# Techniques for Tracking Microbial Community Structure and Function in Natural Environment and Engineered Systems

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**Abstract:** ***Microbial community activity impact on changes in environment and bioprocesses efficiency, however their structure and function are also strongly affected by the condition of their surrounding environment. Understanding mechanisms underlying microbes-environments interactions would allow the predictions of environmental changes and optimization of existing microbe-engineered systems. Therefore linking microbial community structure to function and environmental changes has been one of the biggest challenges for scientific committee in this last decade. Addressing such challenge requires microbial population analysis under various conditions over time and space. Thus, it is important to master and comprehend appropriate methods for analyzing microbial community diversity, structure and function in various environments. In this paper number of techniques is presented, and their advantages and disadvantages are evaluated.***

**Keywords:** Microbial community, structure, function, analysis

## 1. Introduction

Over the last decade, the challenge for linking microbial community diversity and function to bioprocesses effectiveness, and in some extend environmental changes have led to an increasing interest on seeking to more powerful methods for analyzing microbial population diversity and function[1]. As a result, lots of improvements have been made on methods and techniques for analyzing microbial community diversity and function. From the classic, fastidious, imprecise and time consuming cultivation-dependent technique, now the highly throughput, powerful and fast biological molecular methods are mostly used, though the latter presents also some drawbacks and still need more improvements. To date, there is no single technique available that can catch the entire diversity of a microbial community [2, 1]. Biases are introduced at each treatment step, and only an iterative process where culturing, *in situ* techniques and PCR-based methods are all used can avoid misleading results, and further our understanding [3]. Any of these techniques present advantages and drawbacks; and choice has to be made according to the question being addressed and the available means.

## 2. Culture-Dependent Techniques

Cultivation-based techniques, as the term implies, have the prerequisite that the microorganism(s) in question can be grown under defined conditions in the laboratory. It can help to identify key populations capable of carrying out specific metabolic processes, and subsequently enhance the understanding of function and structure in microbially-mediated process such as bioleaching. Furthermore, it provides better background material for further development of molecular techniques. However, the majority of microorganisms in these systems are difficult to cultivate or have not yet been cultivated [4]. If restricted only to culture-dependent techniques, our understanding of the microbial ecology and physiology associated with bioleaching would be incomplete and likely biased because environmental factors that influence microbial activity and function, such as resource competition and biotic and abiotic interactions, would not be taken into consideration. Moreover, a complex microbial network can exhibit characteristics that each of its component organisms do not have when studied in isolation. Two techniques are often used: Most Probable Number Counts (MPN) and Plate Counts.

In MPN method, acidophilic microorganisms are enumerated using a statistical approach whereby samples are diluted and inoculated into a series of tubes containing growth media. Following incubation, the tubes are examined and scored for positive or negative growth, and the results are compared with standard tables to determine the most probable number (MPN) of microorganisms present. By varying the growth medium and incubation conditions, it is possible to enumerate different physiological groups of acidophiles. Again, care is required to correctly interpret the data obtained.

Plate Counts is more suitable for heterotrophic microorganisms than autotrophic microorganisms, as agar is mostly used as gelling agent. Nevertheless, the recent development made in cultivation technique (e.g. overlay technique) has enabled the enumeration of most known leaching bacteria using this method [3]. As a traditional and culture-dependent method, the plate count is fast, inexpensive, and can directly provide information on the active microbial population. Limitations include its selectivity and growth conditions such as temperature, pH.

## 3. PCR-based Molecular Methods

Over the last years, PCR-based techniques have been increasingly used to identify and quantify microorganisms in the environment and bioprocesses such as bioleaching process [3, 5]. Most of them are based on the extraction of DNA from a culture, a bioreactor or an environmental sample, followed

by the amplification of DNA using the Polymerase Chain Reaction (PCR), and finally an analysis of the DNA amplification products [6]. In most of the cases, the *16S ribosomal RNA* gene (*16S rRNA*) of prokaryotes (Bacteria and Archaea) is targeted, but also functional genes coding for key enzymes of particular metabolic interest have been analyzed (e.g. the *rus* gene coding for rusticyanin in *At. ferrooxidans*)[3]. The 16S ribosomal RNA gene is common to all organisms, though the molecule is somewhat smaller (*16S*) in prokaryotes than in eukaryotes (*18S*). It is a highly conserved molecule (e.g., it shares a high degree of sequence identity among all organisms) that is made up of regions of near identity interspersed with regions of high sequence variability. The regions of the *16S rRNA* that are nearly identical in all organisms are targets for PCR primers, to facilitate the amplification of the gene from all microorganisms – the so-called universal primers. Universal primer pairs are available to amplify nearly the entire *16S rRNA* gene from bacteria and archaea, as well as internal portions of the gene. In contrast, the regions of high variability can serve as targets for species-specific PCR primers. In the following, several PCR-based techniques for the identification of microorganisms are presented.

