

# Antarctic microbial mats: treasure-troves of new *Flavobacterium* diversity

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## Abstract

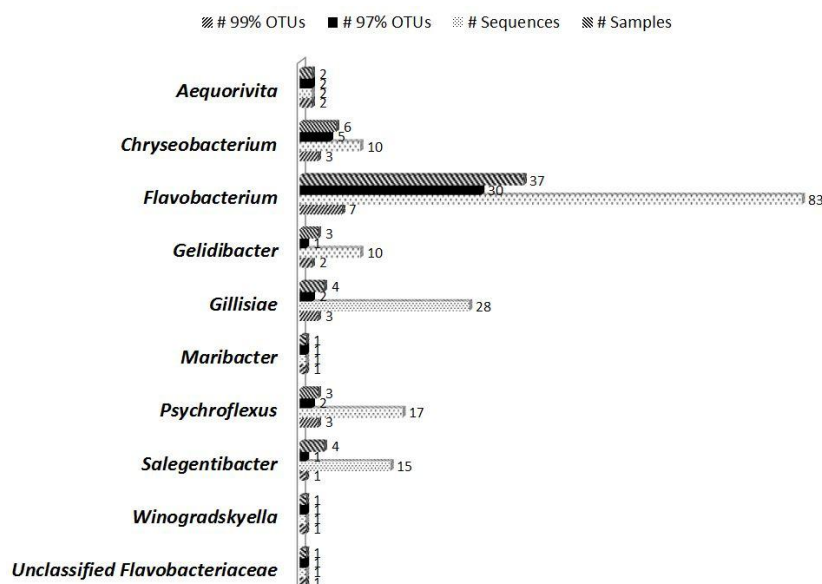
Antarctica is the most isolated continent on our planet: it is surrounded by oceans and has been separated from the nearest continent South America by the 1.800 km wide Drake Passage since 17 to 32 million years [1]. It has been cold since at least 15 million years and isolated by the Atlantic Circumpolar Current in the oceans and the clockwise atmospheric circulation that together impede inflow from lower latitudes [2] and reduce the chances of non-native organisms to reach the continent [3]. Antarctica is largely ice-covered with just 0.32 % of its surface area being ice-free [4]. The extremely low temperatures with freeze-thawing cycles, varying light conditions with high UV irradiation, strong winds and desiccation and varying salinity and nutrient levels impose extreme conditions on the continent that place considerable physiological stress on living organism [4]. Eukaryotic diversity is relatively limited while microorganisms are more numerous and diverse, yet relatively less well known. Their study is of interest because it will yield baseline data for future reference and may provide novel extremophilic organisms of biotechnological use (for example [5]).

As part of a project funded by the Belgian Science Policy service to assess the distribution of microorganisms in Antarctica, we performed a large-scale campaign to isolate heterotrophic bacteria from microbial mats from various terrestrial and aquatic habitats in several locations in Antarctica. Starting from at least 1 g of material, dilution series were set up and plated on four media (Marine agar, R2A, 10 times diluted R2A and Peptone Yeast Extract Glucose medium) and incubated aerobically at three temperatures (4, 15 and 20°C). We obtained several hundred isolates per sample and isolates were first screened by (GTG)<sub>5</sub>-PCR, a whole-genome repetitive element (rep-PCR) fingerprinting method that permitted to recognize similar isolates and make an initial grouping. Representative strains were then identified by partial 16S rRNA gene sequencing and comparison with the RDP database. The results revealed that *Flavobacteriaceae* were very regularly isolated from these Antarctic samples on all media and at all temperatures used [6] and *Flavobacterium* was the most frequently recovered genus of the family (Fig. 1).

With about 90 named species, the genus *Flavobacterium* is a large genus that has grown particularly in recent years with 42 species being described over the last 5 years. Our finding of flavobacteria is in agreement with the observation that many *Flavobacterium* species originate from relatively cold habitats and 15 were first described from Antarctica

(www.bacterio.cict.fr). We initiated a more detailed characterization of a selection of 74 of our *Flavobacterium* isolates originating from samples near the Princess Elisabeth Station (Sør Rondane Mountains), Pourquoi-Pas Island, the Transantarctic Mountains and Skarvsness, Syowa. The completion of the 16S rRNA gene sequence revealed 15 groups of isolates that had internal sequence similarities of at least 97% and that were distinct from other species. This was also confirmed by a phylogenetic analysis of the *gyrB* gene, a more variable protein-encoding gene that gave a more resolved phylogenetic grouping [7].

