MICROBIAL RESOURCE MANAGEMENT: THE ROAD TO GO FOR ENVIRONMENTAL BIOTECHNOLOGY

W. Verstraete, L. Wittebolle, T. van de Wiele and N. Boon
Laboratory of Microbial Ecology and Technology, Ghent University, Belgium

K. Heylen, B. Vanparys and **P. de Vos**
Laboratory of Microbiology, Ghent University, Belgium

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Contents

Summary

The breakthroughs in microbiology have allowed us to come to terms with the microbial resources present in culture collections and in natural environments. The challenge at present is to manage these microbial resources, particularly when one deals with open systems where the dynamics of microbial ecology are predominant. Hence, to properly address the aspects of Microbial Resource Management (MRM), one needs to handle the questions of who is there, who is doing what with whom and how can one adjust, control and/or steer these mixed cultures and communities. It is argued that microbial ecologists and environmental microbiologists need to address a new mind-set. The Beijerinck axioma that all microorganisms are everywhere should not be presumed to be generally valid. The Darwin based niche assembly concept needs to be supplemented with the neutral theory of Hubbell. The Pareto 80/20 principle is also applicable to the macro-economies of microbial communities. Finally, the concept of a "stable" microbial community should be replaced by that of a cooperative community continuum. Overall, MRM is at the basis of a number of new developments in domains such as environmental safety and health, renewable energy production, closing environmental cycles and providing new materials. Specific examples such as, for example, pro-active

immuno-stimulation by means of drinking water, electro-microbiology, decreasing global warming by implementation of methanotrophs and generation of nano-biocatalysts are discussed.

1. Introduction

The environmental "super challenges" of the 21st century have become quite clear in the last five years. There is the climate change due to the vast increase in the production of greenhouse gases, there is the need for a renewable energy supply and there is the constant threat of pandemics such as the Asian flu, the mad cow disease, the outbreak of *Legionella* etc. But most importantly, one has to come to terms with the exploitation of our planet, since our resources are getting scarce. Particularly the supply of drinking water will increasingly become an issue of conflict.

In this context, it is important to review to what extent microbial ecology, particularly the management of the various kinds of microbial resources, can offer great new potentials to address these super challenges. Indeed, we are surrounded with a wide variety of microorganisms in the soil used to grow food, in the water we drink and in the air we breathe. These very diverse microbial communities constitute "a metagenome of knowledge" on how to function. This metagenome also extends to the microbial communities on and inside our body. Through their metabolic actions, they are major players in terms of our health and well being but also in terms of environmental sustainability.

2. Microbial Resources: "quoi de neuf ?" (What is new?)

Microbial culture collections currently contain more than a million different strains and thus are a testimony of the efforts made for the conservation of biodiversity and the desire to make these potentials available to the public [see also – *The Importance of Culture Collections and Gene Banks*]**.** To what extent these collections can and need to be expanded is debatable, since it is generally accepted that microorganisms tend to act not alone but in association with others, it is obvious therefore that at present considerable effort should be devoted to the preservation and collection of novel microbial associations in natural samples and enrichment cultures. However, preservation of the habitats in which they thrive is also needed. Up to now, attention has mainly be focused on various unique sites, such as hot springs or pristine places (e.g. the Arctic/Antarctic region). The latter, for instance, has given rise in the last decade to an enormous expansion in the knowledge of novel polar microbial taxa, which in turn has led to industrial applications, such as cold-adapted enzymes, anti-freeze products and strains capable to bioremediate in cold soils. We should explore new frontier habitats such as the deep sea, the deep underground and the deep intestine. Indeed, such environments harbor a wealth of putatively useful processes and products.