### 3.1 Clone library

Clone library is a powerful method to address the biodiversity and to identify new species, PCR products can be cloned and the *16S rRNA* gene of the various clones in the clone library can be sequenced. The similarities of the sequences can then be shown in a phylogenetic tree, to address the phylogenetic affiliation of the microorganisms in the sample. This approach has been chosen to analyze the microbial communities in natural, acidic environments and bioleaching operations [7, 8]

### 3.2 Restriction fragment length polymorphism (RFLP) and terminal restriction fragment length polymorphism (T-RFLP)

Also known as amplified ribosomal DNA restriction analysis (ARDRA), RFLP is a tool used to study microbial diversity that relies on DNA polymorphisms. For use of this technique, however, *16S rRNA* gene sequence information is required. In general, PCR-amplified rDNA is digested with base pairs cutting restriction enzyme [9]. Different lengths are detected using agarose or non-denaturing polyacrylamide gel electrophoresis (PCGE) in the case of community analysis [10]. To obtain useful results, one must ensure digestion completeness and the reproducibility of the RFLP banding pattern[11]. This method is useful for detecting structural changes in microbial communities but not as a measure of diversity or detection of specific phylogenetic groups [12, 13]. Banding patterns in diverse communities become too complex to analyze using RFLP since a single species could have four to six restriction fragments [10]. Perhaps by using a six-base cutting enzyme, the number of restriction fragments per species could be reduced, thereby increasing the resolution of this method. T-RFLP follows the same principle as RFLP except that the complete *16S rRNA* genes in a sample are amplified using a fluorescently labeled primer to yield a mixture of labeled 16S rRNA genes. The amplification products are digested with restriction enzymes to produce labeled terminal restriction enzyme fragments (T-RFs), which are then denatured, and the single-stranded T-RFs are separated by electrophoresis under denaturing conditions (e.g., in the presence of urea). Comparison of the migration time of the T-RFs to internal standards, labeled with a different fluorochrome, allows accurate sizing of the fragments to within ±1 nucleotide. T-RFLP fingerprints are often used to track spatial and temporal changes in microbial diversity.

### 3.3 Automated ribosomal intergenic spacer analysis (ARISA) and ribosomal intergenic spacer analysis (RISA)

These techniques are DNA-based community fingerprinting methods that relies on DNA polymorphisms. Unlike ARDRA, the method is based on the length polymorphism of the ribosomal intergenic spacer region between the *16s* and *23srRNA* genes rather than the 16S gene itself [14, 15]. The non-coding ribosomal internal spacer region is variable in both sizes and nucleotide sequence even within closely related strains, implying higher resolution of this method [16, 17]. The method has been successfully used to characterize, classify, and type strains, as well as to fingerprint simple communities and mixed populations [16, 18]. Ribosomal intergenic spacer analysis (RISA) exploits variability in the length of the internal transcribed spacer regions of *rRNA* genes to sort samples rapidly into operational taxonomic units (OTUs). Members of different species may share the same ITS fragment size [19]. Although ARISA assays a different taxonomic resolution than species level, it is a consistent measure of community composition. Consequently, differences between two OTU assemblages directly reflect changes in species composition. In RISA, the sequence polymorphisms are detected using silver stain while in ARISA the forward primer is fluorescently labeled and is automatically detected [14]. Both methods provide highly reproducible bacterial community profiles but RISA requires large quantities of DNA, is more time consuming, silver staining is somewhat insensitive and resolution tends to be low [14]. ARISA increases the sensitivity of the method and reduces the time but is still subject to the traditional limitations of PCR [11].

### 3.4 Denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE)

DGGE and TGGE are two similar methods for studying microbial diversity. TGGE uses the same principle as DGGE except the gradient is temperature rather than chemical denaturants [20]. These techniques were originally developed to detect point mutations in DNA sequences [21]. In the DGGE method, portions of the 16S rRNA gene containing the variable regions are amplified using archaeal or bacterial primers with a “GC clamp”. The resulting PCR products can be separated in denaturing gradient gel electrophoresis (DGGE) which allows a separation of DNA fragments of the same length but different base-pair sequences. Bands in the DGGE can be excised and the *16S rRNA* gene sequenced to address the phylogenetic affiliation of the organisms. Theoretically, these methods scan separate DNA with one base-pair difference [22]. Advantages of DGGE and TGGE include being reliable, reproducible, rapid, and somewhat inexpensive; providing concurrent analysis of multiple samples; and having the ability to follow changes in microbial

populations [20]. Limitations of DGGE/TGGE include PCR biases [23], laborious sample handling, as this could potentially influence the microbial community, and variable DNA extraction efficiency [20]. It is estimated that DGGE can only detect 1–2% of the microbial population representing dominant species present in an environmental sample [24]. In addition, DNA fragments of different sequences may have similar mobility characteristics in the polyacrylamide gel. Therefore, one band may not necessarily represent one species [25] and one bacterial species may also give rise to multiple bands because of multiple *16S rRNA* genes with slightly different sequences [25].

