Chapter 1 Management of Microbial Resources in the Environment: A Broad Perspective

Abdul Malik, Farhana Masood, and Elisabeth Grohmann

Abstract The earth contains a huge number of largely uncharacterized Bacteria and Archaea. Microbiologists are struggling to summarize their genetic diversity and classify them, which has resulted in heated debates on methods for defining species, mechanisms that lead to speciation and whether microbial species even exist. New molecular microbiological techniques allow for environmental screening to determine the presence of nucleic acids in environmental samples. These molecular genetic techniques allow screening for organisms that could be maintained in culture along with those that cannot be identified by standard non molecular means as they cannot be cultured. Although not allowing the description of specific organisms, this technique permits determining numbers and lineages of microorganisms in environmental samples, notably phylogenetic relationships and genetic similarity to sequences in established databases. Recent progress has revealed that the capture of genetic resources from complex microbial communities allows the discovery of a richness of new enzymatic diversity that had not previously been imagined. This new diversity, constitutes a large potential of new and improved applications in industry, medicine, agriculture, bioenergy etc., and promises to facilitate in a significant manner, our transition to a sustainable society, by contributing to the transition to renewable sources of energy, chemicals and materials, the reduction of pollutant burdens. Hiding within the as-yet-undiscovered microorganisms are cures

E. Grohmann

A. Malik (🖂) • F. Masood

Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, Uttar Pradesh 202002, India e-mail: ab_malik30@yahoo.com; farhanamasud4@gmail.com

Division of Infectious Diseases, University Medical Center Freiburg, Hugstetter Strasse 55, Freiburg 79106, Germany e-mail: elisabeth.grohmann@uniklinik-freiburg.de

for diseases, means to clean polluted environments, new food sources, and better ways to manufacture products daily used in modern society. This chapter focuses on the structural and functional diversity of the microbes around the globe, which are the primary and richest source of natural genetic resources that can be utilized for the improvement of agriculture, food production, and human health as well as for the welfare of the environment and ecosystems.

Keywords Microbial resources • Environment • Genetic diversity • Molecular tools • Metagenomics

1 Introduction

Microorganisms are a highly diverse group of organisms and constitute about 60% of the Earth's biomass (Singh et al. 2009). In aquatic environments, such as the oceans, the number of microbial cells has been estimated to be approximately 1.2×10^{29} , while in terrestrial environments, soil sustains as many as $4-5 \times 10^{30}$ microbial cells (Singh et al. 2009). Owing to such enormous numbers, microorganisms are essential components of the Earth's biota and represent a large unexplored reservoir of genetic diversity. Understanding this unexplored genetic diversity is a high-priority issue in microbial ecology from perspectives such as global climate change and the greenhouse effect. In fact, all organisms in the biosphere either directly or indirectly depend on microbial activities. In soil ecosystems, microorganisms are pivotal in suppressing soil-borne plant diseases, promoting plant growth, and in promoting changes in vegetation (Garbeva et al. 2004). An understanding of microbial dynamics and their interactions with biotic and abiotic factors is indispensable in bioremediation techniques, energy generation processes, and in pharmaceuticals, food, chemical and mining industries.

A plethora of biochemical and molecular methods have been applied to reveal the microbial community composition over time and space in response to environmental changes. These new approaches allow linkage between ecological processes in the environment with specific microbial populations and help us to answer important questions in microbial ecology such as what factors and resources govern the enormous genetic and metabolic diversity in an environment. 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.

2 Microbial Resources

Microbial culture collections currently contain more than a million different strains (Verstraete et al. 2007) 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. 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 been 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 (van Trappen et al. 2005) which in turn has led to industrial applications such as cold-adapted enzymes (Siddiqui and Cavicchioli 2006), anti-freeze products (Gilbert et al. 2004) and strains capable to bioremediate in cold soils (Margesin et al. 2003). 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.

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 showed potentials for the production of anti-cancer drugs (Yamada et al. 2004) and aquifers polluted with chloro-organics which have yielded very interesting halo-respiring micro-organisms (de Wildeman and Verstraete 2003; Smidt and de Vos 2004). 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.

3 Storage of Microbial Resources

For the effective conservation and utilization of microbial resources which have been found, all developed countries have established microbial culture collections, some of which have history up to 100 years. They take sound management mechanisms, act relatively independently, and have strong research capabilities. Not only microbial resources are available in the culture collection, technical services can also be provided. Microbial resource centers (MRCs) harbour collections of culturable organisms (e.g. algae, bacteria, fungi, including yeasts, protozoa and viruses), their replicable parts (e.g. genomes, plasmids, viruses, cDNAs), viable but not yet culturable organisms, cells and tissues, databases containing molecular, physiological and structural information relevant to these collections and allied bioinformatics.

