BA/01

AMBIO - Results

Antarctic microbial biodiversity: the importance of geographical and ecological factors

DURATION OF THE PROJECT
15/12/2008 - 30/06/2011

BUDGET
857.900 €

KEYWORDS
Antarctica, Microbial diversity, biogeography, bacteria, cyanobacteria, microalgae, endemism, cosmopolitanism.

CONTEXT

Microbial organisms dominate most Antarctic ecosystems and play a crucial role in their functioning and primary productivity. Compared with temperate and tropical regions and despite their ecological importance, little is known about Antarctic microbial diversity and its geographical distribution. This is due to the lack of systematic sampling and geographical coverage, and the problems associated with species definition, cryptic diversity and cultivability. As a result, we largely lack the 'baseline' data needed to observe possible future changes in microbial diversity and taxonomic composition due to ecosystem change and/or human introductions.

Most 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. This approach also lacked stability because of the plasticity of the morphology. Molecular tools enabled studies based on the SSU rRNA gene, and have shown a quite different view of the diversity and the existence of not-yet cultivated genotypes. In contrast to phenotypic markers, the genotypic based approaches have a more fine-grained taxonomic resolution and reflect the evolutionary history of the organisms. Molecular-based approaches also have a considerable potential for the study of the geographical distribution of microorganisms. This is important, because it is still unclear whether geographic isolation is present in microorganisms, and hence whether they exhibit a biogeography at all. This 'ubiquity hypothesis' was first formulated by Baas-Becking (1934) and states that 'everything is everywhere, but the environment selects'. It is underlain by the assumption that the vast population sizes of micro-organisms drive ubiquitous dispersal and make local extinction virtually impossible. However, various recent studies suggest that micro-organisms, do display restricted geographic ranges and that endemism is possible.

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. This is due to its extreme isolation with respect to the rest of the world, resulting from its geographic setting and the nature of ocean and atmospheric currents as well as of the scattered occurrence of terrestrial oases along the continental margins. 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 the biodiversity and community structures of Antarctic environments (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'. 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.

OBJECTIVES

In the present project, we aimed 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 used a **polyphasic approach** combining morphologic characterization by microscopy with molecular techniques in order to reveal the diversity of bacteria, 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 in parallel on environmental samples and isolated strains in culture allows us to obtain a more complete image of the diversity.

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CONCLUSIONS

a) Bacteria diversity

Nine samples were used to study the **culturable bacterial diversity** by plating on different types of media and incubation at three relatively low temperatures. A total of 3806 isolates were obtained. They were first characterized by comparison of whole-genome fingerprints (rep-PCR) and this allowed them to be grouped into about 1400 unique rep-types. Very few of these comprised isolates from more than one sample. To identify these organisms, the 16S rRNA gene of a representative of each type was sequenced partially or in full. The diversity recovered belonged to four major phyla, *Actinobacteria*, *Bacteroidetes*, *Proteobacteria* and *Firmicutes.* Isolates belonging to the phylum *Deinococcus-Thermus* were only recovered from four samples. Many potential new species or new genera were documented among the isolates. Although most genera recovered were reported previously from Antarctica, for 30 out of the 83 genera, this was not the case. Moreover, several isolates belonged to genera that at present contain only one species and even one strain. Comparison with sequences from public databases indicates that an important number (42.2%) of species recovered seem to be restricted to Antarctica. However, it is known that only about 5% of all bacterial species are currently present in databases and this number may therefore come down in the future. It does suggest that in Antarctica both cosmopolitan taxa as well as taxa with limited dispersal and which evolved in isolation occur.

A selection of ***Flavobacterium*** isolates recovered was studied in more detail using phylogeny of the 16S rRNA gene and the *gyrB* gene, as well as biochemical and chemotaxonomic approaches. The data revealed new strains from the Antarctic *Flavobacterium micromati* as well as twelve potential new species among our isolates. A PCR test using specific 16S rDNA primers was developed and used to detect these species in the community DNA of 32 Antarctic samples. This test can be used in the future to investigate the distribution of these species in environmental samples.

b) Cyanobacterial diversity

Different molecular methods were used to study the **cyanobacterial diversity** in strains (5) and environmental samples (95). Five Oscillatoriaceae **strains** from four continental samples were isolated. Sequence analysis of these strains allowed the finding of 2 OTUs, not revealed by other molecular techniques (clone libraries and DGGE) stressing the importance of a polyphasic approach to unveil the microbial diversity of an environmental sample.

The **uncultivated diversity** was studied using clone libraries and DGGE. **Clone libraries** gave quite a large range of richness depending on the samples, from 2 to 12 OTUs (OTUs defined at a threshold of 98.5 % 16S rRNA similarity). A Detrended Correspondence Analysis (DCA) was run with data from clone libraries from 20 samples of Prydz Bay, the Transantarctic Mountains, Shackelton Range and the Antarctic Peninsula and revealed that the OTU composition is geographically structured as each region has a more or less unique flora. The 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. The **DGGE** bands from a subset of 56 samples were sequenced and grouped into 33 OTUs. They showed different patterns of distribution: most of them (60%) were geographically and ecologically widespread. The rest seemed to be restricted to the "cold biosphere" (polar and alpine habitats). Among the latter, 5 OTUs seem to be endemic to Antarctica.

