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# The *gyrB* gene is a useful phylogenetic marker for exploring the diversity of *Flavobacterium* strains isolated from terrestrial and aquatic habitats in Antarctica.

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## Abstract

Within the phylum *Bacteroidetes*, the *gyrB* gene, encoding for the B subunit of the DNA gyrase, has been used as phylogenetic marker for several genera closely related to *Flavobacterium*. The phylogenies of the complete 16S rRNA gene and the *gyrB* gene were compared for thirty-three Antarctic *Flavobacterium* isolates and twenty-three type strains from closely related *Flavobacterium* species. *GyrB* gene sequences provided a higher discriminatory power to distinguish between different *Flavobacterium* groups than 16S rRNA gene sequences. The *gyrB* gene is therefore a promising molecular marker for elucidating the phylogenetic relationships among *Flavobacterium* species and should be evaluated for all the other type strains of described *Flavobacterium* species. Combining the phylogeny of both genes, the new Antarctic *Flavobacterium* strains constitute fifteen *Flavobacterium* groups, including at least thirteen potentially new species together with one group of isolates probably belonging to the species *F. micromati* and one group close to *F. gelidilacus*.

Keywords: *gyrB* gene, 16S rRNA gene, *Flavobacterium*, molecular marker, diversity

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## 1. Introduction

Heterotrophic bacterial communities in Antarctica are highly diverse in aquatic (Bowman et al., 2000; Van Trappen et al., 2002) as well as in terrestrial (Aislabie et al., 2006; Babalola et al., 2009) habitats. A genus that has been isolated often from these environments is *Flavobacterium* (Brambilla et al., 2001; Humphry et al., 2001; Van Trappen et al., 2002) and several novel *Flavobacterium* species were described from Antarctic habitats (*F. gelidilacus*, *F. gillisiae*, *F. hibernum*, *F. micromati*, *F. psychrolimnae*, *F. xanthum*) or other cold environments (*F. xinjangense* and *F. omnivorum*). Other *Flavobacterium* species have been mainly isolated from freshwater fish (*F. branchiophilum*, *F. columnare*, *F. psychrophilum*), temperate freshwater (*F. aquatile*, *F. flevense*, *F. saccharophilum*) and from soil (*F. johnsoniae*, *F. pectinovorum*). Most *Flavobacterium* species are psychrotolerant and as they are able to hydrolyse several carbohydrates and biomacromolecules such as gelatine, casein and starch, they might be of biotechnological importance (Bernardet and Bowman, 2006).

The family *Flavobacteriaceae* (phylum *Bacteroidetes*) as well as the genus *Flavobacterium* have been revised and added to repeatedly over the years (Vandamme et al., 1994; Bernardet et al., 1996; Bernardet et al., 2002). *Flavobacterium* was created in 1923 for all bacteria that formed yellow or orange pigmented colonies and weakly produced acid from carbohydrates (Bergey et al., 1923). This broadly defined and taxonomically heterogeneous group was further refined using phenotypic characteristics (Holmes et al., 1984) and the determination of guanine plus cytosine (G+C) content (Reichenbach, 1989). The introduction of 16S rRNA oligonucleotide catalog (Paster et al., 1985), DNA-rRNA hybridisation data (Bauwens and De Ley, 1981; Segers et al., 1993; Vandamme et al., 1994) and sequence data (Woese et al., 1990; Gherna and Woese, 1992) changed the family and the genus further and provided the framework for the present classification. Currently, strains are assigned to the genus *Flavobacterium* (including 71 species to date) based on fatty acid analysis, G+C content and a number of morphological and phenotypical characteristics following the proposal of Bernardet et al. (1996) in combination with 16S rRNA gene sequence analysis (Bernardet et al., 2002; Bernardet and Bowman, 2006).