Some interesting recent discoveries are e.g. the anaerobic ammonium oxidation (Anammox reaction) which in the sea converts ammonium and nitrite to dinitrogen gas, the Archaea-Bacteria consortia that oxidize methane anaerobically by means of sulfate reduction and the pH 11-12 tolerant humus degrading bacteria in the gut of soil eating termites. Most importantly, not only these "natural" habitats are of value, but also a number of sites altered by industrial actions, often unwanted, are now to be earmarked as "resources" of microbial diversity. Examples are sites with acid mine drainage, which recently delivered potentials for the production of anti-cancer drugs and aquifers polluted with chloro-organics which have yielded very interesting halo-respiring microorganisms. It becomes obvious that not only the maintenance of microbial culture collections can be justified, but just as well the preservation of special sites, as sources of ongoing microbial evolution, selection and development of special microbial interactions, processes and products. We even should consider the need to declare a selection of these polluted industrial sites, which are in full evolution of new microbial consortia and potentials, as microbial resources worth to be preserved for the benefit of science and society.

3. Microbial Resource Management (MRM)

3.1. Three key strategies in MRM

Microorganisms acting as consortia are comparable with human beings working together in organizations and firms requiring human resource management. To put the basics of MRM in perspective one needs to know:

- Who is there? The genomic methods currently allow the listing of the various organisms present. One can, for instance, enumerate series of up to 10,000 bacterial species present in the soil and up to 1,000 species active in the digestive tract. Besides the molecular analysis of microbial communities, one should also explore new ways to isolate new species by new cultivation approaches.
- Who is doing what? There are a variety of new approaches to identify the actuators. Particularly powerful are 16S rRNA probing in combination with micro autoradiography, Stable Isotope Probing, and micro-arrays. All these methods will allow making up the "catalogues" of the species involved in the different processes. Yet, they will only give a limited picture of the overall "happiness" and "functioning" of the microbial consortia involved.
- Who is doing what with whom? To deal with complex groups of living entities, it is required to know the interrelationships. We need to learn about the standard type exchanges between different groups of organisms, particularly about types of "trading" of electron donors and/or acceptors. We can particularly learn from existing well functioning multi-species groups. Such apparently successful consortia are for instance a group of 4 bacteria converting daidzeine to equol, a mix of *Dehalobacter* sp. and *Dehalococcoides* sp., degrading chlorinated ethanes in soil and a group of methanotrophs capable to efficiently convert in a stable way methane to polyhydroxybutyrate. Yet, progress in this domain leaves much to be desired: the Anammox process probably depends on a partnership in which the Planctomycetes are the major actors but the microbial associates need to be identified. *Legionella pneumophila* is omnipresent in the environment, but is very fastidious in pure culture and therefore probably lives as a necrotroph on as yet unidentified partner bacteria. The role and identity of the protozoa in the biohydrogenation of fatty acids in the rumen is still a large enigma.

3.2. Three missing links in MRM

The knowledge about MRM is still very limited. Important questions for these microbial species are related to the "economics" of living apart or together.

- What is the minimum differential in delta G to switch to another partner or to another metabolism? The auto/mixotrophic growth of *Thioploca* on oxygen resp. nitrate illustrates that organisms very carefully optimize their energy gain in terms of the available electron acceptor.
- How important is cross-feeding between species and what is the prevalent transport mechanism between the partners ? Examples are the studies on syntrophic methanogenic consortia and the need for physical cell-to-cell contact in order to have efficient interspecies hydrogen diffusion.
- The architectural configuration of cells in flocs, granules and biofilms. Clearly, the microbial habitats are also having their three dimensional divisions in cells, channels of food supply and canals for release of end products. Excellent examples of such configurations are microbial granules and biofilms.

These missing links relate to a number of crucial processes for which the microbial resource manager should acquire skills in order to engineer them effectively. For instance, we need to deal with phenomena of mobility and chemotaxis and consequently the spatial and temporal changes as they occur between cells in a 3D microsite. Hence, the discipline of "micrometrics" should be launched in microbial ecology. The aspects of movement and transport of microorganisms in air, water and particularly in soil are crucial in the context of genetic exchange, biodiversity (see further), and coordinated functioning such as for instance that of ammonium oxidizing and nitrite oxidizing bacteria. There is the still equivocal concept that microbial flocs grow in such a way that they acquire advective flow inside the cell conglomerate and thus profit from advantageous food supply. Validation of the latter putative driving force can be useful to engineer processes such as the so-called active surface protonation of flocs (cells actively pump out protons to clump better), the production of dinitrogen in static water layers and the recycling of nutrients in the form of proteinaceous microbial flocs in aquaculture.