### 3.5 Single-stranded DNA conformation polymorphism (SSCP)

SSCP is based on the amplification of a small, variable region of the 16S rRNA gene, and also relies on sequence-specific secondary structure for the separation of the resulting PCR products [26]. In this case, however, the small product is denatured and allowed to renature during electrophoresis, which affects the mobility of the products through the gel. The renaturation occurs in a sequence-dependent manner, thus allowing the separation of unique genes within a community. In SSCP, the PCR products are labeled with a fluorescent molecule during PCR (using a primer synthesized with a fluorochrome attached to the 5′ end), and are standardized (e.g., given a “size”) by comparison with known size standards labeled with a different fluorochrome [26]. This allows for comparison of SSCP products with those of known acidophiles, or with products from clones, to identify microorganisms within a community. SSCP has been used to measure succession of bacterial communities [27], differentiate between pure cultures of soil microorganisms, and monitor bacterial population changes in bioreactor [29]. SSCP analysis should, in principle, be easier to carry out than DGGE or TGGE, asno primers with GC-clamp or specific apparatus for gradient gels are required [3]. A limitation of the method, in addition to potential PCR bias, however, is that a single bacterial species may yield several bands due to the presence of several operons or more than one conformation of the single-stranded PCR amplifications [10].

### 3.6 Randomly amplified polymorphic DNA polymerase chain reaction

(RAPD-PCR) is a simple and rapid method for determining genetic diversity and similarity in various organisms [30]. This technique is based on random amplification of DNA sample with random primers; the PCR products are analyzed with electrophoresis stained with ethidum bromide; and the gel images are analyzed with imaging systems. Then RAPD bands are scored as binary presence or absence characters, to assemble a matrix of RAPD phenotypes. The percentage of polymorphic bands is utilized to measure genetic diversity. Main advantages of the RAPD technology include suitability for work on anonymous genomes, applicability to problems where only limited quantities of DNA are available, efficiency and low expense. Nevertheless, the short primers result in low repetition. Besides PCR bias, RAPD technique is limited by the fact that there no possibility to distinguish whether a DNA segment is amplified from a locus that is heterozygous (1 copy) or homozygous (2 copies). Co-dominant RAPD markers, observed as different-sized DNA segments amplified from the same locus, are detected only rarely.

### 3.7 Amplified fragment length polymorphism (AFLP)

AFLPs are PCR-based markers for the rapid screening of genetic diversity. The key feature of AFLP-PCR is its capacity for simultaneous screening of many different DNA regions distributed randomly throughout the genome. In essence, AFLP methods allow PCR amplification to detect polymorphisms of genomic restriction fragments. AFLP markers have proven useful for assessing genetic differences among individuals, populations, and independently evolving lineages, such as species [31]. The main disadvantage of AFLP-PCR is the difficulty in identifying homologous markers (alleles), rendering this method less useful for studies that require precise assignment of allelic states, such asheterozygosity analysis. However, because of the rapidity and ease with which reliable, high-resolution marks can be generated, AFLPs are emerging **as** a powerful addition to the molecular toolkit of ecologists and evolutionary biologists [32].

### 3.8 RT-PCR

All the previous approaches are based on the 16S rRNA gene. Though, they are rapid and generally reliable approaches, they only indicate the presence of the microorganism containing that gene. To get information on the active microorganisms of a sample, RNA-based analyses should be performed [3]. To detect RNA by PCR methods, and thus employ the community analysis techniques described before, a viral enzyme is employed to transcribe RNA into DNA (referred to as copy DNA or cDNA) in a process known as reverse transcription (or transcriptase) PCR (RT-PCR). Following conversion of RNA isolated from a sample into cDNA, PCR amplification is carried out as normal. This product can then be subjected to further analysis by any of the previously described techniques to investigate the active microbial community. RT-PCR can also be employed on genes other than the 16S rRNA genes, but much greater care needs to be taken as messenger RNA is notoriously unstable relative to ribosomal RNA. This technique has been largely used in gene expression analysis.

## 4. Nucleic acid hybridization and fluorescent in situ hybridization (FISH)

Nucleic acid hybridization using specific probes is an important qualitative and quantitative tool in molecular bacterial ecology investigation [33]. These hybridization techniques can be performed on extracted DNA and/or RNA. Oligonucleotide or polynucleotide probes designed from known sequences ranging in specificity from domain to species can be tagged with markers at the 5’-end [34, 35]. Fluorescent markers commonly used include derivatives of fluorescein or rhodamine. Quantitative dot-blot hybridization is used to measure the relative abundance of a certain group of microorganisms. The sample is lysed to release all nucleic acids, and then RNA sequences of interest are quantified relatively to total RNA by dot-blot hybridization with specific and universal oligonucleotide primers. The relative abundance may represent changes in the abundance in the population or changes in the activity and hence the amount of