Although DNA-DNA hybridisations (DDH) are the golden standard for species identification (Stackebrandt et al., 2002), these experiments are technically challenging, laborious and time-consuming. Sequence analysis of 16S rRNA genes is used for prokaryotic classification (Rossello-Mora and Amann, 2001) to provide a tentative identification. It can often limit the number of DDH experiments required. Nevertheless, the 16S rRNA gene has a limited resolving power at species level (Fox et al., 1992; Probst et al., 1998). Within the genus *Flavobacterium*, values of 97.2– 98.7% 16S rRNA sequence similarity are found between distinct *Flavobacterium* species (Bernardet and Bowman, 2006). As protein-encoding genes evolve faster, they are considered more appropriate for phylogenetic analysis of closely related species. Within the genus *Flavobacterium*, protein-encoding genes have not yet been used for detailed phylogenetic study. The *gyrB* gene was found to be a successful marker for phylogenetic analysis in several groups in other phyla e.g. *Acinetobacter* (*Proteobacteria*) (Yamamoto and Harayama, 1996) and *Micromonospora (Actinobacteria)* (Kasai et al., 2000), but also in the phylum *Bacteroidetes* in the genus *Marinilabilia* and related taxa (Suzuki et al., 1999). In these studies, phylogenetic analysis based on the *gyrB* gene sequences was shown to be consistent with DNA-DNA hybridization and phenotypic comparison (Yamamoto and Harayama, 1996). Suzuki et al (2001) applied *gyrB* gene sequencing to study the phylogenetic relationships of marine isolates within the phylum *Bacteroidetes* and included two *Flavobacterium* species. In addition, more *gyrB* sequences from *Flavobacterium* species are becoming available in the frame of genome projects (Duchaud et al., 2007).

In a previous study of aquatic and terrestrial microbial mats in Antarctica, several *Flavobacterium* strains were isolated that showed low similarity with described *Flavobacterium* species, based on the partial or full 16S rRNA gene sequences (Peeters et al., submitted). In the present study, we determined the *gyrB* gene sequence of thirty-three of these new Antarctic isolates and of the type strains of related *Flavobacterium* species to study the diversity of our isolates in more detail and to elucidate the usefulness of *gyrB* as a phylogenetic marker for phylogeny in the genus *Flavobacterium*. We also compared with the phylogeny based on the near complete 16S rRNA gene sequences.

## 2. Methods

### 2.1 Strains used

The *Flavobacterium* strains studied here (Table 1) were obtained as part of a large study into the diversity of heterotrophic bacteria in microbial mats from Antarctica (Peeters et al., submitted). The samples used in that study originated from a terrestrial sample, taken in the close neighbourhood of the Princess Elisabeth Station in Utsteinen, Dronning Maud Land (Peeters et al., 2011a), and microbial mat samples from lakes in the Transantarctic Mountains (Peeters et al., 2011b), the Schirmacher Oasis and on Pourquoi-Pas Island (Antarctic Peninsula) (for details see Table 1). In these previous studies, isolates were first grouped by rep-PCR fingerprinting and representatives of all rep-types were tentatively identified by full or partial 16S rRNA gene sequencing (Peeters et al., 2011a & 2011b; Peeters et al., submitted). Several of these strains were identified as *Flavobacterium* and thirty-three of them were used in this study (Table 1). To elucidate their phylogenetic relationships, type strains of closely related *Flavobacterium* species were also included (Table 2).

**2.2 16S rRNA gene sequence analysis**

The complete 16S rRNA gene sequences of four Antarctic *Flavobacterium* isolates were available from previous studies (Peeters et al., 2011a & 2011b). The 16S rRNA genes of the remaining twenty-nine Antarctic *Flavobacterium* isolates were only partially sequenced (400 bp) (Peeters et al., submitted). These sequences were completed in this study (accession numbers listed in Table 1) using the same method as described before (Vancanneyt et al., 2004). A multiple sequence alignment of all complete 16S rRNA gene sequences was made using BioNumerics (v 5.1.) software package (Applied-Maths) and a region of 912 bp, containing good sequence data for all strains, was delimited for further analysis. After visual inspection, distances were calculated using the Kimura-2 correction. A neighbour joining dendrogram (Saitou and Nei, 1987) was constructed and bootstrapping analysis was performed using 500 bootstrap replicates. A maximum likelihood dendrogram was calculated by the program PhyML (Guindon and Gascuel, 2003). The reliability of the tree was checked using the approximate Likelihood Ratio Test method (aLRT) (Anisimova and Gascuel, 2006).