In conclusion, these sequence analyses point towards the existence of environmental and geographic limitations on the distribution of the cyanobacterial OTUs. Thus, both cosmopolitan and potentially endemic distributions were observed.

c) Microalgal diversity

The **cultivable diversity of coccal green algae** was studied in samples from 33 lakes in maritime and continental Antarctica. The 14 distinct chlorophycean and trebouxiophycean lineages observed were compared with the sequences present in GenBank and point to a wide phylogenetic diversity of apparently endemic Antarctic lineages at different taxonomic levels. Two taxa were detected in most regions, suggesting that they are widely dispersed over Antarctica. Most of the studied taxa (10 out of 14) however were only retrieved from one ice-free region. A molecular clock was applied and calibrated using absolute ages estimated by setting the split of Chlorophyta and Streptophyta at 700 and 1500 Ma. On this basis, the majority (16/26) of the lineages have estimated ages between 17 and 84 Ma and likely diverged from their closest relatives around the time of the opening of Drake Passage, while some lineages with longer branch lengths have estimated ages (330 to 708 Ma), that precede the break-up of Gondwana. The variation in branch length points to several independent but rare colonisation events.

In **diatoms**, the **cultivable diversity** was studied in the globally distributed species complex *Pinnularia borealis*. The time-calibrated molecular phylogeny based on concatenated *rbcL* and LSU (D1-D2 region) sequence data showed a divergence of the Continental Antarctic lineages from a western European lineage around 7.67 Ma. Combined, the findings in **green algae** and **diatoms** are in agreement with patterns found in multicellular organisms and they support the 'glacial refugia hypothesis', which states that long-term survival took place which resulted in a specific Antarctic flora and fauna.

d) Geographic patterns in Antarctic microbial diversity
A comparison of the **uncultured diversity** in 41 samples revealed that conductivity and variables related to salinity significantly explain differences in the community structure of **diatoms, green algae, and cyanobacteria**. A **variation partitioning** analysis of 41 samples in which all microbial groups were studied revealed that geographical variables were more important in the eukaryotic microorganisms compared with the prokaryotes. If these differences between the different taxonomic groups are confirmed, the contrasting patterns observed between prokaryotes and eukaryotes are likely related to life cycle characteristics (e.g. formation of spores, resting stage, sexual versus asexual phase). Hence, we hypothesize that findings from one particular microbial group cannot be generalized to microbes as a whole. A 454 pyrosequening analyses will enable us to test further this hypothesis. What we already found is that **cultivation and culture-independent approaches are complementary in exploring the diversity of a particular habitat**, because some cultivated taxa were not detected using the 454 pyrosequencing analysis.

CONTRIBUTION OF THE PROJECT TO A SUSTAINABLE DEVELOPMENT POLICY

Our biodiversity analyses have revealed a considerable diversity. Depending on the microbial group (bacteria, cyanobacteria or microalgae), and based mostly on SSU rRNA sequences, a number of species new to science and possibly unique to Antarctica were identified. These findings demonstrate the large value of Antarctica as a relatively unexplored territory that represents an immense resource for biotechnological, biomedical and environmental and applications. The discovery that Antarctic lakes are dominated by endemic microbial organisms has important implications for the conservation of these ecosystems. More in particular, the identification of Antarctic Specially Protected Areas (ASPAs) was traditionally based on the diversity/presence of multicellular organisms. Because microorganisms, together with a few mosses, lichens, two flowering plants and a number of small invertebrates, are the only permanent inhabitants, they should be an additional criterion for the delineation of ASPAs. For example, an endemic, as yet unidentified diatom species occurs in a few lakes in the Larsemann Hills. The presence of this taxon is likely related to the fact that some of the lakes acted as glacial refugia during past glacial maxima. The protection of this region should thus be a priority. It is also apparent that we have only just caught 'the tip of the iceberg' of the biodiversity that inhabits Antarctica. Further studies are needed and will undoubtedly yield even more novel organisms and insights. In view of the anticipated increased effects of global warming (such as rising temperature, increased desiccation, changes in UV radiation and snow/ice cover), it seems urgent to further assess particularly the impact of global change on Antarctic biota. Indeed, in addition to endemics, the total microbial biota is important for the ecosystem functioning and might be impacted by future climate change effects. Moreover, particular care should be taken to avoid the introduction of alien species from outside Antarctica, and also between different regions of the continent and the sub-Antarctic islands. This is one of the priorities for the Committee for Environmental Protection (CEP).

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