepared according to the manufacturer's instructions. The arginine dihydrolase, β-galactosidase (ONPG) and urease results were recorded from the API 20NE kit. Results of the comparative physiological characterization for the most closely related *Flavobacterium* species are given in Table 1 and in the species descriptions.

The 16S rRNA gene sequence of strain T13(2019)^T was analysed as described previously [11]. PCR-amplification of the 16S rRNA gene was carried out with primers 27F (5'-GAGTTTGATCMTGGCTCAG-3') and 1492R (5'-ACGGYTACCTTGTACGACTT-3') [12]. The 16S rRNA gene

Table 1. Long-chain fatty acid composition (%) of strain T13(2019)^T and type strains of related *Flavobacterium* species. TR, traces (<1%) and –, not detected

Strains: 1, T13(2019)^T; 2, *F. chungangense* KACC 13353^T. All data from this study were obtained from cells grown on TSA at 28 °C for 24 h.

Fatty acid	1	2
Unknown 11.543*	0.9	2.0
C _{12:1} AT 11–12	–	TR
C _{13:1} AT 12–13	0.8	TR
Unknown 13.566*	–	5.3
iso C _{14:0}	–	TR
C _{14:0}	0.8	TR
Iso-C _{15:1} G	2.8	2.2
Iso-C _{15:0}	23.6	24.4
Anteiso-C _{15:0}	3.1	2.3
C _{15:1} ω6c	1.6	6.2
C _{15:0}	5.9	13.5
Iso-C _{16:1} h	–	1.0
Iso-C _{16:0}	–	TR
C _{16:0}	8.4	2.4
Iso-C _{15:0} 3-OH	7.9	6.6
C _{15:0} 3-OH	1.2	2.2
iso C _{17:1} ω9c	4.9	3.7
Unknown 16.580*	–	TR
Iso-C _{17:0}	1.4	TR
C _{17:1} ω8c	0.7	2.0
C _{17:1} ω6c	1.2	4.7
Iso-C _{16:0} 3-OH	1.1	1.6
C _{16:0} 3-OH	2.8	1.0
Iso-C _{17:0} 3-OH	11.7	7.4
C _{18:1} ω5c	0.7	–
C _{17:0} 2-OH	0.7	TR
C _{17:0} 3-OH	–	TR
Summed feature 1 †	–	TR
Summed feature 2 †	1.3	TR
Summed feature 3 †	12.3	5.3

*Unknown fatty acids; numbers indicate the equivalent chain length

†Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 1 comprised C_{13:0} 3-OH and/or iso-C_{15:1} h; Summed feature 2 comprised iso-C_{16:1} l and/or C_{14:0} 3-OH; summed feature 3 comprised C_{16:1} ω7c and/or C_{16:1} ω6c.