**2.3. *GyrB* gene sequence analysis**

For *Flavobacterium johnsoniae*, *F. aquatile* and *Myroides odoratus* the *gyrB* sequences were available in the EMBL database (Table 2). For the other strains used, the *gyrB* sequences were determined in this study. DNA preparation was carried out as described by Baele et al. (2003). Primers were designed in Kodon 3.5 using all available *gyrB* sequences from *Flavobacterium* and species from closely related genera (*Bacteroides*, *Cytophaga*, *Flexibacter*, *Terrimonas*, *Porphyrobacter*, *Parabacteroides*, *Salinibacter* and *Prevotella*) in the EMBL database (Sept. 2009). A *gyrB* segment of about 1200 bp long was obtained with primers *gyrB*-241F (5'-GAYACCGGWCGTGGTATTCC-3') and *gyrB*-1588R (5'TCDAYATCGGCATCACACAT-3') which were used both for amplification and sequencing reactions. For amplification, the reaction mix (50 µl) consisted of 5 µl GeneAmp® 10x PCR buffer (Applied Biosystems), 5 µl dNTP's (2mM), 0.5 µl of the forward and reverse primer (50 µM), 1 µl Taq Polymerase (1 U/µl), 33 µl MilliQ water and 5 µl template DNA. After an initial denaturation step (95°C for 5 min), 3 cycles of pre-amplification (95°C for 1 min, 55°C for 2 min 15 sec and 72°C for 1 min 15 sec) and 25 cycles of amplification (95°C for 35 sec, 55°C for 1 min 15 sec and 72°C for 1 min 15 sec) were performed, finishing with 72°C for 7 min. PCR products were purified using a Nucleofast 96 PCR clean up membrane system (Machery-Nagel, Germany) and a Tecan Workstation 200. The sequencing PCR is performed as described before (Vancanneyt et al., 2004). Sequence assembly and phylogenetic analysis was performed with the BioNumerics (v 5.1.) software package (Applied-Maths) using a region of 1006 bp, containing good sequence data for all strains. The multiple alignment was verified by comparison with an alignment of the corresponding aminoacids. After visual inspection of the sequence alignments, distances were calculated using the Kimura-2 correction. A neighbour joining dendrogram (Saitou and Nei, 1987) was constructed and bootstrapping analysis was performed using 500 bootstrap replicates. A maximum likelihood dendrogram was calculated by the program PhyML (Guindon and Gascuel, 2003). The reliability of the tree was checked using the approximate Likelihood Ratio Test method (aLRT) (Anisimova and Gascuel, 2006). Accession numbers of the *gyrB* gene sequence of the *Flavobacterium* strains and the type strains of the *Flavobacterium* species are listed in Table 1 and 2, respectively.

## 3. Results and Discussion

158 This study was set out to resolve the relationships of thirty-tree Antarctic *Flavobacterium*
159 strains that were previously characterized by partial 16S rRNA gene sequencing and found
160 to represent several potentially novel groups. We completed the 16S rRNA gene sequences
161 for all strains and performed a phylogenetic analysis including also the type strains of
162 twenty-three related or Antarctic *Flavobacterium* species. Neighbour joining and maximum
163 likelihood trees (Fig. 1 and S1) showed a similar topology with the *Flavobacterium* isolates
164 forming fifteen groups, labelled *Flavobacterium* sp. 1 to 15. *Flavobacterium* sp. 13 and
165 *Flavobacterium* sp. 5 were located close to, respectively, *F. micromati* and *F. gelidilacus* with
166 99.8 and 99.0% sequence similarity to the respective type strain. It is well known that
167 because of its high conservation, the 16S rRNA gene sequence has limited resolving power
168 at species level (Rossello-Mora and Amann, 2001). Indeed, there are examples of distinct
169 species with identical or nearly identical 16S rRNA gene sequences (Fox et al., 1992; Probst
170 et al., 1998), micro heterogeneity of the 16S rRNA genes within one species (Bennasar et al.,
171 1996) or single organisms with two or more 16S rRNA genes with relatively high sequence
172 divergence (Nübel et al., 1996). In the genus *Flavobacterium*, several new species have been
173 described with rather high 16S rRNA gene sequence similarity e.g. the type strains of
174 *F. weaverense* and *F. segetis* share 98.9% 16S rRNA gene sequence similarity, yet they have
175 a DNA-DNA hybridization value of only 34 % (Yi and Chun, 2006). Because protein-encoding
176 genes are generally less conserved (Ochman and Wilson, 1987), they may be more
177 appropriate for phylogenetic analysis of closely related species. Several protein-encoding
178 genes such as *glnA*, *recA* and *hsp60* have been used for typing and taxonomical purposes
179 within genera in the *Bacteroidetes* (Gutacker et al., 2002; Sakamoto et al., 2010). In this
180 study, the *gyrB* gene, encoding for the B subunit of the DNA gyrase was selected because it
181 was previously used successfully to distinguish between closely related taxa affiliated with
182 the genus *Flavobacterium* (Suzuki et al., 1999, 2001). Izumi et al (2003) reported on the use
183 of *gyrB* primers in a PCR-RFLP analysis for the genotyping of *Flavobacterium psychrophilum*
184 and Suzuki et al (1999) designed *gyrB* primers to study the phylogenetic relationship for the
185 genus *Marinilabilia* (*Bacteroidetes*) and related taxa. We tested all primers reported in these
186 studies *in silico* on the *gyrB* sequences available from related genera and from the complete
187 genome of *Flavobacterium johnsoniae* DSM 2064 and found considerable mismatches with
188 all groups included in the comparison. Therefore, more general primers were designed
189 based on the available sequence information.