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Biographical Sketches

W. Verstraete is born on April 25, 1946 in Beernem (Belgium). He graduated in 1968 from the Gent University as bio-engineer. He followed a summer course on Soil Microbiology at the Pasteur Institute of Paris. In 1971, he obtained a Ph D degree in the field of microbiology at the Cornell University, Ithaca (USA).Since 1971, he works at the Gent University, first as assistant and since 1979 as professor and head of the Laboratory of Microbial Ecology and Technology (LabMET - Faculty of Bioscience Engineering).

His R & D has a central theme: processes mediated by microbial mixed cultures. His team deals with microbial transformations in waters and soils and the gastro-intestinal tract. A variety of biotechnological processes, based on microbial consortia, are subject to R&D at LabMET.

He received in 1975 the Intermediair prize for a review article entitled "Environmental hygiene from a microbial-ecological perspective". In 1976, he received from the Belgian Comité of the International Association for Water Quality (IAWQ) the prize for the design of a treatment plant dealing with concentrated wastewaters. In 1982, he received the prize of the Technological Institute of the Royal Society of Flemish Engineers for his work in the field of anaerobic digestion. In 1997, he was awarded the Francqui chair at the University of Louvain-la-Neuve. In 1999, he received the Altran prize (Fr) for his research about nitrogen removal technologies in wastewaters. In 2002, he received an 8 years' appointment as Honorary Professor in the Advanced Wastewater Management Centre of the University of Queensland, Brisbane, Australia. In 2005, he was awarded by an international jury the Five Yearly Prize for Applied Sciences of the Flemish Science Foundation. In 2006, he was awarded at the Beijing World

Congress 2006 by the International Water Association (IWA) the prestigious Imhoff Award for his contribution in the domain of water biotreatment. He also received the “Excellence in Review” award for the expertise in reviewing papers for the scientific journal “Environmental Science and Technology”.

W. Verstraete was co-chairman of the Working Party on Environmental Biotechnology of the European Federation for Biotechnology (EFB) (1989 -1993) and was General-Secretary of the European Environmental Research Organization (EERO) (1991-1997). He was a member of the OECD Workgroup on Environmental Biotechnology (1990-1998). In 1993, he was elected to the Fellowship of the International Institute of Biotechnology. In 1991, 1997 and 2004 he co-chaired the International Symposium on Environmental Biotechnology of the EFB at Ostend (B). In 1994 he chaired the International Symposium on Anaerobic Digestion of the International Water Association (IWA) at Cape Town (SA). In 2001, he was for the second time chairman of this international event, this time organised in Antwerp. He was president of the Royal Society of Flemish Engineers (Koninklijke Vlaamse Ingenieursvereniging) for the period 1997-1999. He is a member of the Royal Academy of Sciences and Arts of Belgium. He was chairman of the Belgian branch of the IWA (2000-2005) and of the Centre of Environmental Studies of the Ghent University (2000-2005). W. Verstraete is appointed member of the Technical Commission for Soil Protection of the Netherlands. He is a member of the Flemish Council for Scientific Affairs (VRWB). He is also member of the scientific council of the Belgian food industry (FEVIA).

W. Verstraete has field experience with respect to design and operation of drinking water production plants (slow sand filtration), aerobic wastewater treatment (in particular with respect to nitrificationdenitrification), anaerobic digestion of wastewaters and sludges, solid state fermentation of organic residues and bioremediation processes of soils and sediments. He has also gained experience in various aspects of pre- and probiotics used in human and animal nutrition.