3.1 Role of Culture Collections

The major culture collections throughout the world have as their primary commission, the preservation and distribution of germplasm that has been demonstrated to have significance to the microbiology community. The importance of a particular strain may be as a reference for medical or taxonomic research, as an assay organism for testing or screening, or as an essential component of a patent application for a product or process in which it is involved. Alternatively, the strain may be placed in a collection with reference to the publication in which it was cited as part of the investigation. This latter form of deposition is essential on account of the inherent transience of researchers and their research programs, making it possible for later investigators to repeat or advance published research that would be impossible in the absence of the strains involved. As mentioned above, the many national reference and service collections have succeeded in preserving, for later generations of microbiologists, many of the private and specialized collections of microorganisms that may represent an entire career of one microbiologist. In other cases, however, the acquisition of a collection of strains may well result from a change in the direction of the research program in a scientist's laboratory.

The major culture collections of the world also serve as centers for excellence in research in systematics and taxonomy. In large part the identification and characterization of strains is an integral function of collections, and the availability of a large collection of strains is essential for this type of research. Culture collections that have contract identification services are also continually searching for faster and more reliable methods to characterize unknown strains for their clients. In many cases, the strains maintained in any collection will directly reflect the taxonomic interests of the curators, in terms of the depth and breadth of particular taxonomic groups.

3.2 Bioinformatics and Culture Collection

The meticulous and thorough characterization of microorganisms, the storage and analysis of the generated information and its intelligent interrogation will provide us with microbial solutions to critical challenges of the twenty first century. It is imperative that scientists, researchers in biosystematics and taxonomy employ modern tools of informatics and data processing to make best use of our microbial resources. The elements of taxonomy such as species description, development of identification keys, scientific nomenclature, treatment of morphological, nutritional and physiological traits, are increasingly being computerized to meet the challenge.

The process of storage of genetic information with digital techniques for archiving, interpreting and quantifying of data in artificial systems is an important feature of bioinformatics (Wuyts et al. 2001, 2002). Microbial taxonomists and curators must take full advantage of the available technology that has been so ably adopted in other biological fields. Sequence data available on the web for many years for public access and utilization (http://www.ncbi.nih.gov/Genbank/GenbankSearch.html); EMBL (http://www.ebi.ac.uk/embl/) are two key resources.

However, Bridge et al. (2003) suggested that up to 20% of publicly available, taxonomically important DNA sequences for three randomly chosen groups of fungi were probably incorrectly named, chimaeric, of poor quality or incomplete for reliable comparison. MRCs have a role to play, in providing information based on authentic and stable strains with validated sequence data. MRCs need to harness the new bioinformatics technologies and begin with networking processes to establish a global network. The groundwork can be laid by stimulating collaborative molecular taxonomic research and novel database development.

4 Microbial Resource Management (MRM)

In order to deal with MRM in environmental practice, a number of approaches are currently in use. We can at present link high throughput/quick scan molecular analyses with performance data. We can for instance line up data obtained by DGGE, T-RFLP, micro-arrays, etc., to biomolecular databases resp. to diagnoses/prediction/prevention/control (Denef et al. 2004; van der Gast et al. 2006). Yet, although the "omic" methods are capable to provide an "avalanche" of information, the latter is difficult to interpret. In case one is interested in straightforward information on process performance and end product output, the process engineer will continue to find the conventional physical/chemical parameters to be the best source of information. However, in case the in-depth questions about the coherence of the microbial community are of interest, then the molecular analyses can provide information, which is not so much of direct tactical importance but rather essential in terms of strategic considerations on the behavior of the "health" of the microbial community.

Verstraete et al. (2007) developed the Microbial Resource Management (MRM) concept. This is a practical mindset that has been developed as a concept to solve practical problems through the use of microorganisms. For this reason, the inception of MRM was shortly followed by a complementary set of tools to deal with it (Marzorati et al. 2008) which come in the form of a three stage analysis independent of the technique and its settings. These tools help to provide an ecological and predictive value of the analysis which incorporates the structure and diversity of the microbial community being examined.

The most popular molecular fingerprinting techniques including temperature gradient gel electrophoresis (TGGE) and denaturing-gradient gel electrophoresis (DGGE), terminal-restriction fragment length polymorphism (t-RFLP), 16S rRNA gene clone libraries, and length heterogeneity-polymerase chain reaction (LH-PCR) are commonly used to study the structure and composition of the microbial communities. Interpreting and comparing this type of data became easier due to the initiation of MRM and the three-stage tool set developed by Marzorati et al. (2008). The parameters within the tool set include range weighted richness (Rr), dynamics (Dy), and functional organization (Fo). When using these tools in combination, they can provide us with an ecological interpretation of the raw data describing the