As expected for a more variable housekeeping gene, the distance between the *Flavobacterium* groups and the type strains is significantly higher in the *gyrB* gene dendrogram (Fig. 2, S2) in comparison with the 16S rRNA gene dendrogram (Fig. 1, S1, Table 3)). The threshold for species definition has been suggested to be 98.7 to 99.0% 16S rRNA gene sequence similarity by Stackebrandt and Ebers (2006) whereas for the *gyrB* phylogeny this is less well documented. Suzuki et al (2001) reported that the proposed limit for species identity, the 70% DNA reassociation value corresponds with 88.8% *gyrB* sequence similarity in the subset of the *Bacteroidetes* they studied, whereas several other studies revealed a wide range of interspecies similarity values (60.0-89.0% *gyrB* gene sequence similarity within the genus *Helicobacter* (*Epsilonproteobacteria*) (Hannula and Hanninen, 2007), 75.4-95.0% within the genus *Bacillus* (*Firmicutes*) (Wang et al., 2007), 85.0-97.5% within the genus *Aeromonas* (*Gammaproteobacteria*) (Yanez et al., 2003), 77.5-97.6% within the genus *Gordonia* (*Actinobacteria*) (Kang et al., 2009), 89.5-98.2% within the genus *Kribbella (Actinobacteria*) (Kirby et al., 2010), and 70.1-98.7% within the genus *Streptococcus* (*Firmicutes*) (Itoh et al., 2006)). Among the type strains of the *Flavobacterium* species investigated in this study, the interspecies *gyrB* sequence similarity values varied from 79.1% between *F. aquatile* and *F. reichenbachii* to 94.9% between *F. xanthum* and *F. omnivorum* .

The phylogenetic trees based on the *gyrB* sequences (Fig. 2, S2) show that the groups found in the 16S rRNA gene dendrogram (Fig. 1, S1) were confirmed. The Antarctic *Flavobacterium* groups generally showed lower *gyrB* gene sequence similarity with neighbouring groups and species which confirmed their status as potentially new species. *Flavobacterium* sp. 13 and sp. 5, which in the 16S rRNA gene phylogeny were closely related to *F. micromati* and *F. gelidilacus*, respectively, also group with these species in the *gyrB* phylogeny. Both groupings are well supported, however, the *gyrB* similarity of *Flavobacterium* sp. 13 to *F. micromati* LMG 21919 (97.0%) is higher than that of *Flavobacterium* sp. 5 to *F. gelidilacus* LMG 21477 (91.9%). *Flavobacterium* sp. 13 probably belongs to *F. micromati* that was originally isolated from microbial mats in Antarctic lakes (Van Trappen et al., 2004) as were the isolates of *Flavobacterium* sp. 13 (Table 1). *Flavobacterium* sp. 5 probably represents a new species in view of the rather low *gyrB* gene sequence similarity with *F. gelidilacus* in comparison with the higher similarity values obtained between some type strains.

Nevertheless, the precise relation to *F. gelidilacus*, another species from Antarctic microbial mats (Van Trappen et al., 2003), remains to be investigated further.

The similarities within the delineated *Flavobacterium* groups are generally very high for the 16S rRNA gene sequences (Table 3). The *gyrB* sequences were mostly also very similar within groups and ranged from 97.2 to 100% (Table 3). In *Flavobacterium* sp. 2, sp. 8 and sp. 13 (Fig. 2, S2) subclusters were observed with 97.2 to 99.0% sequence similarity. In other genera, comparable high intraspecies *gyrB* gene sequence similarities were observed, e.g. 98.5-100% *gyrB* gene sequence similarity within the genus *Streptomyces* (*Actinobacteria*) (Hatano et al., 2003), 97.4-100% within the genus *Aeromonas* (*Gammaproteobacteria*) (Yanez et al., 2003), 95.0-100% within the genus *Bacillus* (*Firmicutes*) (Wang et al., 2007) and 94.6-100% within the genus *Helicobacter* (*Epsilonproteobacteria*) (Hannula and Hanninen, 2007).