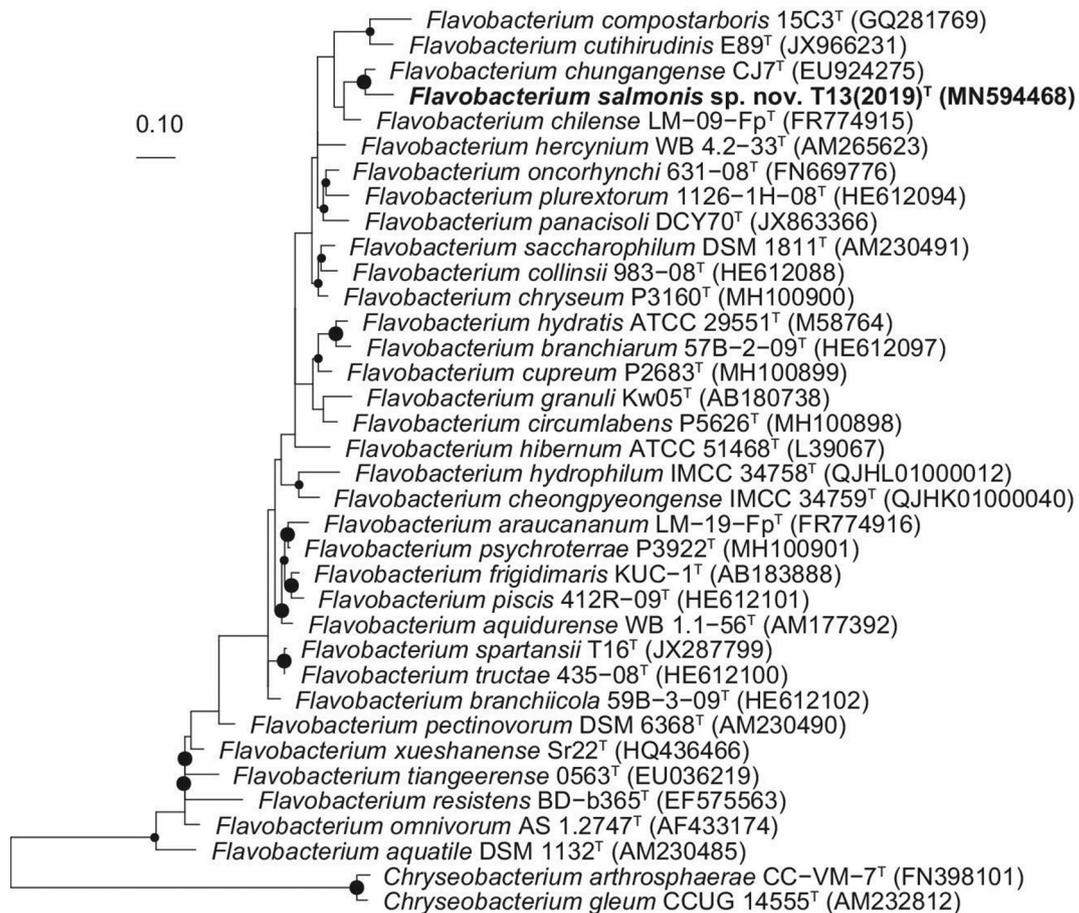


Fig. 1. Phylogenetic tree showing the phylogenetic relationship of strain T13(2019)^T to all closest related *Flavobacterium* type strains. The tree represents a consensus tree based on the Maximum-likelihood, Neighbour-joining, and Maximum-parsimony trees calculated in ARB using the LPTs 132 database. Circles at nodes indicate which nodes were present in two (small circles) or three (large circles) of the three trees. Bar: 0.01 nucleotide substitutions per nucleotide position.

was sequenced in two Sanger sequencing reactions using primers 27F and E786F (5'-GATTAGATACCCTGGTAG-3') as sequencing primers. Partial sequences were manually corrected based on the electropherograms and merged to a continuous sequence stretch using MEGA 7. The sequenced 16S rRNA gene fragment of strains T13(2019)^T represents a continuous stretch of 1430 nt spanning 16S rRNA gene positions 47 to 1505 (numbered according to the *E. coli rrnB* [13]). Next related type strains were determined using the EzBio-Cloud 16S database including 16S rRNA gene sequences of type strains of all validated bacterial species [14].

Phylogenetic trees were calculated using the software package ARB release 5.2 [15] and the corresponding 'All species living tree project' (LTPs [16]; database release LTPs132 [17]). Type strain sequences not yet included in the database were implemented into the database using the quick add mode after alignment with the SILVA Incremental Aligner (SINA; v1.2.11 [18]). The alignment including all type strain 16S rRNA gene sequence of the genus *Flavobacterium* and the required outgroup type strain was checked manually. Phylogenetic trees were calculated with the maximum-likelihood

method using RAxML version 7.04 [19] with GTR-GAMMA and rapid bootstrap analysis, the maximum-parsimony method using DNAPARS v 3.6 [20], and the neighbour-joining method using ARB Neighbour-joining and the Jukes-Cantor correction [21]. The phylogenetic analysis based on 16S rRNA gene sequences between gene sequence positions 84 and 1400 (numbered according to the *E. coli rrnB* numbering [13]) which was covered by all *Flavobacterium* type strain 16S rRNA gene sequences. The phylogenetic trees based on 100 re-samplings (bootstrap analysis [22]). A consensus tree was generated from the three phylogenetic trees using the respective ARB tool [15].