RNA content [34]. *In situ* hybridization can be also conducted at the cellular level. This method referred to fluorescent in situ hybridization (FISH), provides valuable spatial distribution information on microorganisms in environmental samples. Traditionally, radioactive isotopes were used to label oligonucleotide probes, but recently fluorescent probes are often preferred. The samples are fixed to increase permeability of the cells but still maintaining cellular structure and integrity. The sample can either be attached to microscope slides or hybridized in suspension. Fluorescently labeled primers are added and allowed to hybridize, excess is washed away and the hybridized cells detected [36]. FISH method has been used successfully to study the spatial distribution of bacteria in biofilms [37]. However, with respect to sensitivity, some limitations to the standard FISH method that prevents detection of cells with low ribosome content have been noted. Low physiological activity was often correlated with low ribosome content per cell, therefore slow-growing or starving cells may not be detected [38]. To overcome this limitation, FISH has adopted a tyramime signal amplification technique, which allowed the analysis of slow-growing microorganisms [39]. Another limitation of in situ hybridization or hybridization of nucleic acids extracted directly from environmental samples is the lack of sensitivity. Unless sequences are present in high copy number, i.e. from dominant species, they probably will not be detected. PCR eliminates this problem. DNA extracted directly from the environment can act as a template for PCR or mRNA can be reverse-transcribed into cDNA and then amplified [39, 40].

## 5. Microarrays Techniques

Similar to the situation in which microprocessors have increased the speed of computation, microarray based genomic technologies have revolutionized genetic analyses of biological systems [41]. Microarray-based technology has become a robust genomic tool to detect, track, and profile hundreds to thousands of different microbial populations simultaneously in complex biological processes and environments such as bioleaching operations, soils and sediments [42, 43]. Based on the types of probes arrayed, microarrays used in environmental studies can be divided into three major classes [44, 45]: functional gene arrays (FGAs), community genome arrays (CGAs), and phylogenetic oligonucleotide arrays (POAs). FGAs contain probes corresponding to genes encoding key enzymes involved in various biogeochemical, ecological and environmental processes, such as carbon fixation, nitrification, denitrification, sulfate reduction, and contaminant degradation. Both PCR-amplified DNA fragments and oligonucleotides derived from functional genes can be used to fabricate FGAs [46]. To avoid confusion, the former are referred to as PCR product-based FGAs, whereas the latter are referred to as oligonucleotide-based FGAs. These types of arrays are useful in studying the physiological status and functional activities of microbial communities in natural environments [45]. CGAs are constructed using whole genomic DNA isolated from pure-culture microorganisms and can be used to describe a microbial community in terms of its cultivable component [44]. Phylogenetic oligonucleotide arrays are constructed with short synthetic oligonucleotides from rRNA genes and can be used for phylogenetic analyses of microbial-community composition and structure in environmental samples [47]. Like DNA–DNA hybridization, the use of microarrays has the advantage that it is not confounded by PCR biases and microarrays can contain thousands of target gene sequences. However, it can only detect the most abundant species. In general, the species need to be cultured, but in principle cloned DNA fragments of unculturables could be used. The diversity has to be minimal or enriched cultures used; otherwise cross-hybridization can become problematic. Using genes or DNA fragments instead of genomes on the microarray offers the advantages of eliminating the need to keep cultures of organisms growing as genes can be cloned into plasmids or PCR used to continually amplify the DNA fragments. In addition, fragments would increase the specificity of hybridization over the use of genomes and functional genes in the community could be assessed [48].

## 6. Emerging molecular methods (Omic approaches)

Traditional molecular fingerprinting methods (e.g. denaturing/temperature gradient electrophoresis, single-strand-conformation polymorphism [17], and (terminal) restriction fragment length polymorphism) and Sanger sequencing of clone libraries using the *16S rRNA* gene have been applied for several years to study microbial community structure and function in various environments and bioprocesses [43]. However, these techniques are time-consuming, relatively low-throughput and fraught with drawback such as PCR bias which may lead to misleading results and inappropriate interpretations [23]. To overcome such obstacles for studying microbial community structure and function in natural and artificial settings, microarrays techniques have been developed [41, 48]. However these techniques are also wrought with limitations since they are built using known gene sequences retrieved from public databases such as NCBI and EMBL. Although they are regularly updated, and the covered gene and genome sequences increase exponentially[41, 49, 50], adapting microarray technology for use in environmental studies still presents numerous challenges in terms of probe design, the coverage of gene sequences, specificity, sensitivity and quantification capability [47, 51, 52]. Hence, to further understand complex biological system response to environmental changes, the need arises to combine information from different levels of molecular profiling to create a full picture of a system's behavior [1]. Nevertheless, the recent advances in sequencing technology, with the development of high-throughput technologies from Illumina/Solexa, ABI/SOLiD, 454/Roche, and Helicos[53, 54], have dramatically decreased the cost and increased the yield of sequence data generated, making it feasible to rapidly sequence tens to hundreds of amplicon samples on a single run[1, 55]. The ability to process large numbers of samples is important as it allows the simultaneous examination of temporally and spatially resolved samples, which provides increased statistical power for correlation analyses [1]. These advances in sequencing technology together with key complementary techniques such as imaging, isotope labeling and chemical analyses have led to the emergence of new and powerful molecular methods for microbial community analysis referred to as integrated omic approaches that are constituted of metagenomics, transcriptomics, metaproteomics and metabolomics approaches [1].