It should be noted that all *Flavobacterium* groups studied here, comprised several rep-types (Peeters et al., submitted) and the strains were chosen to represent this diversity. The topology of the neighbour joining and the maximum likelihood dendrogram were slightly different for the 16S rRNA gene compared with the *gyrB* gene (Fig. 1, 2, S1 and S2), as has been observed also for other groups (Yamamoto and Harayama, 1996). However, overall, the phylogeny of the 16S rRNA (Fig. 1, S1) and *gyrB* (Fig. 2, S2) gene were similar and confirmed the division of the Antarctic strains in fifteen groups, one probably belonging to *F. micromati* and one close to *F. gelidilacus*. The other thirteen *Flavobacterium* groups formed separate groups in both the 16S rRNA gene and the *gyrB* gene phylogeny and probably represent new species. However, additional characterisation is necessary to confirm this and to describe them as new species.

In conclusion, this study showed that within the genus *Flavobacterium*, the *gyrB* gene has a higher discriminatory power than the 16S rRNA gene. In comparison with the 16S rRNA gene sequence, the sequence similarities for the *gyrB* gene between the delineated groups are significantly lower whereas inside the different groups they are still very high. Although there are differences in topology in the dendrograms based on either gene, the same groups of Antarctic *Flavobacterium* strains were recovered. Thus, the *gyrB* gene is a promising molecular marker to elucidate the phylogenetic relationships among *Flavobacterium* species and should be evaluated for all the other *Flavobacterium* species described. The phylogeny

252 of both the 16S rRNA gene and the *gyrB* gene, showed that the Antarctic *Flavobacterium*
253 isolates studied here represent at least thirteen potentially new species. These will be
254 studied in more detail with various methods to confirm this and describe these groups
255 appropriately.

256

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440 *omnivorum* sp. nov., novel psychrophiles from the China No. 1 glacier. *Int J Syst Evol Microbiol* **53**:
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447 Table 1 Strain numbers, accession numbers and isolation source of the Antarctic *Flavobacterium* isolates used.
448 The 16S rRNA gene sequences marked with an asterisk were determined in previous studies (Peeters et al.,
449 2011a & 2011b).