Figure 1. Bar chart showing the relative recovery of the different genera of the family *Flavobacteriaceae*, expressed as the number of Operational Taxonomic Units (OTUs) delineated at 99% 16S rRNA gene sequence similarity (# 99% OTUs), or 97% (# 97% OTUs), the number of sequences and the number of different samples (out 9 studied) that a genus was recovered from.



With a view to establishing whether these groups might represent separate species, we characterized representative strains by means of morphological, physiological and biochemical tests using traditional procedures and API 20E and API 20NE miniaturized systems. We also performed fatty acid analysis of the strains. Our results confirmed that one of the groups is *Flavobacterium micromati*, a species described from

Antarctic mats. The other groups can be differentiated from each other and from existing species by means of a combination of several phenotypic characteristics. We are currently performing DNA-DNA hybridizations to verify whether they indeed represent novel species for which new species can be proposed.

We also were interested in the geographic distribution of our isolates. There is an on-going discussion regarding the distribution of micro-organisms which are traditionally regarded as cosmopolitan because of their very small size, vast populations and easy dispersal [8]. We verified the distribution of our isolates by comparing their 16S rRNA sequence with sequences from public databases and verifying the geographic origin of highly similar sequences (>99% sequence similarity). For most of our isolates in general we did deduce a cosmopolitan distribution from the fact that similar sequences originated from locations in quite different places worldwide [6]. This is different for *Flavobacterium*. While we recovered flavobacteria from 7 of 9 samples tested, a particular group was mostly only recovered from one sample; only four groups were found in two different samples. This observation is not surprising given the limited sampling effort and number of isolates studied. Comparison of the 16S rRNA sequences of the isolates with sequence databases, including environmental datasets, revealed very few hits outside Antarctica, Arctic or alpine locations.

It would therefore seem that most of our Antarctic *Flavobacterium* isolates are *at present* restricted to Antarctica. This conclusion is only tentative because the deduced geographic distribution depends on the comprehensiveness of the databases. It is generally accepted that knowledge on bacterial diversity and ecology is currently still limited from many habitats on our planet and in particular from many extreme habitats including Antarctica. Some of these groups might thus be more widespread than currently available data indicate.

Our finding of a large number of potentially new flavobacterial species from microbial mats in various Antarctic locations demonstrates that these habitats represent a rich source of new *Flavobacterium* diversity. The preliminary observation that these groups are restricted to Antarctica further emphasizes the importance and urgency of cataloguing Antarctic biodiversity in view of growing signs of climate change and increasing human activities on the South Pole [9].

## References

- [1] Lyle M., Gibbs S., Moore T.C., Rea D.K., 2007. Late Oligocene initiation of the Antarctic circumpolar current: Evidence from the South Pacific. *Geology*, 35:691–694
- [2] Bargagli R., 2005. Antarctica: Geomorphology and climate trends. *In* *Antarctic Ecosystems: Environmental Contamination, Climate Change, and Human Impact*, Springer-Verlag, Berlin, pp 1–41
- [3] Pearce D.A., Bridge P.D., Hughes K.A., Sattler B., Psenner R., Russell N.J., 2009. Microorganisms in the atmosphere over Antarctica. *FEMS Microbial Ecology*, 69:143–157
- [4] Convey P., Gibson J.A.E., Hillenbrand C.D., Hodgson D.A., Pugh P.J.A., Smellie J.L., Stevens M.I., 2008. Antarctic terrestrial life - challenging the history of the frozen continent? *Biological Reviews*, 83:103–117
- [5] Rojas J.L., Martin J., Tormo J.R., Vicente F., Brunati M., Ciciliato I., Losi D., Van Trappen S., Mergaert J., Swings J., Marinelli F., Genilloud O., 2009. Bacterial diversity from benthic mats of Antarctic lakes as a source of new bioactive metabolites. *Marine Genomics*, 2:33–41
- [6] Peeters K., Verleyen E., Hodgson D.A., Convey P., Ertz D., Vyverman W., Willems A., 2012. Heterotrophic bacterial diversity in aquatic microbial mat communities from Antarctica. *Polar Biology*, 35:543–554
- [7] Peeters K., Verscheure S., Willems A., 2011. The *gyrB* gene is a useful phylogenetic marker for exploring the diversity of *Flavobacterium* strains isolated from terrestrial and aquatic habitats in Antarctica. *FEMS Microbiology Letters*, 321:130–140
- [8] Fenchel T., Finlay B.J., 2004. The ubiquity of small species: Patterns of local and global diversity. *Bioscience*, 54:777–784
- [9] Convey P., 2011. Antarctic terrestrial biodiversity in a changing world. *Polar Biology*, 11:1629–1641