### 6.1 Metagenomics

Alsoreferred to as environmental and community genomics, metagenomicsis the random sequencing of genomic DNA extracted directly from a microbial community inhabiting a natural or engineered environment [1]. This approach goes beyond 16S rRNA gene based characterization of microbial communities, by providing a second tier of technical innovation that facilitates study of the physiology and ecology of environmental microorganisms [55]. The ultimate goal of metagenomics is to reconstruct large genome fragments or complete genomes from community members, but to date this has only been possible in relatively low diversity environments [56].

### 6.2 Metatranscriptomics

Correspondingly to metagenomics, metatranscriptomics refers to the expressed subset of genes within a microbial community at a certain point in time. Hence, *metatranscriptomics* is the technique that is employed to obtain a sequence-based expression profile [57, 58]. So it involves the sequencing of reverse transcribed mRNA extracted from a microbial community and provides a way to measure in situ gene expression [1, 57]. In contrast to metagenomics, which provides an inventory of the community gene pool, metatranscriptomics identifies which of those genes are being transcribed in a given ecological context, including under experimentally manipulated conditions [57, 58]. Metatranscriptomics reads need to be mapped against reference genomes or a metagenome from the same environment, so that differential gene expression levels can be calculated.

### 6.3 Metaproteomics

By analogy, metaproteomics is referred to as microbial community proteomics, is the characterization of the protein complement of a microbial community under a given set of conditions at a specific point in time. Metaproteome analysis in contrasted environmental situations should allow (1) tracking new functional genes and metabolic pathways and (2) identifying proteins preferentially associated with specific stresses [59]. Although metatranscriptomics provides insight into gene expression and activity, additional levels of cellular localization and regulation occur at the protein level and therefore the signal from the transcriptome and the proteome can be substantially different. In metaproteomics, proteins are extracted from a mixed microbial community sample, followed by fractionation, separation using liquid chromatography or two dimension polyacrylamide gel (2D PAGE) and detection with tandem mass spectrometry (MS/MS)[60]. In order to characterize the proteins detected, the amino acid sequences need to be known and thus a comprehensive dataset of proteins or metagenomics data is essential [1].

### 6.4 Metabolomics

Metabolomics is a recent, yet well-established approach for analyzing and profiling the whole low molecular weight organic metabolites to determine the biological pathways of a given microbial community under a given condition [61]. It provides a qualitative and quantitative measure of all low molecular-weight molecules involved in metabolic reactions and required for the maintenance, growth and normal function of a microbial community [1]. As in metaproteomics, mass spectrometry (MS) techniques, because of their sensitivity and selectivity, have become methods of choice to characterize metabolomes. Metabolomics fluxes are not regulated by gene expression alone since one transcript can direct the production of multiple proteins, post-translational modification can alter the location and function of proteins, and the chemical environment can affect protein function [62]. Changes at the metabolome are expected to be amplified relative to changes at the transcriptome or proteome level, and thus the metabolome can provide informative details about key metabolic pathways.

The combined use of these meta-omic approaches, together with advanced visualization and isotope labeling techniques can generate high-throughput data sets, which can be used to discover potential biomarkers, such as deferentially expressed genes (DEGs) and dramatically changed metabolites [1]. They allow also the identification of major biological pathways such as iron and sulfur oxidation pathways as well as heavy metal resistance, which are useful to reveal potential mechanisms of microbial dissolution of metal sulfides in the case of bioleaching research era for example. Such information's would provide a fundamental understanding of the processes occurring in ecological, environmental and microbial-mediated processes, and obviously make it possible, on one hand to optimize existing biological operations and steer the microbial community towards enhanced bioprocesses performances, and on the other hand to link microbial community structure to function and to environmental and ecological changes [1].

## 7. Conclusion

The necessity to understand, master and monitor microbial community structure and function in both natural environment and engineered systems, have led to the conception and implementation of tools and techniques for microbial community analysis. Advances on molecular methods have made it possible to effectively access microbial diversity, particularly with regard to detection of uncultivable and fastidious microbial species and those present in low abundance. Although huge improvements have been made, none of these techniques, including the recent high throughput methods candraw a full and accurate picture of microbial community structure and functions and the interactions within. Therefore, it is difficult to state whether one technique of studying microbial diversity is better than another. Each of them has advantages and drawbacks. So it is recommended to use avariety of tests with different endpoints and degrees of resolution in order to avoid dealing with misleading informations.

## References

[1] Vanwonterghem, P. D. Jensen, D. P. Ho, D. J. Batstone and G. W. Tyson, "Linking microbial community structure, interactions and function in anaerobic digesters using new molecular techniques," Current Opinion in Biotechnology, vol. 27, p. 55–64, 2014.