structure of the community. This has been demonstrated successfully in various environments over the last few years including the human gut (Grootaert et al. 2009; van den Abbeele et al. 2010); wastewater treatment systems (Wittebolle et al. 2008, 2009a, b; Vlaeminck et al. 2009); prebiotics and human gut microbial diversity (Marzorati et al. 2010b; Possemiers et al. 2010); microbial community related to celiac health issues (Schippa et al. 2010); drinking water (Lautenschlager et al. 2010); anaerobic digestion (Carballa et al. 2011; Pycke et al. 2011); aquaculture (Oi et al. 2009; De Schryver et al. 2010, 2011; Crab et al. 2009; Prol-Garcia et al. 2010); these above mentioned studies, which commonly used fingerprinting techniques such as DGGE, fatty acid methyl ester (FAME), clone libraries, and t-RFLP, have helped us to elucidate unknown characteristics of natural prokaryotic ecosystems in these various areas utilizing MRM. The first parameter, Rr, was originally introduced to establish a technique-specific range of values which indicate the richness and genetic diversity (based on the polymorphism of the 16S rRNA gene sequence) of species within an indigenous bacterial community (Marzorati et al. 2008). Rr was based on the DGGE gel patterns derived from the GC content and positioning of the sequences from complex microbial communities. A high Rr value indicates an environment with a high carrying capacity, an environment that can host several species with a wide GC variability. It has been successfully used with rRNA intergenic spacer analysis (RISA; Rojas-Oropeza et al. 2010), TGGE (Schippa et al. 2010), and clone libraries (Marzorati et al. 2010a).

Dy, the second parameter in the tool set, was used to determine the rate of change within the same community over a fixed time interval. It refers to the number of species, on average, that are detected to be of significance in a given environment at a certain time point, thus providing a large picture of the dynamics within a community. Dy can be used as a standalone parameter as seen previously in a study looking at the changing community during bioaugmentation of activated sludge (Bathe et al. 2009).

The third complementary parameter is the functional organization (Fo; Marzorati et al. 2008). This parameter initially was designed to determine the resulting action of which microorganisms were suited to the ongoing environmental-microbiological interactions. This should inevitably give them a selective advantage over the other bacteria, thus increasing their dominance among the other species in the microbial community being examined. Similarly, Fo was successfully used to demonstrate changes in evenness in various areas of research including wastewater and MFCs. Fo was renamed as community organization (Co), as a parameter that describes the microbial community in terms of degrees of evenness.

4.1 Advances in Microbial Resource Management

The recent development of new technologies providing high-throughput, low-cost sequencing methods has provided us with alternatives including Lynx Therapeutics' Massively Parallel Signature Sequencing (MPSS; Reinartz et al. 2002), 454

pyrosequencing (Edwards et al. 2006), Illumina (Solexa) sequencing (Whiteford et al. 2009), and ABI SOLiD (Sequencing by Oligonucleotide Ligation and Detection) sequencing (Valouev et al. 2008). These technologies are more sensitive than the traditional DGGE and other fingerprinting methods and provide us with a broader taxonomic coverage of the unknown and often unculturable microbial communities. Therefore, the basic parameters of the MRM tool set have to be reengineered and adapted to provide a universal platform with which to compare and contrast this new immense amount of data. Rr, based on community fingerprinting methods, was often limited in comparing richness in complex communities due to their low detection limit (Bent et al. 2007). These techniques often considerably underestimate the actual richness of communities and hence the actual diversity, which is why we must now be more vigilant in the face of new sequencing technologies. In fact, high throughput sequencing provides a much deeper insight of the actual diversity of the analyzed microbial community. As a result, the analysis provides not only the sequences of the dominant microorganisms but also of all those less abundant microorganisms normally not detected with the above mentioned fingerprinting techniques due to the low detection limit.

To know about the specific community dynamics a new technique, stable isotope probing (SIP) combined with DGGE is used. SIP is based on an ultracentrifugation step that fractionates a microbial sample previously incubated with, the compound of interest which was isotopically labelled (Neufeld et al. 2007). The result is a separation of the microbial sample according to its weight, which is dependent on its isotope content. SIP is an excellent technique to identify key microbial players in mixed communities and to look at a specific process in a controlled environment. With the key players identified, their physiology and ecology can be investigated, therefore providing important tools for MRM. Through SIP, we can determine which microorganisms are responsible for the process of interest in addition to interactions within the community providing us with information on the functional organization of the community.

5 Utilization of Microbial Resources

In modern times, as biotechnology advances constantly, microorganisms have been applied to all aspects of industrial and agricultural production. Microorganisms have generated enormous social and economic benefits. It mainly refers to green chemistry and engineering, environmental bioremediation, renewable energy, natural medicine, food production and processing. Developing agricultural microbial resources is of vital importance. In recent years, research and development on new agricultural production technology have made great progress. It is mainly represented by microbial feed, microbial fertilizers, microbial pesticides, and microbial food.