Strain T13(2019)^T shared highest 16S rRNA gene sequence similarity with the type strains of *Flavobacterium chungangense* (98.44%) followed by *Flavobacterium tructae* and *Flavobacterium spartansii* (both 98.22%); sequence similarities to two further type strains were also above 98.0%, *Flavobacterium plurextorum* and *Flavobacterium pectinovorum*. All respective species were considered in the phylogenetic analysis. Strain T13(2019)^T clustered without high bootstrap

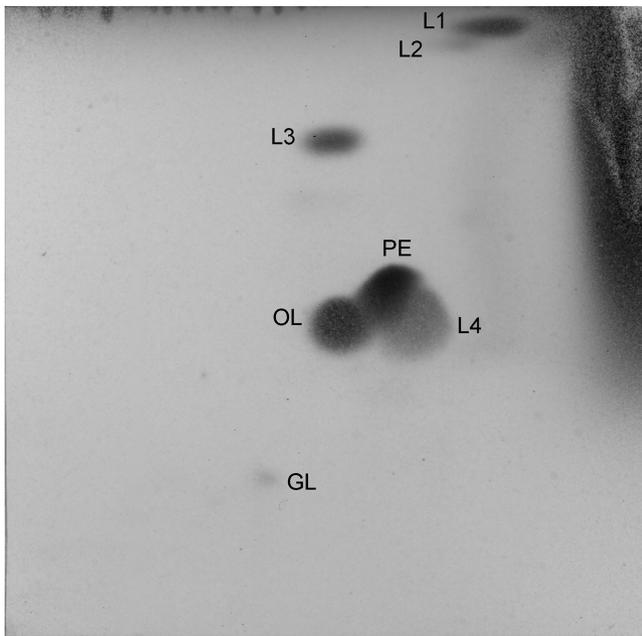


Fig. 2. Two-dimensional thin layer chromatogram showing the total polar lipids of strain T13(2019)^T. Lipids were visualized by spraying with ethanolic (5%) molybdato-phosphoric acid and developing at 160 °C. PE, phosphatidylethanolamine; OL, ornithine lipid; L1-L4, unidentified polar lipids lacking a functional group; GL, unidentified glycolipid.

support but in all calculated trees with the type strain of *F. chungangense* (Fig. 1)

The whole genome sequencing of strains T13(2019)^T and *F. chungangense* CJ7^T was carried out with a NextSeq 500 instrument using the Nextera XT DNA library preparation kit (Illumina) and a 2×150 bp paired-end protocol, leading to 4040711 read pairs (201× average sequencing depth, 323 bp average insert size) for strain T13(2019)^T, and 1256559 read pairs (69× average sequencing depth, 384 bp average insert size) for strain CJ7^T. Sequenced reads were trimmed and clipped using AlienTrimmer [23], corrected using musket [24], and next assembled using SPAdes [25]. The draft genome of strain T13(2019)^T has a total size of 5862835 bps, represented in 138 scaffolds, with L50 and N50 values of 8 and 250437 bps, respectively, and a G+C content of 33.71%. Scaffold sequences were processed with Prokka [26] for gene prediction and annotation, which led to the detection of 5030 coding genes, 59 tRNAs and three rRNAs.

The average nucleotide identity (ANI) was estimated using fastANI [27] between the genome assemblies of strains T13(2019)^T and *F. chungangense* CJ7^T, but also against all the publicly available *Flavobacterium* type strain genome sequences. As expected, the most closely related type strain genome were those of *F. chungangense* CJ7^T (95.19%), followed by *F. limi* THG-AG6^T (84.75%) (Table S1, Fig. S1, available in the online version of this article). Corresponding pairwise *in silico* DNA–DNA hybridization (*is*DDH) percentages were 58.50% [55.7, 61.3] and 28.20% [25.8, 30.7], respectively

(formula two with confidence interval [28]). Although the estimated ANI against *F. chungangense* CJ7^T is quite close to the 95% species delineation cut-off value, the DDH measures (i.e. both estimate and confidence interval) are below the associated 70% cut-off.

In the course of the genome sequence comparisons (Fig. S1), it was shown, that the type strains of *F. tractae* and *F. spartansii* shared 100% 16S rRNA gene sequence similarities and ANI values of 97.21% and 97.59% and *is*DDH values of 80.40% [77.5%, 83%].

For biomass production of strain T13(2019)^T subjected to analyses of quinones, polar lipids, and polyamine cells were grown in PYE broth (0.3% peptone from casein, 0.3% yeast extract, pH 7.2) at 28 °C with shaking. Biomass used for extraction of quinones and polar lipids was harvested at the stationary growth phase whereas biomass used to extract polyamines was harvested at the late exponential growth phase.