[2] Dahllöf, "Molecular community analysis of microbial diversity," Current Opinion in Biotechnology, vol. 13, p. 213–217, 2002.
[3] D. B. Johnson and K. B. Hallberg, "Techniques for detecting and identifying acidophilic mineral-oxidizing microorganisms," in Biomining, Springer, 2007, pp. 237-261.
[4] D. B. Johnson, "Selective solid media for isolating and enumerating acidophilic bacteria," Journal of Microbiological Methods, vol. 23, no. 2, p. 205–218, 1995.
[5] P. R. Hirsch, T. H. Mauchine and I. M. Clark, "Culture-independent molecular techniques for soil microbial ecology," Soil Biology & Biochemistry, vol. 42, pp. 878-887, 2010.
[6] Schippers, "Microorganisms involved in bioleaching and nucleic acid-based molecular methods for their identification and quantification," in Microbial processing of metal sulfides, E. R. Donati and W. Sand, Eds., Houten, 2007, pp. 3-33.
[7] Chen, X. Liu and W. Liu, "Application of clone library analysis and real-time PCR for comparison of microbial communities in a low-grade copper sulfide ore bioheap leachate," J Ind Microbiol Biotechnol, vol. 36, p. 1409–1416, 2009.
[8] E. González-Toril, A. Garcia-Moyano and R. Amils, "Phylogeny of prokaryotic microorganisms from the Tinto River," in Proceedings of the 16th International Biohydrometallurgy Symposium, Cape Townown, 2005.
[9] J. L. Kirk, L. A. Beaudette, M. Hart, P. Moutoglisc, J. N. Klironomos, H. Lee and J. T. Trevors, "Methods of studying soil microbial diversity," Journal of Microbiological Methods , vol. 58, p. 169– 188, 2004.
[10] J. M. Tiedje, S. Asuming-Brempong, K. Nusslein, T. L. Marsh and S. J. Flynn, "Opening the black box of soil microbial diversity," Appl Soil Ecol, vol. 13, p. 109– 122, 1999.
[11] B. Liu, G. Jia, J. Chen and G. Wang, "A Review of Methods for Studying Microbial Diversity in Soils," Pedosphere, vol. 16, no. 1, pp. 18-24, 2006.
[12] W. T. Liu, T. L. Marsh, H. Cheng and L. J. Forney, "Characterisation of microbial diversity by determining terminal restriction length polymorphisms of genes encoding 16S rDNA," Applied and Environmental Microbiology , vol. 63, pp. 4516-4522, 1997.
[13] Yang, W. Shi, M. Wan, Y. Zhang, L. Zou, J. Huang, G. Qiu and X. Liu, "Diversity of bacterial communities in acid mine drainage from the Shen-bu copper mine, Gansu province, China," Electronic Journal of Biotechnology, vol. 11, no. 1, pp. 107-118, 2008.
[14] M. M. Fisher and E. W. Triplett, "Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities," Appl Environ Microbiol, vol. 65, p. 4630–4636, 1999.
[15] J. Borneman and E. W. Triplett, "Molecular microbial diversity in soils from eastern Amazonia: Evidence for unusual microorganisms and microbial population shifts associated with deforestation," Applied and Environmental Microbiology, vol. 63, pp. 2647-2653, 1997.
[16] M. L. Nagpal, K. F. Fox and A. Fox, "Utility of 16s-23s rRNA spacer region methodology: How similar are interspace regions within a genome and between strains for closely related organisms?," Journal of Microbial Methods, vol. 33, pp. 211-219, 1998.
[17] R. F. Bergamo, M. T. M. Novo, R. V. Verissimo, L. C. Paulino, N. C. Stoppe, M. I. Z. Sato, M. G. P, P. I. Prado, O. Garcia and L. M. M. Ottoboni, "Differentiation of Acidithiobacillus ferrooxidans and A. thiooxidans strains based on 16S-23S rDNA spacer polymorphism analysis," Res Microbiol , vol. 155, p. 559–567, 2004.
[18] L. Ranjard, E. Brothier and S. Nazaret, "Sequencing bands of RISA fingerprints for the characterization and the microscale distribution of soil bacterial populations responding to mercury spiking," Applied and Environmental Microbiology, vol. 66, pp. 5334-5339, 2000.
[19] L. Ranjard, F. Poly, J. C. Lata, C. Mougel, J. Thioulouse and S. Nazaret, "Characterization of bacterial and fungal soil communities by automated ribosomal intergenic spacer analysis fingerprints: Biological and methodological variability," Applied and Environmental Microbiology, vol. 67, pp. 4479-4487, 2001.
[20] G. Muyzer, "DGGE/TGGE a method for identifying genes from natural ecosystems," Curr Opin Microbiol, vol. 2, p. 317–322., 1999.
[21] G. Muyzer and K. Smalla, "Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis in microbial ecology," Antonie van Leeuwenhock, vol. 73, p. 127– 141, 1999.
[22] K. M. Miller, T. J. Ming, A. D. Schulze and R. E. Withler, "Denaturing gradient gel electrophoresis (DGGE): A rapid and sensitive technique to screen nucleotide sequence variation in populations," BioTechniques, vol. 27, pp. 1016-1030, 1999.
[23] F. V. Wintzingerode, U. B. Gobel and E. Stackebrandt, "Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis," FEMS Microbiol Rev , vol. 21, p. 213– 229., 1997.
[24] S. J. MacNaughton, J. R. Stephen, A. D. Venosa, G. A. Davis, Y. J. Chang and D. C. White, "Microbial population changes during bioremediation of an experimental oil spill," Appl Environ Microbiol, vol. 65, p. 3566–3574, 1999.
[25] Gelsomino, A. C. Keijzer-Wolters, G. Cacco and J. D. Van Elsas, "Assessment of bacterial community structure in soil by polymerase chain reaction and denaturing gradient gel electrophoresis," J Microbiol Methods, vol. 38, p. 1 –15, 1999.
[26] M. Orita, Y. Suzuki, T. Sekiya and K. Hayashi, "A rapid and sensitive detection of point mutations and genetic polymorphisms using polymerase chain reaction," Genomics, vol. 5, p. 874– 879., 1989.
[27] S. Peters, S. Koschinsky, F. Schwieger and C. C. Tebbe, "Succession of microbial communities during hot composting as detected by PCR-single-strand-conformation-polymorphism-based genetic profiles of small-subunit rRNA genes," Appl Environ Microbiol, vol. 66, p. 930– 936, 2000.
[28] F. Schwieger and C. Tebbe, "A new approach to utilize PC-Rsingle-strand-conformation-polymorphism for 16S rRNA gene-based microbial community analysis," Appl Environ Microbiol, vol. 64, p. 4870– 4876, 1998.
[29] E. Zumstein, R. Moletta and J. J. Godon, "Examination of two years of community dynamics in an anaerobic bioreactor using fluorescence polymerase chain reaction