As people seize natural resources crazily and over depend on fossil energy, issues such as severe energy depletion, resources shortage and environmental pollution have come up. Industrial production and discharge by traditional chemical method are also one of the major reasons for environmental pollution. A sustainable society should be less dependent on unsustainable resources and pollution caused by fossil resources should be reduced. It's vital to make full use of the abundant natural resources, to replace backward, polluted chemical industry with innovative and advanced bioeconomy.

5.1 Environmental Management

Microbial resources will become an important force in solving the environmental problems. Microbes play an important role in water environment, such as purification and pollution. Stabilization lagoons and bio-membranes are two classic approaches dealing with polluted water. When the polluted water is pumped into the reaction pool, microbes in it transform organics into inorganics by degradation, nitrification and also photosynthesis. Usually several pools are used together, including facultative anaerobic, anaerobic and aerobic ones. For bio-membranes, various kinds of microbes will attach to the membrane and form an ecosystem, which results in a very high speed of the degradation of organic matter and also in a very high quality of the water obtained.

Waste is an unavoidable by product of human activities. Rapid population growth, urbanization and industrial growth have led to increase the quantity and complexity of generated waste and severe waste management problems in most cities of third world countries. The large quantity of waste generated necessitates a system of collection, transportation and disposal. Landfill and composting methods could be used in dealing with solid waste. Landfilling involves the controlled disposal of wastes on or in the earth's mantle. Landfills are used to dispose of solid waste that cannot be recycled and is of no further use, the residual matter remaining after solid wastes have been pre-sorted at a materials recovery facility and the residual matter remaining after the recovery of conversion products or energy. It is by far the most common method of ultimate disposal for waste residuals. Many countries use uninhabited land, quarries, mines and pits as landfill sites. Biological reprocessing methods like composting and anaerobic digestion are natural ways to decompose solid organic waste. Composting is nature's way of recycling organic wastes. Composting is a method of decomposing waste for disposal by microorganisms (mainly bacteria and fungi) to produce a humus-like substance that can be used as a fertilizer. This process converts waste which is organic in nature to inorganic materials that can be returned to the soil as fertilizer i.e. biological stabilization of organic material in such a manner that most of the nutrient and humus that are so necessary for plant growth are returned to the soil.

5.2 Energy Development

Microorganisms will play an irreplaceable role in the process of searching for new energy, compounding new energy and energy re-synthesis. Modification and utilization

of the existing microorganisms and the exploration of new microorganisms resources for renewable bio-energy manufacturing are the new novel perspectives. By the year 2120, 3.6% of electric power and 6–7% of the total energy will come from renewable resources (Lakó et al. 2008).

The waste generated in the process of industrial and agricultural production, such as crop straw, weeds, manure, sludge, organic industrial waste, garbage, etc., can be used as raw materials, and transformed into combustible gas or liquid bio-fuels by the microorganisms. Fatty acid esters (fatty acid methyl ester or ethyl ester) are major components of biodiesel. The current bio-diesel limited to the sources of animal fats or vegetable oil has certain limitations in its development. New technologies that transform the biomass directly into fatty acid ester through microbial fermentation need to be developed, at the same time, microbial resources, such as microalgae which can synthesize oil naturally, yeast, etc., should be explored.

The main component of biogas is methane, which is the product of organic matter decomposed by microorganisms under strict anaerobic conditions. Methane fermentation can release about 90% of the chemical energy in organic matter, which can be transformed into mechanical energy, electrical energy and heat energy. At present, gas as a fuel source has been transported around the world through pipelines for domestic and industrial use or converted to methanol as a supplementary fuel for internal combustion engines.

5.3 Bio-chemical Refining

The biorefining concept is an analogue of today's petroleum refineries producing multiple fuels and products from petroleum. By combining chemistry, biotechnology, engineering and systems approach, biorefinery could produce food, fertilizers, industrial chemicals, fuels, and power from biomass (Kamm and Kamm 2004). With the rapid consumption of fossil resources and the increasingly serious issue of the environment security, the refining of bio-based chemicals has become more and more popular. Many chemical companies are increasing their investments to produce 'green' chemicals through the utilization of microbial genes and enzyme resources as well as the biotransformation method in place of bio-chemical conversion. Bio-based chemicals have many types, including biological ethylene, optically pure D-or L-lactic acid, 1, 3-propanediol, 1, 4-butanediol, 3-hydroxy-propionic acid, acrylic acid, n-butanol, butyric acid, succinic acid, adipic acid, etc. They are not only important chemicals, but also important chemicals after being transformed. In addition, microbes can utilize biomass to synthesize lots of compounds that possess potential application value. As a result, further exploiting microbial resources and accelerating the biorefinery of the above chemicals will play an important role in development of bio-economy. Besides, exploration of the genes and enzymes from microorganisms and replacement of chemical transformation by bio-transformation are also important future directions.

