Methods of microbial community analysis: Introduction to the symposium

Convenor: Kornelia Smalla

Federal Biological Research Centre for Agriculture and Forestry, Messeweg 11/12, D-38104 Braunschweig, Germany

During the past decade, the application of new methods to study microbial communities has greatly improved our abilty to analyze microbial communities but at the same it became evident how little we know about their structural and functional diversity. More and more bacteria are recognized as multicellular organisms and intercellular communication has become one of the fields in microbial ecology attracting a lot of attention and revolutionizing our view of how bacteria operate. Thus, to adequately study microbes in complex microbial communities, new techniques allowing us to analyze microbes at the community-level and without prior isolation, rather than the traditional single-cell, pure culture analyses, are required. The dilemma that only a small portion of bacteria are readily accessible by standard cultivation techniques and that bacterial cells might lose the ability to grow on solid media in response to environmental stress could partly be overcome by analyzing community DNA or RNA directly extracted from environmental samples or by in situ microscopy. The use of the 16S rRNA gene as a molecular marker is now an established method for determining phylogenetic relationships and for analyzing ecosystems mainly based on cloning of 16S rDNA fragments amplified from directly extracted nucleic acids. While the cloning and sequencing strategies are rather labour and cost intensive the recently developed fingerprinting techniques, such as denaturing gradient gel electrophoresis (DGGE), single strand conformation polymorphism (SSCP) or terminal restriction analysis (t-RFLP) of PCR-amplified 16S rDNA have the potential required to analyze large numbers of samples in a top-to-bottom approach. In situ analysis of microbes has been facilitated by combining advanced microscopic techniques with a range of molecular approaches such as fluorescently labeled probes, reporter genes or in situ PCR. The objective is to gain information on the population structure, compartmentalization of bacterial communities, specific gene expression and transfer without disturbing the complex interaction, e.g. within a biofilm. Methods available to analyze the functional diversity and potential of complex microbial communities are clearly less developed than tools to analyze their structural diversity. The easily-produced substrate utilization profiles using BIOLOG microtiter plates allow for intensive spatial and temporal analysis of microbial communities. However, the approach is biased towards fast-growing bacteria and thus the metabolic fingerprints are unlikely to resemble the in situ metabolic potential. All techniques have their own limitations, in particular when they are applied to complex microbial communities. Biases and limitations need to be carefully checked. Multiphasic approaches should be used to study microbial communities.

It was a great honour to be asked to organize a symposium on "Methods of microbial community analysis" and this was indeed an ideal opportunity to provide a platform for a critical evaluation of the present state of the art in methods of microbial community

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analysis. At ISME 8 five experts in the field who have made valuable contributions to the progress of microbial community analysis gave an overview of the present state of the art, and at the same time critically evaluated different new methods of microbial community analysis. Frans de Bruijn, East Lansing (Michigan, U.S.A.) presented various applications of PCR-mediated genomic fingerprinting of bacteria using repetitive sequences as targets for primers and critically discussed the relative utility and resolution of these techniques. The recent progress made in the in situ study of bacteria in biofilms through combinations of molecular approaches with fluorescence microscopy was presented by Soren Molin, Technical University of Denmark. An overview on genetic fingerprinting techniques for microbial community analysis was given by Gerard Muyzer from The Netherlands Institute for Sea Research. Erko Stackebrandt, German Strain Culture Collection, Braunschweig reviewed the contribution of rDNA clone libraries as part of the big picture. The final contribution was given by Jay Garland on the potentials and limitations of BIOLOG for microbial community analysis. Four of these seminars are summarized here.