(PCR) single-strand conformation polymorphism analysis," Environ Microbiol, vol. 2, p. 69– 78, 2000.
[30] J. G. K. Williams, A. R. Kubelik, K. J. Livak, J. A. Rafalski and S. V. Tingey, "(1991) DNA polymorphisms amplified by arbitrary primers are useful genetic markers," Nucleic Acids Research , vol. 18, pp. 6531-6535, 1991.
[31] P. Vos, R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper and M. Zabeau, " 1995. AFLP: A new technique for DNA fingerprinting," . Nucleic Acids Research, vol. 23, pp. 4407-4414, 1995.
[32] V. Mueller and V. Wolfenbarger, "AFLP genotyping and fingerprinting," Trends in Ecology & Evolution, vol. 14, pp. 389-394, 1999.
[33] C. Guo, W. Sun, J. B. Harsh and A. Ogram, "Hybridization analysis of microbial DNA from fuel oil-contaminated and noncontaminated soil," Microb Ecol , vol. 34, p. 178– 187, 1997.
[34] J. Theron and T. E. Cloete, "Molecular techniques for determining microbial diversity and community structure in natural environments," Crit Rev Microbiol, vol. 26, p. 37– 57, 2000.
[35] B. S. Griffiths, K. Ritz, N. Ebblewhite and G. Dobson, "Soil microbial community structure: effects of substrate loading rates," Soil Biol Biochem, vol. 31, p. 145– 153, 1999.
[36] M. Head, J. R. Saunders and R. W. Pickup, "Microbial evolution, diversity and ecology: a decade of ribosomal RNA analysis of uncultivated microorganisms," Microb Ecol, vol. 35, p. 1– 21, 1998.
[37] T. Thurnheer, R. Gmur and B. Guggenheim, "Multiplex FISH analysis of a six-species bacterial biofilm," Journal of Microbiological Methods, vol. 56, pp. 37-47, 2004.
[38] R. I. Amann, W. Ludwig and K. H. Schleifer, "Phylogenetic identification and in situ detection of individual microbial cells without cultivation," Applied and Environmental Microbiology, vol. 59, pp. 143-169, 1995.
[39] Pernthaler, J. Pernthaler and R. Amann, "Pernthaler, A., Pernthaler, J. andFluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria," Applied and Enwironmental Microbiology, vol. 68, pp. 3094-3101, 2002.
[40] D. van Elsas and A. Wolters, "Polymerase chain reaction (PCR) analysis of soil microbial DNA," in Molecular Microbial Ecology Manual, A. D. L. Akkermans, J. D. van-Elsas and F. J. de-Bruijn, Eds., Boston, Kluwer Academic Publishing, 1995, p. 2.7.2 1 –2.7.2 10.
[41] Z. He, J. G. Terry, C. W. Schadt, L. Wu, J. Liebich, S. C. Chong, Z. Huang, W. Wu, B. Gu, P. Jardine, C. Criddle and J. Zhou, "GeoChip: a comprehensive microarray for investigating biogeochemical, ecological and environmental processes," International Society for Microbial Ecology, vol. 1, p. 67–77, 2007.
[42] Y. Liang, Z. He, L. Wu, Y. Deng, G. Li and J. Zhou, "Development of a Common Oligonucleotide Reference Standard for Microarray Data Normalization and Comparison across Different Microbial Communities," Applied and Environmental Microbiology, vol. 76, no. 4, p. 1088–1094, 2010.
[43] Y. Liu, H. Yin, Y. Liang, L. Shen, Y. Liu, X. Fu, B. Ngom, W. Zeng, G. Qiu and X. Liu, "Changes in the composition of an acid mine drainage microbial community upon successive transfers in medium containing low-grade copper sulfide," Bioresource Technology , vol. 102, p. 9388–9394, 2011.
[44] Zhou, "Microarrays for bacterial detection and microbial community analysis," Curr. Opin. Microbiol., vol. 6, p. 288–294, 2003.
[45] J. Zhou and D. K. Thompson, "Challenges in applying microarrays to environmental studies," Curr. Opin. Biotechnol., vol. 202–204, p. 13, 2002.