Quinones and polar lipids were extracted applying the integrated procedure reported by Tindall [29, 30] and Altenburger *et al.* [31]). HPLC equipment was described by Stolz *et al.* [32]. Strain T13(2019)^T revealed a quinone system containing exclusively MK-6 which is in line with the description of the family *Flavobacteriaceae* [33]. Strain T13(2019)^T exhibited a polar lipid profile consisting of the major lipids phosphatidylethanolamine, an ornithine lipid and unidentified polar lipids L3 and L4 lacking a functional group (Fig. 2). Additionally, moderate to minor amounts of unidentified lipids L1 and L2 and a glycolipid were detected.

Polyamines were extracted and derivatized as reported by Busse and Auling [34] and analysed according to Busse *et al.* [35]. In the polyamine pattern of strain T13(2019)^T *sym*-homospermidine [32.0 μmol (g dry weight)⁻¹], spermidine [7.35 μmol (g dry weight)⁻¹], spermine [6.2 μmol (g dry weight)⁻¹], cadaverine [3.1 μmol (g dry weight)⁻¹] and putrescine [0.8 μmol (g dry weight)⁻¹] were detected. A polyamine pattern with the predominant polyamine *sym*-homospermidine was also listed as a characteristic in the description of the family *Flavobacteriaceae* [33].

Fatty acid analysis was performed on strain T13(2019)^T and on the type strain of *F. chungangense* KACC 13353^T (see Table 2) according to Kämpfer and Kroppenstedt [36] after growth on TSA at 28 °C for 24 h using a HP gas chromatograph HP 6890 with a Sherlock MIDI software version 2.11. Identification of the fatty acids was performed with the TSBA peak naming table version 4.1.

The fatty acid profile of strain T13(2019)^T was similar to those of *Flavobacterium* spp. reference strains (Table 2). They showed a typical, complex fatty acid profile consisting of major amounts of iso-C_{15:0}, iso-C_{15:0} 3-OH, iso-C_{17:0} 3-OH, C_{15:0}, summed feature 3 (C_{16:1} ω7c and/or iso-C_{15:0} 2-OH) and various hydroxylated fatty acids in smaller amounts, among them iso-C_{16:0} 3-OH and C_{15:0} 3-OH.

Table 2. Physiological results and differentiating features of strains *F. salmonis* T13(2019)^T and the type strains of the most closely related *Flavobacterium* species

Strains: 1, T13(2019)^T; 2, *F. chungangense* KACC 13353^T and 3, *F. tractae* 435-08^T and 4, *F. spartansii* T16^T; Data for taxa 1 and 2 from this study. ND, Not determined; (+), weakly positive.

Strain	1	2	3	4*
Origin	Fish culture water sample	Freshwater lake sample	Diseased rainbow trout	Diseased feral Chinook salmon
Year of isolation	2013	2009	2014	2014
Country	Chile	South Korea	Spain	USA
Flexirubin	+	–*	+*	+
Acid produced from				
Sucrose	+	+	–	–
D-Mannitol	–	–	–	–
L-Arabinose	–	+	–	–
Maltose	(+)	+	+	+
D-Xylose	–	–	–	–
Trehalose	–	–	+	+
Cellobiose	–	–	+	+
D-Mannose	–	+	+	v
Hydrolysis of				
oNP-β-D-galactopyranoside	(+)	+	–	ND
pNP-β-D-glucuronide	–	–	–	ND
pNP-phosphoryl-choline	–	–	+	ND
Assimilation of:				
N-acetyl-D-galactosamine	–	–	(+)	+
N-acetyl-D-glucosamine	–	–	+	ND
L-Arabinose	+	–	–	–
D-Fructose	–	+	–	–
Gluconate	–	–	–	–
Sucrose	+	–	–	–
Trehalose	–	–	+	ND
D-Xylose	–	–	–	ND
L-Malate	–	+	–	ND
Pyruvate	–	–	–	ND
L-Alanine	–	–	–	ND
L-Ornithine	–	–	–	–
Other physiological tests*				
Gliding motility	+	–	–	+
Temperature growth range (°C)	10–30	5–35	4–30	4–22
pH tolerance range	6.5–9.5	5–8	6.5–9.0	6–8
NaCl tolerance range (%)	0–1	0–4	0–1	0–2

Continued

Table 2. Continued

Strain	1	2	3	4*
Nitrate reduction	+	–	+	–
Lipase (C14)	+	–	–	–

*Data obtained from Kim et al. [40] for *F. chungangense*, Zamora et al. [3] for *F. tructae* and Loch & Faisal [4] for *F. spartansii* T16^T.