5.4 Industrial Enzymes and Biocatalysts

The variety of microorganisms able to degrade natural and synthetic organic compounds can be used for applications in environmental biotechnology as well as in industrial synthetic chemistry. In particular, the latter approach to use enzymes for biotransformation is of growing interest. Biotransformations are chemical reactions that are catalyzed by microorganisms in terms of growing or resting cells or that are catalyzed by isolated enzymes. Because of the high stereo- or regioselectivity combined with high product purity and high enantiomeric excesses, biotransformations can be technically superior to traditional chemical synthesis. The use of biotransformations for industrial synthetic chemistry is an interdisciplinary, and therefore very exciting, field that needs the close cooperation of microbiologists, molecular biologists, chemists, and engineers. Besides classical methods, new technologies including the screening for non-culturable microorganisms and high throughput screening techniques are speeding up the discovery of new biocatalysts. The key of biotransformation is to develop highly efficient, highly selective biological catalysts, which can be realized by combining basic genomic technology with high-throughput screening technology and genome database mining. Meanwhile, further improvement and optimization of the directed evolution technology is another important force.

5.5 Utilization of Microbial Resources in Extreme Environments

The microbial resources in extreme environments which have a unique type of genes, special physiological mechanisms and metabolic products, are a kind of treasure-house of new resources. Enzymes from extreme environments have a very strong catalytic effect on environmental friendly products, and can be applied to a variety of special reaction systems. Psychrophilic enzymes can reduce the energy consumption in industry, while thermophilic enzymes are the important source of thermo stable enzymes and leaching bacteria. Its application in food, energy, environment, metabolic projects, mineral exploration, etc. can provide opportunities for the development of new chemicals, drugs and biological products.

6 Recent Development in Microbial Resource Utilization

6.1 Isolation and Microorganism Culture Technology and Metagenomics Technology

Microorganisms play an important role in recycling of materials and life continuance, their diversity is used to monitor and predict environmental changes. Lots of unknown microorganisms have never been cultured. In recent years, scientists have developed several new methods to isolate and culture microorganisms. The key point of acquiring new functional microorganisms is to create high throughput, rapid and efficient technologies towards isolation and culture of microorganisms.

Meanwhile, genomics and modern molecular biology technologies are getting more mature. These modern technologies gradually infiltrate into the entire field of life sciences. They also represent new research methods for microbiological research. A recently developed metagenomic approach employs cloning of the total microbial genome, the so-called 'metagenome', directly isolated from natural environments in culturable bacteria such as *Escherichia coli* (Beja et al. 2000; Handelsman et al. 1998; Rondon et al. 1999, 2000) and discovering novel microbial resources (Handelsman 2004). The basis of metagenomic approach originated from the molecular ecological studies of microbial diversity, indicating that majority of micro-organisms in nature was not cultivated by standard cultivation techniques. In addition, the combination of phylogenetic marker screening of metagenomic libraries and genomics could reveal the physiology of as-yet-uncultured microorganisms, only identified by culture-independent studies (Quaiser et al. 2002; Liles et al. 2003).

A series of engineering systems and technical platforms have been built for genetic engineering, protein engineering, metabolic engineering, synthetic biology, and bioprocess studies. In 1990s, with the development of gene function studies by applying functional genomics, molecular biology, molecular pathology and cell biology, the number of targets for drug screening has been growing in an unprecedented rate. High-throughput screening (HTS) is one of the newest techniques used in drug design and may be applied in biological and chemical sciences. This method, due to utilization of robots, detectors and software that regulate the whole process, enables a series of analyses of chemical compounds to be conducted in a short time and the affinity of biological structures which is often related to toxicity to be defined. The HTS method is more frequently utilized in conjunction with analytical techniques such as NMR or coupled methods e.g., LC-MS/MS.

6.2 Combinatorial Biochemistry Technology

Combinatorial biochemistry and combinatorial biosynthesis make use of microorganisms to synthesize a wide range of compounds at the gene level. Combinatorial biosynthesis has been successfully employed for e.g. carotenoid- and antibiotic polyketide-producing micro-organisms. It involves interchanging secondary metabolism genes between micro-organisms to create unnatural gene combinations or hybrid genes. Novel metabolites can be made due to the effect of new enzymemetabolic pathway combinations or to the formation of proteins with new enzymatic properties. Combinatorial biosynthesis technology can help us to discover new compounds. Combinatorial biosynthesis combined with genetic engineering and high-throughput screening technology will make it possible to carry out drug development in vivo by microorganisms via modern biology and chemistry. By this approach, we will find better strategies to synthesize new drugs and get more compounds with new structure.