[46] Y. Wu, D. K. Thompson, G. Li, R. A. Hurt, J. M. Tiedje and J. Zhou, "Development and evaluation of functional gene arrays for detection of selected genes in the environment," Appl. Environ. Microbiol, vol. 67, p. 5780–5790, 2001.
[47] S. K. Rhee, X. Liu, L. Wu and S. C. Chong, "Detection of Genes Involved in Biodegradation and Biotransformation in Microbial Communities by Using 50-Mer Oligonucleotide Microarrays," Appl. Environ. Microbiol, vol. 70, no. 7, pp. 4303-4317, 2004.
[48] E. A. Greene and G. Voordouw, "Analysis of environmental microbial communities by reverse sample genome probing," J. Microbiol. Methods, vol. 53, p. 211–219, 2003.
[49] Z. He, Y. Deng, J. D. V. Nostrand, Q. Tu, M. Xu, C. L. Hemme, X. Li, L. Wu, J. G. Terry, Y. Yin, J. Liebich, C. H. Terry and J. Zhou, "GeoChip 3.0 as a high-throughput tool for analyzing microbial community composition, structure and functional activity," International Society for Microbial Ecology, vol. 4, p. 1167–1179, 2010.
[50] Z. He, Y. Deng and J. Zhou, "Development of functional gene microarrays for microbial community analysis," Current Opinion in Biotechnology, vol. 23, no. 1, pp. 49-55, 2012.
[51] Loy, A. Lehner, N. N. Lee, J. Adamczyk, H. Meier and J. Ernst, "Oligonucleotide microarray for 16S rRNA gene-based detection of all recognized lineages of sulfate-reducing prokaryotes in the environment," Appl Environ Microbiol , vol. 68, pp. 5064-5081, 2002.
[52] Wu, X. Liu, C. W. Schadt and J. Zhou, "Microarray-based analysis of subnanogram quantities of microbial community DNAs by using whole-community genome amplification," Appl Environ Microbiol , vol. 72, p. 4931–4941, 2006.
[53] Morozova and M. A. Marra, "Applications of next-generation sequencing technologies in functional genomics," Genomics , vol. 92, p. 255–264, 2008.
[54] W. J. Ansorge, "Next-generation DNA sequencing techniques," New Biotechnology, vol. 25, no. 4, p. 195–203, 2009.
[55] J. Shendure and H. Ji, "Next-generation DNA sequencing," Nature Biotechnology, vol. 26, pp. 1135 - 1145, 2008.
[56] J. Handelsman, "Metagenomics: Application of Genomics to Uncultured Microorganisms," Microbiol. Mol. Biol. Rev., vol. 68, no. 4, pp. 669-685, 2004.
[57] C. Su, L. Lei, Y. Duan, K. Q. Zhang and J. Yang, "Culture-independent methods for studying environmental microorganisms: methods, application, and perspective," Appl Microbiol Biotechnol , vol. 93, pp. 993-1003, 2012.
[58] S. M. Gifford, S. Sharma, M. R. K. Johanna and M. A. Moran, "Quantitative analysis of a deeply sequenced marine microbial metatranscriptome," International Society for Microbial Ecology, vol. 5, p. 461–472, 2011.

[59] P.-A. Maron, L. Ranjard, C. Mougel and P. Lemanceau, "Metaproteomics: A New Approach for Studying Functional Microbial Ecology," Microbial Ecology, vol. 53, p. 486–493, 2007.
[60] W. Zhang, F. Li and L. Nie, "Integrating multiple 'omics' analysis for microbial biology: application and methodologies," Microbiology , vol. 156, p. 287–301, 2010.
[61] J. G. Bundy, M. P. Davey and M. R. Viant, "Environmental metabolomics: a critical review and future perspectives," Metabolomics, vol. 5, p. 3–21, 2009.
[62] C. Y. Lin, M. R. Viant and R. S. Tjeerdema, "Metabolomics: methodologies and applications in the environmental sciences," J Pesticide Sci, vol. 31, pp. 245-251, 2006.

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