| Species | Strain no | Accession no 16S rRNA gene | Accession no *gyrB* gene | Isolation source |
|---|---|---|---|---|
| *Flavobacterium* sp. 1 | R-40838 | FR682718* | FR772324 | terrestrial microbial mat, Utsteinen nunatak, Antarctica |
| | R-40949 | FR772055 | FR772296 | terrestrial microbial mat, Utsteinen nunatak, Antarctica |
| *Flavobacterium* sp. 2 | R-36233 | FR682719* | FR772292 | terrestrial microbial mat, Utsteinen nunatak, Antarctica |
| | R-36668 | FR772052 | FR772293 | terrestrial microbial mat, Utsteinen nunatak, Antarctica |
| | R-36669 | FR772053 | FR772294 | terrestrial microbial mat, Utsteinen nunatak, Antarctica |
| | R-36523 | FR772054 | FR772295 | terrestrial microbial mat, Utsteinen nunatak, Antarctica |
| *Flavobacterium* sp. 3 | R-41499 | FR772077 | FR772318 | aquatic microbial mat, Schirmacher Oasis, Antarctica |
| *Flavobacterium* sp. 4 | R-38377 | FR772072 | FR772313 | aquatic microbial mat, Pourquoi-Pas Island, Antarctica |
| | R-37599 | FR772073 | FR772314 | aquatic microbial mat, Pourquoi-Pas Island, Antarctica |
| | R-38423 | FR772067 | FR772308 | aquatic microbial mat, Pourquoi-Pas Island, Antarctica |
| | R-40835 | FR772071 | FR772312 | aquatic microbial mat, Pourquoi-Pas Island, Antarctica |
| | R-36964 | FR691441* | FR772322 | aquatic microbial mat, Forlidas Pond, Antarctica |
| *Flavobacterium* sp. 5 | R-38388 | FR772056 | FR772297 | aquatic microbial mat, Pourquoi-Pas Island, Antarctica |
| *Flavobacterium* sp. 6 | R-38274 | FR772058 | FR772299 | aquatic microbial mat, Pourquoi-Pas Island, Antarctica |
| | R-38352 | FR772069 | FR772310 | aquatic microbial mat, Pourquoi-Pas Island, Antarctica |
| *Flavobacterium* sp. 7 | R-38477 | FR772059 | FR772300 | aquatic microbial mat, Pourquoi-Pas Island, Antarctica |
| *Flavobacterium* sp. 8 | R-40837 | FR772060 | FR772301 | aquatic microbial mat, Pourquoi-Pas Island, Antarctica |
| | R-38313 | FR772065 | FR772306 | aquatic microbial mat, Pourquoi-Pas Island, Antarctica |
| | R-38503 | FR772061 | FR772302 | aquatic microbial mat, Pourquoi-Pas Island, Antarctica |
| | R-41504 | FR772062 | FR772303 | aquatic microbial mat, Pourquoi-Pas Island, Antarctica |
| *Flavobacterium* sp. 9 | R-38294 | FR772063 | FR772304 | aquatic microbial mat, Pourquoi-Pas Island, Antarctica |
| | R-38296 | FR772064 | FR772305 | aquatic microbial mat, Pourquoi-Pas Island, Antarctica |
| *Flavobacterium* sp. 10 | R-38392 | FR772074 | FR772315 | aquatic microbial mat, Pourquoi-Pas Island, Antarctica |
| *Flavobacterium* sp. 11 | R-37608 | FR772076 | FR772317 | aquatic microbial mat, Pourquoi-Pas Island, Antarctica |
| *Flavobacterium* sp. 12 | R-38474 | FR772057 | FR772298 | aquatic microbial mat, Pourquoi-Pas Island, Antarctica |
| | R-38373 | FR772070 | FR772311 | aquatic microbial mat, Pourquoi-Pas Island, Antarctica |
| *Flavobacterium* sp. 13 | R-40832 | FR772078 | FR772319 | aquatic microbial mat, Forlidas Pond, Antarctica |
| | R-36976 | FR772080 | FR772323 | aquatic microbial mat, Forlidas Pond, Antarctica |
| | R-36963 | FR691440* | FR772321 | aquatic microbial mat, Forlidas Pond, Antarctica |
| | R-36961 | FR772079 | FR772320 | aquatic microbial mat, Forlidas Pond, Antarctica |
| *Flavobacterium* sp. 14 | R-38349 | FR772068 | FR772309 | aquatic microbial mat, Pourquoi-Pas Island, Antarctica |
| *Flavobacterium* sp. 15 | R-38420 | FR772066 | FR772307 | aquatic microbial mat, Pourquoi-Pas Island, Antarctica |
| | R-37612 | FR772075 | FR772316 | aquatic microbial mat, Pourquoi-Pas Island, Antarctica |

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452 Table 2 *Flavobacterium* species included in this study. Accession numbers for newly determined sequences are shown in bold.