6.3 Directed Evolution Technology

The development of industrial biotechnology focusing on biocatalysis needs to exploit new efficient biocatalysts. These biocatalysts should meet the demands of new catalytic activity and high productivity. They can adapt to unsuitable environments and satisfy industrial development. To this end, we can utilize microbial resources to develop new enzymes. And we can artificially modify enzymes' biological activity in accordance with special needs.

In recent years, with the development of directed evolution technology, there is no need to solve the protein's three dimensional structure and enzymatic mechanism in advance. The natural evolution mechanism of artificial simulation (random mutation, recombination, natural selection) modified enzyme genes *in vitro*. It can directly select for enzyme mutants whose function might meet special requirements. By this way, we can get such enzymes in a few days or weeks, much quicker than millions of years nature needs to achieve it. It is an important method of finding novel bioactive molecules and biotransformation pathways. The newborn directed evolution technology has greatly expanded the range of protein engineering research and application. It has opened up a new way for enzyme's structure and function research. Meanwhile, it gradually demonstrates its vitality in the fields of industry, agriculture, medicine, etc.

6.4 Biological Information Technology

Modern biological technologies result in an enormous accumulation of biological data, including microbial strain resources and related genetic resources. They enable us to restudy biological problems on basis of the whole genome, which will lead to major scientific discoveries. The huge amount of data generated from genomics research (transcriptomics, proteomics, and metabolomics) is what traditional methods cannot do, so bioinformatics came into being. Future development direction is to utilize ongoing biological information technologies and develop new software algorithms.

7 Conclusions

Microorganisms are an almost unlimited source of metabolic capabilities ready to be exploited for multiple purposes. A combination of several techniques should be applied to interrogate the diversity, function, and ecology of microorganisms. Culture-based and culture-independent molecular techniques are neither contradictory nor excluding and should be considered as complementary. An interdisciplinary systems approach embracing several "omics" technologies to reveal the interactions between genes, proteins, and environmental factors will be needed to provide new insights into environmental microbiology. Environmental metagenomic libraries have proved to be great resources for new microbial enzymes and antibiotics with potential applications in biotechnology, medicine, and industry respectively. Massive construction of metagenomic libraries and development of high throughput screening technologies will be necessary to obtain valuable microbial resources. Development of multi-"omics" approaches will be a high-priority area of research in the coming years. Microbial resource management is 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.

References

- Bathe S, Schwarzenbeck N, Hausner M (2009) Bioaugmentation of activated sludge towards 3-chloroaniline removal with a mixed bacterial population carrying a degradative plasmid. Bioresour Technol 100:2902–2909
- Beja O, Suzuki MT, Koonin EV, Aravind L, Hadd A, Nguyen LP, Villacorta R, Amjadi M, Garrigues C, Javanovich SB, Feldman RA, Delong EF (2000) Construction and analysis of bacterial artificial chromosome libraries from a marine microbial assemblage. Environ Microbiol 2:516–529
- Bent SJ, Pierson JD, Forney LJ, Danovaro R, Luna GM, Dell'anno A, Pietrangeli B (2007) Measuring species richness based on microbial community fingerprints: the emperor has no clothes. Appl Environ Microbiol 73:2399–2401
- Bridge PD, Roberts PJ, Spooner BM, Panchal G (2003) On the unreliability of published DNA sequences. New Phytol 160:43–48
- Carballa M, Smits M, Etcherbehere C et al (2011) Correlations between molecular and operational parameters in continuous lab scale anaerobic reactors. Appl Microbiol Biotechnol 89:303–314
- Crab R, Kochva M, Verstraete W, Avnimelech Y (2009) Bio-flocs technology application in overwintering of tilapia. Aquacult Eng 40:105–112
- De Schryver P, Sinha AK, Kunwar PS, Baruah K, Verstraete W, Boon N, De Boeck G, Bossier P (2010) Poly-beta-hydroxybutyrate (PHB) increases growth performance and intestinal bacterial range weighted richness in juvenile European sea bass, *Dicentrarchus labrax*. Appl Microbiol Biotechnol 86:1535–1541
- De Schryver P, Dierckens K, Thi QQ, Amalia R, Marzorati M, Bossier P, Boon N, Verstraete W (2011) Convergent dynamics of the juvenile European sea bass gut microbiota induced by poly-beta-hydroxybutyrate. Environ Microbiol 13:1042–1051
- de Wildeman S, Verstraete W (2003) The quest for microbial reductive dechlorination of C_2 to C_4 chloroalkanes is warranted. Appl Microbiol Biotechnol 61:94–102
- Denef V, Park J, Tsoi T, Rouillard J, Zhang H, Wibbenmeyer JA, Verstraete W, Gulari E, Hashsham SA, Tiedje JM (2004) Biphenyl and benzoate metabolism in a genomic context: outlining genome-wide metabolic networks in *Burkholderia xenovorans* LB400. Appl Environ Microbiol 70:4961–4970
- Edwards RA, Rodriguez-Brito B, Wegley L, Haynes M, Breitbart M, Peterson DM, Saar MO, Alexander S, Alexander EC Jr, Rohwer F (2006) Using pyrosequencing to shed light on deep mine microbial ecology. BMC Genomics 7:57