Fatty acid profiles as well as 16S rRNA gene sequence analyses show unambiguously that the strain T13(2019)^T is affiliated with the genus *Flavobacterium*.

On the basis of the observed phenotypic differences, the results of the ANI calculations and the differences in 16S rRNA gene sequences, we propose a novel *Flavobacterium* species.

DESCRIPTION OF FLAVOBACTERIUM SALMONIS SP. NOV.

Flavobacterium salmonis (sal.mo'nis. L. gen. n. *salmonis* of salmon, since it was first discovered in salmon).

Cells are Gram-staining-negative, gliding, strictly aerobic long rods, 0.8–1.0 µm in diameter and 2.0–3.0 µm in length. Colonies grown on tryptone soy agar are circular, convex and yellowish and on TYES agar plates are orange, circular and translucent colonies and do not adhere to agar medium. The flexirubin test is positive and Congo red is not absorbed. Optimal temperature for growth is 25 °C but growth is observed at 10–30 °C but not at 4 and 36 °C. Oxidase and catalase activities are positive.

Good growth occurs on plates prepared with marine agar, nutrient agar, tryptone soy agar, Columbia agar (no haemolysis production), R2A, and Mueller-Hinton, but not on thiosulfate-citrate-bile salts-sucrose. Cells grow in the presence of 0–1% NaCl, regardless of the culture media employed in this test. Optimal pH for growth is 7–8 on TSA and 5–7 in TYES broth; growth occurs at pH 6.5–8.5 and 5–9 in TSA and TYES broth, respectively. Growth occurs on casein plates. Hydrolysis of aesculin, gelatine and amylase production are observed, but neither Tween 80 hydrolysis nor DNase production. The strain produces diffuse agar brown pigmentation after 4–5 d of incubation on L-tyrosine agar plates.

Acid is produced from D-glucose, maltose, cellobiose (weak) and sucrose, but not from L-arabinose, lactose, trehalose, D-xylose, D-mannitol, dulcitol, salicin, D-adonitol, inositol, sorbitol, raffinose, rhamnose, methyl-D-glucoside, erythritol, melibiose and D-arabitol. No H₂S- and indole production, and also no activities of urease, arginine-dihydrolase, lysine-decarboxylase, and ornithine-decarboxylase are observed. L-Arabinose (weak), cellobiose (weak), D-glucose, maltose, rhamnose and sucrose are utilized as carbon sources. The utilization of N-acetyl-D-galactosamine, p-arbutin, N-acetyl-D-glucosamine, gluconate, D-fructose,

D-galactose, D-mannitol, D-mannose, melibiose, D-ribose, sorbitol, trehalose, D-xylose, pyruvate, acetate, cis-aconitate, adipate, D-adonitol, inositol, azelate, L-alanine, L-ornithine β-alanine, L-aspartate, citrate, fumarate, glutarate, L-histidine, 3-hydroxybenzoate, 4-hydroxybenzoate, DL-3-hydroxybutyrate, 4-hydroxybutyrate, L-leucine, L-malate, D-maltitol, mesaconate, oxoglutarate, phenylacetate, propionate, putrescine, L-phenylalanine, suberate and L-tryptophane are negative, according to the method of Kämpfer et al. [37]. Hydrolyses aesculin, oNP-β-D-galactopyranoside (oNP=ortho-nitrophenyl), pNP-alpha-D-glucopyranoside, pNP-β-D-glucopyranoside, and L-glutamate-gamma-3-carboxy-pNA. pNP-β-D-glucuronide (pNP=para-nitrophenyl), L-alanine-pNA (pNA=para-nitroanilide), p-phosphoryl-choline, Bis-pNP-phosphate, pNP-phenylphosphonate and L-proline-pNA. pNP-β-D-xyloside are not hydrolysed.

Major polyamine is *sym*-homospermidine. In the quinone system MK-6 is predominant. Major polar lipids are phosphatidylethanolamine, ornithine lipid and the unidentified polar lipids L1, L3 and L4 lacking a functional group. Moderate to minor amounts of unidentified lipid L2 and a glycolipid are present, as well.

Major fatty acids are iso-C_{15:0}, iso-C_{15:0} 3-OH, iso-C_{17:0} 3-OH, C_{15:0}, summed feature 3 (C_{16:1} ω7c and/or iso-C_{15:0} 2-OH). Various hydroxylated fatty acids in smaller amounts, among them iso-C_{16:0} 3-OH, C_{16:0} 3-OH and C_{15:0} 3-OH are detected.