| Species | Strain no | Accession no. 16S rRNA gene | Accession no. *gyrB* gene | Isolation source | reference |
|---|---|---|---|---|---|
| *Flavobacterium antarcticum* | LMG 25319$^T$ | FM163401 | **FR774016** | terrestrial sample from the Antarctic | (Yi et al., 2005) |
| *Flavobacterium aquatile* | LMG 4427$^T$ | AM230485 | AB034225 | deep well, Kent, England | (Bernardet et al., 1996) |
| *Flavobacterium degerlachei* | LMG 21915$^T$ | AJ557886 | **FR774017** | microbial mats in Antarctic lakes | (Van Trappen et al., 2004) |
| *Flavobacterium flevense* | LMG 8328$^T$ | D12662 | **FR774018** | freshwater lake, The Netherlands | (Bernardet et al., 1996) |
| *Flavobacterium frigidarium* | LMG 21010$^T$ | AF162266 | **FR774019** | marine sediment, Antarctica | (Humphry et al., 2001) |
| *Flavobacterium frigoris* | LMG 21922$^T$ | AJ557887 | **FR850657** | Microbial mats in Antarctic lakes | (Van Trappen et al., 2004) |
| *Flavobacterium fryxellicola* | LMG 22022$^T$ | AJ811961 | **FR774020** | microbial mats in Antarctic lakes | (Van Trappen et al., 2005) |
| *Flavobacterium gelidilacus* | LMG 21477$^T$ | AJ440996 | **FR774021** | microbial mats in Antarctic lakes | (Van Trappen et al., 2003) |
| *Flavobacterium gillisiae* | LMG 21422$^T$ | U85889 | **FR774014** | Antarctic coastal sea ice | (McCammon and Bowman, 2000) |
| *Flavobacterium glaciei* | LMG 25320$^T$ | DQ515962 | **FR774022** | China No.1 glacier | (Zhang et al., 2006) |
| *Flavobacterium hibernum* | LMG 21424$^T$ | L39067 | **FR774023** | freshwater Antarctic lake | (McCammon et al., 1998) |
| *Flavobacterium johnsoniae* | LMG 1340$^T$ | AM230489 | AB034222 | soil or mud, Rothamsted or Cambridge, England | (Bernardet et al., 1996) |
| *Flavobacterium limicola* | LMG 21930$^T$ | AB075230 | **FR774015** | freshwater sediments | (Tamaki et al., 2003) |
| *Flavobacterium micromati* | LMG 21919$^T$ | AJ557888 | **FR774024** | microbial mats in Antarctic lakes | (Van Trappen et al., 2004) |
| *Flavobacterium omnivorum* | LMG 21986$^T$ | AF433174 | **FR774025** | China No. 1 glacier | (Zhu et al., 2003) |
| *Flavobacterium psychrolimnae* | LMG 22018$^T$ | AJ585428 | **FR774026** | microbial mats in Antarctic lakes | (Van Trappen et al., 2005) |
| *Flavobacterium psychrophilum* | LMG 13179$^T$ | AB078060 | **FR774027** | kidney of salmon | (Bernardet et al., 1996) |
| *Flavobacterium reichenbachii* | LMG 25512$^T$ | AM177616 | **FR774028** | hard water rivulet, Germany | (Ali et al., 2009) |
| *Flavobacterium succinicans* | LMG 10402$^T$ | AM230492 | **FR774029** | eroded fin of salmon, Washington | (Bernardet et al., 1996) |
| *Flavobacterium swingsii* | LMG 25510$^T$ | AM934651 | **FR774030** | hard water rivulet, Germany | (Ali et al., 2009) |
| *Flavobacterium tegetincola* | LMG 21423$^T$ | U85887 | **FR774031** | Antarctic cyanobacterial mat | (McCammon and Bowman, 2000) |
| *Flavobacterium xanthum* | LMG 8372$^T$ | AF030380 | **FR774032** | pool mud, Syowa, Antarctica | (McCammon and Bowman, 2000) |
| *Flavobacterium xinjiangense* | LMG 21985$^T$ | AF433173 | **FR774033** | China No. 1 glacier | (Zhu et al., 2003) |
| *Myroides odoratus* | NBRC 14945$^T$ | M58777 | AB034239 | urine and serum specimen | (Vancanneyt et al., 1996) |

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454 **Table 3** Within group similarity, closest related species and corresponding sequence similarity for the different Antarctic *Flavobacterium* groups based on the 16S rRNA and
455 the *gyrB* gene phylogeny. Antarctic *Flavobacterium* groups for which no within group similarity is listed consists of one strain.

