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ANTARCTIC MICROBIAL BIODIVERSITY :

THE IMPORTANCE OF GEOGRAPHICAL AND ECOLOGICAL FACTORS

"AMBIO"

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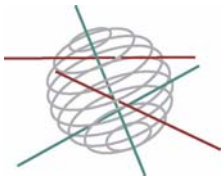

Biodiversity

FINAL REPORT PHASE 1
SUMMARY

ANTARCTIC MICROBIAL BIODIVERSITY: THE IMPORTANCE OF GEOGRAPHICAL AND ECOLOGICAL FACTORS

"AMBIO"

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High latitude ecosystems are particularly sensitive to climate change (e.g. Quayle et al. 2002) and direct human activity (pollution, physical damage, introduction of alien species; Robinson et al. 2003). Microbial organisms dominate most Antarctic ecosystems (including coastal and inland lakes, meltwater streams, cryoconites, …) and play a crucial role in their functioning; they form the base of the food web, are the main actors in the biogeochemical cycles, and mediate bioerosion (Vincent, 1988; Friedmann, 1993). Moreover, their fossil remains and biogeochemical markers provide a sensitive testimony of past environmental change (Verleyen et al. 2004a,b; Hodgson et al. 2005a,b).

Compared with temperate and tropical microbial diversity and despite their ecological importance, little is known about Antarctic microbial diversity and its geographical distribution (Sabbe et al. 2003; Ellis-Evans, 1996; Gibson et al., 2006; Taton et al. 2006a; Stackebrandt et al. 2004; Hughes et al. 2004). This is underlain by various causes, in particular the lack of systematic sampling and geographical coverage, and the problems associated with species definition, cryptic diversity and cultivability (Sabbe et al., 2003; Taton et al. 2003, Van Trappen et al., 2002; Gibson et al, in press). As a result, we largely lack the 'baseline' data needed to understand the contribution of various processes that are responsible for the geographical patterns in microbial diversity and composition and to observe possible future changes in microbial diversity and taxonomic composition due to ecosystem change and/or human introductions (Cowan & Tow 2004).

A great deal of the earlier diversity studies were carried out with traditional methods such as isolation of bacterial strains and microscopic identifications of cyanobacteria and protists on the basis of morphological features and 'force-fitting' of names of temperate taxa on the Antarctic ones. The latter bias gave an impression of cosmopolitanism of these taxonomic groups. Since the mid-eighties, molecular taxonomic markers have been increasingly used for genotypic characterisations of strains but also to retrieve directly the microbial diversity in environmental samples. The latter, largely based on ribosomal RNA operon sequences (mostly SSU rRNA, but recently also on the Internally Transcribed Spacer between SSU and LSU rRNA genes), have shown quite a different view of diversity and the existence of not-yet cultivated genotypes. In contrast to phenotypic markers, the genotypic ones are comparable, stable in different environmental conditions and reflect the evolutionary history of the organisms. They also have a considerable potential for the study of the geographical distribution of microorganisms (Amann, 1995; Loisel et al. 2006). It has been shown that molecular methods can sometimes introduce biases (e.g. Speksnijder et al., 2001); it is therefore important to combine both culture based studies and different molecular community analyses.

Antarctica is a prime place to investigate microbial biogeography and to elucidate the roles of historical processes and contemporary environmental conditions shaping microbial diversity and community structure, by virtue of its extreme isolation with respect to the rest of the world, resulting from its geographic position, and the nature of ocean and atmospheric currents; the scattered occurrence of terrestrial oases along the margins of the continent. Furthermore, organisms inhabiting the continent need to

survive in extreme environmental conditions, such as low and extremely fluctuating temperatures, dramatically changing light conditions, high seasonal UV-B loads, and low humidity. Thus, as a whole, the continent bears wide environmental gradients that impose increasing stresses on biodiversity and community structures (Lawley et al. 2004, Gibson et al. 2006). In addition, certain habitats offer some protection from the extreme conditions. For example, liquid water in aquatic environments may act as 'thermal buffer' (Gibson et al. 2006). Moreover, preliminary data on aerosol diversity in the Antarctic Peninsula showed the potential for wide-range transport of microbial diversity, though much of the aerobiota found was of local origin (Hughes et al. 2004).