The type strain T13(2019)^T (=CIP 111411^T=LMG 30298^T=CCM 8798^T) was isolated from Atlantic salmon (*Salmo salar*) fry culture in Chile.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain T13(2019)^T is MN594468. Genome Shotgun project of strain T13(2019)^T has been deposited at DDBJ/ENA/GenBank under the accession number CAIJDJP00000000.

In addition, we propose the reclassification of *Flavobacterium spartansii* as a heterotypic synonym of *Flavobacterium tructae* on the basis of whole genome sequence comparisons and phenotypic properties.

The 16S rRNA sequences of *F. tructae* (HE612100) and *F. spartansii* (JX287799) revealed identity (100%) and ANI values of the genome sequences gave similarities of 97.21%/97.59%, respectively, and *is*DDH values of 80.40% [77.5%, 83%].

A comparison of phenotypic traits (Table 1) also showed high similarities and in many cases identical results between both strains.

Flavobacterium tructae and *Flavobacterium spartansii* were both proposed in 2014 by Zamora *et al.* [3], and Loch *et al.* [4] respectively. Both names appear in the same notification list of validated names [38].

The close genomic relationship was already detected by Garcia-López *et al.* [39] who emended the description of *F. spartansii*. However, a formal proposal was not made at this time.

Here we propose that *Flavobacterium spartansii* is a heterotypic synonym of *Flavobacterium tructae*. The description is that of Zamora *et al.* [3]. Accession numbers of the genome are given by Garcia-López *et al.* [39]. Strain T16^T (=LMG 27337^T=ATCC BAA-2541^T) is an additional strain of *Flavobacterium tructae*.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethical statement

The authors certify that the manuscript does not proclaim any work which requires approval by ethic committee(s).

References

- Bernardet J-F, Bowman JP. Genus I. *Flavobacterium*. In: Krieg NR, Staley JT, Brown DR, Hedlund BP, Paster BJ *et al.* (editors). *Bergey's Manual of Systematic Bacteriology*, vol. 4, 2nd ed. New York: Springer-Verlag; 2011. pp. 112–154.
- Kämpfer P, Lodders N, Marti K, Avendaño-Herrera R. *Flavobacterium chilense* sp. nov. and *Flavobacterium araucanum* sp. nov., isolated from farmed salmonid fish. *Int J Syst Evol Microbiol* 2012;62:1402–1408.
- Zamora L, Vela AI, Sanchez-Porro C, Palacios MA, Moore ER *et al.* *Flavobacterium tructae* sp. nov. and *Flavobacterium piscis* sp. nov., isolated from farmed rainbow trout (*Oncorhynchus mykiss*). *Int J Syst Evol Microbiol* 2014;64:392–399.
- Loch TP, Faisal M. *Flavobacterium spartansii* sp. nov., a pathogen of fishes, and emended descriptions of *Flavobacterium aquidunense* and *Flavobacterium araucanum*. *Int J Syst Evol Microbiol* 2014;64:406–412.
- Chen WM, Guo YP, Kwon SW, Sheu SY. *Flavobacterium piscinae* sp. nov., isolated from a fish pond. *Int J Syst Evol Microbiol* 2019;69:1775–1782.
- Bernardet JF, Bowman JP. Genus *Flavobacterium*. *Bergey's Manual of Systematics of Archaea and Bacteria*; 2015.
- Holt RA, Rohovec JS, Fryer JL. Bacterial cold water. In: Inglis V, Roberts RJ, Bromage NR (editors). *Bacterial Diseases of Fish*. Oxford: Blackwell Scientific Publication; 1993. pp. 3–23.
- Gerhardt P, Murray RGE, Wood WA, Krieg NR. *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology; 1994.
- Bernardet JF, Nakagawa Y, Holmes B. Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *Int J Syst Evol Microbiol* 2002;52:1049–1070.
- Smbert RM, Krieg NR. Phenotypic characterization. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (editors). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology; 1994. pp. 607–654.
- Kämpfer P, Dreyer U, Neef A, Dott W, Busse H-J. *Chryseobacterium defluvii* sp. nov., isolated from wastewater. *Int J Syst Evol Microbiol* 2003;53:93–97.
- Lane DJ. 16S/23S rRNA sequencing nucleic acid techniques in bacterial systematics. In: Stackebrandt E, Goodfellow M (editors). *Nucleic Acid Techniques in Bacterial Systematics*. New York: Wiley; 1991. pp. 115–175.
- Brosius J, Dull T, Sleeter D, Noller H. Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*. *J Mol Biol* 1978;148:107–127.
- Yoon SH, SM H, Kwon S, Lim J, Kim Y *et al.* Introducing EzBioCloud: a taxonomically United database of 16S rRNA and whole genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.
- Ludwig W, Strunk O, Westram R, Richter L, Meier H *et al.* ARB: a software environment for sequence data. *Nucleic Acid Res* 2004;32:1363–1371.
- Yilmaz P, Parfrey Wegener L, Yarza P, Gerken J, Pruesse E *et al.* The SILVA and “All-species Living Tree Project (LTP)” taxonomic frameworks. *Nucleic Acids Res*. 2014;42:D643–D648.
- Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W *et al.* SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acid Res* 2012;35:7188–7196.
- Yarza P, Richter M, Peplies J, Euzebey J, Amann R *et al.* SINA: accurate high throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 2012;28:1823–1829.
- Stamatakis A. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 2006;22:2688–2690.
- Felsenstein J. *PHYLIP (Phylogeny Inference Package) version 3.6*. Distributed by the author. Seattle: Department of Genome Sciences, University of Washington; 2005.
- Jukes TH, Cantor CR. Evolution of the protein molecules. In: Munro HN (editor). *Mammalian Protein Metabolism*. New York: Academic Press; 1969. pp. 21–132.
- Felsenstein J. Confidence limits of phylogenies: an approach using the bootstrap. *Evolution* 1985;39:783–791.
- Crisuolo A, Brisse S. AlienTrimmer: a tool to quickly and accurately trim off multiple short contaminant sequences from high-throughput sequencing reads. *Genomics* 2013;102:500–506.
- Liu Y, Schöder J SB. Musket: a multistage *k*-mer spectrum-based error corrector for Illumina sequence data. *Bioinformatics* 2013;29:308–315.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M *et al.* SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012;19:455–477.
- Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 2014;30:2068–2069.
- Jain C, Rodriguez-R LM, Phillippy AM, Konstantinidis KT, Aluru S. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. *Nat Commun* 2018;9:5114.
- Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.
- Tindall BJ. Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* 1990;66:199–202.
- Tindall BJ. A comparative study of the lipid composition of *Halobacterium saccharovororum* from various sources. *Syst Appl Microbiol* 1990;13:128–130.