- Garbeva P, van Veen JA, van Elsas JD (2004) Microbial diversity in soil: selection microbial populations by plant and soil type and implications for disease suppressiveness. Annu Rev Phytopathol 42:243–270
- Gilbert JA, Hill PJ, Dodd CER, Laybourn-Parry J (2004) Demonstration of antifreeze protein activity in Antarctic lake bacteria. Microbiology 150:171–180
- Grootaert C, van den Abbeele P, Marzorati M, Broekaert WF, Courtin CM, Delcour JA, Verstraete W, van de Wiele T (2009) Comparison of prebiotic effects of arabinoxylan oligosaccharides and inulin in a simulator of the human intestinal microbial ecosystem. FEMS Microbiol Ecol 69:231–242
- Handelsman J (2004) Metagenomics: application of genomics to uncultured microorganisms. Microbiol Mol Biol Rev 68:669–685
- Handelsman J, Rondon MR, Brady SP, Clady J, Goodman RM (1998) Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. Chem Biol 5:245–249
- Lakó J, Hancsók J, Yuzhakova T, Marton G, Utasi A, Rédey Á (2008) Biomass a source of chemicals and energy for sustainable development. Environ Eng Manag J 7:499–509
- Lautenschlager K, Boon N, Wang Y, Egli T, Hammes F (2010) Overnight stagnation of drinking water in household taps induces microbial growth and changes in community composition. Water Res 44:4868–4877
- Liles MR, Manske BF, Bintrim SB, Handelsman J, Goodman RM (2003) A census of rRNA genes and linked genomic sequences within a soil metagenomic library. Appl Environ Microbiol 69:2684–2691
- Kamm B, Kamm M (2004) Principles of biorefineries. Appl Microbiol Biotechnol 64:137-145
- Margesin R, Gander S, Zacke G, Gounot AM, Schinner F (2003) Hydrocarbon degradation and enzyme activities of cold adapted bacteria and yeast. Extremophiles 7:451–458
- Marzorati M, Wittebolle L, Boon N, Daffonchio D, Verstraete W (2008) How to get more out of molecular fingerprints: practical tools for microbial ecology. Environ Microbiol 10:1571–1581
- Marzorati M, Balloi A, de Ferra F, Corallo L, Carpani G, Wittebolle L, Verstraete W, Daffonchio D (2010a) Bacterial diversity and reductive dehalogenase redundancy in a 1,2-dichloroethanedegrading bacterial consortium enriched from a contaminated aquifer. Microb Cell Fact 19:9–12
- Marzorati M, Verhelst A, Luta G, Sinnott R, Verstraete W, Van de Wiele T, Possemiers S (2010b) In vitro modulation of the human gastrointestinal microbial community by plant-derived polysaccharide-rich dietary supplements. Int J Food Microbiol 139:168–176
- Neufeld JD, Vohra J, Dumont MG, Lueders T, Manefield M, Friedrich MW, Murrell JC (2007) DNA stable-isotope probing. Nat Protoc 2:860–866
- Possemiers S, Marzorati M, Verstraete W, Van de Wiele T (2010) Bacteria and chocolate: a successful combination for probiotic delivery. Int J Food Microbiol 141:97–103
- Prol-Garcia MJ, Planas M, Pintado J (2010) Different colonization and residence time of *Listonella* anguillarum and Vibrio splendidus in the rotifer Brachionus plicatilis determined by real-time PCR and DGGE. Aquaculture 302:26–35
- Pycke B, Etcherbehere C, Van de Caveye P, Negroni A, Verstraete W, Boon N (2011) A time course analysis of four full-scale anaerobic digesters in relation to the dynamics of change of their microbial communities. Water Sci Technol 63:769–775
- Qi ZZ, Dierckens K, Defoirdt T, Sorgeloos P, Boon N, Bao ZM, Bossier P (2009) Analysis of the evolution of microbial communities associated with different cultures of rotifer strains belonging to different cryptic species of the *Brachionus plicatilis* species complex. Aquaculture 292:23–29
- Quaiser A, Ochsenreiter T, Klenk H-P, Kletzin A, Treusch AH, Meurer G, Eck J, Sensen CW, Schleper G (2002) First insight into the genome of an uncultivated crenarchaeote from soil. Environ Microbiol 4:603–611
- Reinartz J, Bruyns E, Lin JZ, Burcham T, Brenner S, Bowen B, Kramer M, Woychik R (2002) Massively parallel signature sequencing (MPSS) as a tool for in-depth quantitative gene expression profiling in all organisms. Brief Funct Genomic Proteomic 1:95–104