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| | **16S rRNA gene** | | | | **gyrB gene** | | |
|---|---|---|---|---|---|---|---|
| Antarctic species | within group similarity | nearest neighbour | similarity | Antarctic species | within group similarity | nearest neighbour | similarity |
| *Flavobacterium* sp. 1 | 100% | *Flavobacterium psychrolimnae* LMG 22018$^T$ | 97.3% | *Flavobacterium* sp. 1 | 100% | *Flavobacterium limicola* LMG 21930$^T$ | 86.1% |
| *Flavobacterium* sp. 2 | 100% | *Flavobacterium succinicans* LMG 10402$^T$ | 96.4% | *Flavobacterium* sp. 2 | 98.9-98.8% | *Flavobacterium psychrolimnae* LMG 22018$^T$ | 86.6-86.4 % |
| *Flavobacterium* sp. 3 | | *Flavobacterium succinicans* LMG 10402$^T$ | 97.5% | *Flavobacterium* sp. 3 | | *Flavobacterium hibernum* LMG 21424$^T$ | 87.2% |
| *Flavobacterium* sp. 4 | 99.5-99.2% | *Flavobacterium succinicans* LMG 10402$^T$ | 97.9-97.8% | *Flavobacterium* sp. 4 | 99.8-99.7% | *Flavobacterium degerlachei* LMG 21915$^T$ | 86.9-86.7% |
| *Flavobacterium* sp. 5 | | *Flavobacterium gelidilacus* LMG 21477$^T$ | 99.0% | *Flavobacterium* sp. 5 | | *Flavobacterium gelidilacus* LMG 21477$^T$ | 91.9% |
| *Flavobacterium* sp. 6 | 99.9% | *Flavobacterium swingsii* LMG 25510$^T$ | 97.9-97.8% | *Flavobacterium* sp. 6 | 100% | *Flavobacterium swingsii* LMG 25510$^T$ | 88.6% |
| *Flavobacterium* sp. 7 | | *Flavobacterium tegetincola* LMG 21423$^T$ | 98.2% | *Flavobacterium* sp. 7 | | *Flavobacterium antarcticum* LMG 25319$^T$ | 85.5% |
| *Flavobacterium* sp. 8 | 99.1-100% | *Flavobacterium tegetincola* LMG 21423$^T$ | 97.5-96.9% | *Flavobacterium* sp. 8 | 100-99.0% | *Flavobacterium tegetincola* LMG 21423$^T$ | 85.8-85.7% |
| *Flavobacterium* sp. 9 | 99.9% | *Flavobacterium swingsii* LMG 25510$^T$ | 95.6-95.5% | *Flavobacterium* sp. 9 | 99.4% | *Flavobacterium aquatile* LMG 4008$^T$ | 84.6% |
| *Flavobacterium* sp. 10 | | *Flavobacterium swingsii* LMG 25510$^T$ | 96.1% | *Flavobacterium* sp. 10 | | *Flavobacterium aquatile* LMG 4008$^T$ | 84.2% |
| *Flavobacterium* sp. 11 | | *Flavobacterium aquatile* LMG 4427$^T$ | 97.8% | *Flavobacterium* sp. 11 | | *Flavobacterium swingsii* LMG 25510$^T$ | 82.9% |
| *Flavobacterium* sp. 12 | 100% | *Flavobacterium aquatile* LMG 4427$^T$ | 97.1% | *Flavobacterium* sp. 12 | 99.9% | *Flavobacterium aquatile* LMG 4008$^T$ | 84.1-83.7% |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Flavobacterium* sp. 13 | 99.6-99.4% | *Flavobacterium micromati* LMG 21919$^{T}$ | 99.8-99.4% | *Flavobacterium* sp. 13 | 99.9-97.2% | *Flavobacterium micromati* LMG 21919$^{T}$ | 99.0-96.9% |
| *Flavobacterium* sp. 14 | | *Flavobacterium succinicans* LMG 10402$^{T}$ | 97.7% | *Flavobacterium* sp. 14 | | *Flavobacterium swingsii* LMG 25510$^{T}$ | 84.1% |
| *Flavobacterium* sp. 15 | 100% | *Flavobacterium succinicans* LMG 10402$^{T}$ | 97.2% | *Flavobacterium* sp. 15 | 100% | *Flavobacterium micromati* LMG 21919$^{T}$ | 88.5% |

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Fig. 1 Phylogenetic tree based on neighbour joining analysis of the 16S rRNA gene sequence similarities of the *Flavobacterium* strains and closely related species. New *Flavobacterium* isolates are marked as *Flavobacterium* sp. followed by a number. The numbers at branch nodes are bootstrap values shown as percentages of 500 bootstrap replicates (only values > 50% are shown). Bar represents 1% estimated substitutions.

Fig. 2 Phylogenetic tree based on neighbour joining analysis of the *gyrB* gene sequence similarities of the *Flavobacterium* strains and closely related species. New *Flavobacterium* isolates are marked as *Flavobacterium* sp. followed by a number. The numbers at branch nodes are bootstrap values shown as percentages of 500 bootstrap replicates (only values > 50% are shown). Bar represents 1% estimated substitutions.

Fig. S1 Phylogenetic tree calculated using the maximum likelihood method based on the 16S rRNA gene sequences of the *Flavobacterium* strains and closely related species. Antarctic *Flavobacterium* isolates are indicated as *Flavobacterium* sp. followed by a number. The numbers at branch nodes are the aLRT branch support numbers (only values > 80% are shown). Bar represents 0.02% estimated substitutions.

Fig. S2 Phylogenetic tree calculated using the maximum likelihood method based on the *gyrB* gene sequences of the *Flavobacterium* strains and closely related species. Antarctic *Flavobacterium* isolates are indicated as *Flavobacterium* sp. followed by a number. The numbers at branch nodes are the aLRT branch support numbers (only values > 80% are shown). Bar represents 0.05% estimated substitutions.