In the present project we aim to extend the baseline information of microbial diversity through an integrated and standardized analysis of the microbial diversity of aquatic habitats in terrestrial antarctic environments. We use a polyphasic approach combining morphologic characterization by microscopy with molecular techniques in order to reveal the diversity of bacteria (with special emphasis on Proteobacteria, Bacteroidetes), cyanobacteria and protists (with special emphasis on green algae and diatoms), which have been identified as interesting focal taxa during our earlier studies. To work on environmental samples and isolated strains in culture allows us to unveil the diversity.

After plating the samples on different types of media, different growth characteristics were observed for the isolated bacterial strains. Halophilic organisms were isolated from samples TM2 and LA3, which is in agreement with the saline nature of the lakes of origin. Other organisms seem oligortophic or require media with specific nutrient composition in order to grow.

Grouping by rep-PBR showed more than 700 rep-clusters so far but very few contained isolates from the same sample. This points to the uniqueness of bacterial microflora in each site. Because rep-PCR is a very fine typing technique, it does not permit to define site-specific species. 16S rDNA sequencing may be an answer for such question. However, this technique is showing particularly diverse samples (PQ1 and LA3 with respectively 32% and 21% of the isolates having a unique rep-profile.

In several of the groups, part of the isolates showed low similarity values with neighbouring sequences in the EMBL-database were observed. This points to the presence of potentially new taxa, particularly in the *Bacteroidetes* phylum. Potentially new representatives were also found in the *Deinococci* phylum which has relatively few cultivated representatives.

The results of 16S rDNA sequences from pure cyanobacterial strains, DGGE analysis and clone libraries showed 23 OTUs, 5 of them are endemic to Antarctica and 3 constitute a potentially previously undiscovered diversity. Those three OTUs included sequences from the BB, TM and EA which suggests a flux of microorganisms between these regions. On the other hand, Florlidas Pond (high salinity, strong evaporation) and Lundström Lake (low salinity), both located in the TM and separated by a chain of mountains, share only one

cosmopolitan OTU (OTU44). This dissimilarity in the cyanobacterial composition may be due to the different chemical characteristics of the lakes but also maybe to obstacles to the dissemination.

A Detrended Correspondence Analysis (DCA) was run with data from clone libraries from 20 samples of EA, TM and Antarctic Peninsula revealed that the OTU composition is geographically structured as each region has a more or less unique flora. The observed geographic differences might be underlain by several reasons, such as differences in limnological properties between regions or rather the result from dispersal limitation among cyanobacteria. We can also observe that saline samples are grouped.

Finally, the present data from TM, WO and BB show an impoverished diversity in the number of OTUs compared to the coastal lakes from Larsemann Hills, Vestfold Hills and Lake Fryxell (DV) studied by Taton *et al.* (2003, 2006a).

The high diversity of the green algal isolates suggests that this group successfully colonized the Antarctic continent. Two taxa (II11, VI11), were detected in most regions, suggesting that they are widely dispersed over Antarctica. More detailed data (ITS sequences) is required to characterize these taxa on the species level. Most of the studied taxa (10 out of 14) are only retrieved from one ice-free region, suggesting that the antarctic microalgal diversity remains undiscovered.

The study of antarctic sequences points to a unique antarctic flora. This suggests high rates of endemism compared to the results of the morphological work on terrestrial algae (Broady *et al.* 1996).

As for our phylogenetic studies, and using a range of 700 to 1200 million years, we speculate that the Antarctic taxa have been isolated between 5 and 566 million years ago.

As the full analysis of the DGGE results is not finished yet, the following conclusions should be regarded as preliminary. For the bacteria, the results seem to point to a minor importance of geographical variables, this may be due to the limited resolution of the technique in order to reveal patterns in the bacterial community composition. For cyanobacteria and green algae, different distribution patterns are observed, but more data is needed to refine the analysis.