- Rojas-Oropeza M, Dendooven L, Garza-Avendano L, Souza V, Philippot L, Cabirol N (2010) Effects of biosolids application on nitrogen dynamics and microbial structure in a saline-sodic soil of the former Lake Texcoco (Mexico). Bioresour Technol 101:2491–2498
- Rondon MR, Goodman RM, Handelsman J (1999) The earth's bounty: assessing and accessing soil microbial diversity. Trends Biotechnol 17:403–409
- Rondon MR, August PR, Bettermann AD, Brady SF, Grossman TH, Liles MR, Loiacono KA, Lynch BA, MacNeil IA, Minor C, Tiong CL, Gilman M, Osburne MS, Clardy J, Handelsman J, Goodman RM (2000) Cloning the soil metagenome: a strategy for accessing the genetic and functional diversity of uncultured microorganisms. Appl Environ Microbiol 66:2541–2547
- Schippa S, Iebba V, Barbato M, Di Nardo G, Totino V, Checchi MP, Longhi C, Maiella G, Cucchiara S, Conte MP (2010) A distinctive 'microbial signature' in celiac pediatric patients. BMC Microbiol 17:175
- Siddiqui KS, Cavicchioli R (2006) Cold-adapted enzymes. Annu Rev Biochem 75:403-433
- Singh BK, Campbell CD, Sorenson SJ, Zhou J (2009) Soil genomics. Nat Rev Microbiol 7:756. doi:10.1038/nrmicro2119-c1
- Smidt H, de Vos WM (2004) Anaerobic microbial dehalogenation. Annu Rev Microbiol 58:43-73
- Valouev A, Ichikawa J, Tonthat T, Stuart J, Ranade S, Peckham H, Zeng K, Malek JA, Costa G, McKernan K, Sidow A, Fire A, Johnson SM (2008) A high-resolution, nucleosome position map of *C. elegans* reveals a lack of universal sequence-dictated positioning. Genome Res 18:1051–1063
- Van den Abbeele P, Grootaert C, Marzorati M, Possemiers S, Verstraete W, Gerard P, Rabot S, Bruneau A, El Aidy S, Derrien M, Zoetendal E, Kleerebezem M, Smidt H, Van de Wiele T (2010) Microbial community development in a dynamic gut model is reproducible, colon region specific, and selective for *Bacteroidetes* and *Clostridium* Cluster IX. Appl Environ Microbiol 76:5237–5246
- van der Gast CJ, Jefferson B, Reid E (2006) Bacterial diversity is determined by volume in membrane bioreactors. Environ Microbiol 8:1048–1055
- van Trappen S, Vandecandelaere I, Mergaert J, Swings J (2005) Flavobacterium fryxellicola sp. nov. and Flavobacterium psychrolimnae sp. nov, novel psychrophilic bacteria isolated from microbial mats in Antarctic lakes. Int J Syst Evol Microbiol 55:769–772
- Verstraete W, Wittelbolle L, Heylen K, Vanparys B, de Vos P, van de Wiele T, Boon N (2007) Microbial resource management: the road to go for environmental biotechnology. Eng Life Sci 7:117–126
- Vlaeminck SE, Terada A, Smets BF, Van der Linden D, Boon N, Verstraete W, Carballa M (2009) Nitrogen removal from digested black water by one-stage partial nitritation and anammox. Environ Sci Technol 43:5035–5041
- Whiteford N, Skelly T, Curtis C, Ritchie ME, Lohr A, Zaranek AW, Abnizova I, Brown C (2009) Swift: primary data analysis for the Illumina Solexa sequencing platform. Bioinformatics 25:2194–2199
- Wittebolle L, Vervaeren H, Verstraete W, Boon N (2008) Quantifying community dynamics of nitrifiers in functionally stable reactors. Appl Environ Microbiol 74:286–293
- Wittebolle L, Verstraete W, Boon N (2009b) The inoculum effect on the ammonia-oxidizing bacterial communities in parallel sequential batch reactors. Water Res 43:4149–4158
- Wuyts J, De Rijk P, Van de Peer Y, Winkelmans T, De Wachter R (2001) The European large subunit ribosomal RNA database. Nucleic Acids Res 29:175–177
- Wuyts J, Van de Peen Y, Winkelmans T, De Wachter R (2002) The European database on small subunit ribosomal RNA. Nucleic Acids Res 30:183–185
- Yamada T, Hiraoka Y, Das Gupta TK, Chakrabarty AM (2004) *Rusticyanin*, a bacterial electron transfer protein causes G (1) arrest in J774 and apoptosis in human cancer cells. Cell Cycle 3:1182–1187