31. Altenburger P, Kämpfer P, Makristathis A, Lubitz W, Busse H-J. Classification of bacteria isolated from a medieval wall painting. *J Biotechnol* 1996;47:39–52.
32. Stolz A, Busse H-J, Kämpfer P. *Pseudomonas knackmussii* sp. nov. *Int J Syst Evol Microbiol* 2007;57:572–576.
33. Bernardet JF, Segers P, Vancanneyt M, Berthe F, Kersters K et al. 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 Evol Microbiol* 1996;46:128–148.
34. Busse H-J, Auling G. Polyamine pattern as a chemotaxonomic marker within the Proteobacteria. 1988. *Syst Appl Microbiol* 1988;11:1–8.
35. Busse H-J, Bunka S, Hensel A, Lubitz W. Discrimination of members of the family *Pasteurellaceae* based on polyamine patterns. *Int J Syst Evol Microbiol* 1997;47:698–708.
36. Kämpfer P, Kroppenstedt RM. Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* 1996;42:989–1005.
37. Kämpfer P, Steiof M, Dott W. Microbiological characterisation of a fuel-oil contaminated site including numerical identification of heterotrophic water and soil bacteria. *Microb Ecol* 1991;21:227–251.
38. Oren A, Garrity GM, IJSEM list. Notification list. Notification that new names and new combinations have appeared in volume 64, part 2 of the IJSEM. *Int J Syst Evol Microbiol* 2014;64:1459–1460.
39. Garcia-Lopez M, Meier-Kolthoff JP, Tindall BJ, Gronow S, Woyke T et al. Analysis of 1000 type-strain genomes improves taxonomic classification of *Bacteroidetes*. *Front Microbiol* 2019;10:10.
40. Kim JH, Kim YK, Cha CJ. *Flavobacterium chungangense* sp. nov., isolated from a freshwater lake. *Int J Syst Evol Microbiol* 2009;59:1754–1758.

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