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Application of Molecular Biology in the Wastewater Treatment

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ABSTRACT
Due to the huge structural complexity of granular sludge and the many trophic interactions among the microbial populations required to transform complex organic matter into biogas (CO_2 and CH_4), the microbial ecology of micro ecosystem is still not understood. Microbial ecology techniques, such as 16SrRNA, gene cloning and sequencing. Fluorescence insitu hybridization and denatured gradient gel electrophoresis are now a days the most powerful tool available to access the diversity, abundance and distribution of microorganisms in natural and engineered ecosystems, superceding the restrictions and bias of conventional microbiology technique.
The ability of molecular methods to provide high information not attained by traditional methods. They directly interrogate the genetic information contained by the microorganisms composting the microbial community. The effective use of molecular biology to improve biological treatment processes is that molecular methods most important complements to more traditional measures and quantitative analysis. An appropriate scientific basis of new molecular data exits to effectively use these tools toward increased understanding of complex waste treatment environment. From the literature it has been amply reviewed that the best molecular biology tools to serve as an identity for accessing and quantitatively determining the microbial community, existing in conventional wastewater treatment systems.

INTRODUCTION

Microbiological processes used to treat (or bioremediate) water, wastewater, sludges and contaminated sites are among the most prominent applications of Environmental Biotechnology. Although microbiological processes have achieved worldwide acceptable, they can be improved in terms of their reliability and effectiveness. With the development of Environmental biotechnology, reliable details were identified in vision of microbial communities and their interaction. One of the reasons that microbiological processes suffer from performance deficiencies is that the tools to understand microbial ecology have been severely limited. Recently, however, numerous methods based on molecular biology have begun to breakdown the limitations, offering the opportunity to understand microbial ecology and have a sounder basis for engineering and its control. This development is in appropriate pace, as the microbial processes need to meet unique challenges in the present scenario.

Until recent years, microbial populations within such sludge granules have been intensively monitored mainly with conventional techniques such as specific methanogenic activity and most probable number (MPN) –counting. The internal architecture of granules has been also surveyed with traditional methods such as scanning electron microscopy and the immohistrochemical technique. Nevertheless, one of a fraction of the whole microbial community structure has been clarified so far. This largely attributed to the

limitations of the conventional techniques used to date. Recent cultivation on independent molecular approaches has been, however, overcoming some drawbacks of the techniques, and small subunit ribosome DNA (rDNA) and ribosome RNA (rRNA) based molecular techniques is becoming the most powerful tool for microbial community structural analysis.

Due to the large structural complexity of granular sludge and the many tropic interactions among the microbial populations required to transform complex organic matter into biogas, the microbial ecology is still not well understood molecular ecology techniques such as 16SrRNA gene cloning and sequencing, PCR based methods, quantitative Slot- blotting by hybridization, Fluorescence Insitu Hybridization, and Denaturing Gradient Gel Electrophoresis are nowadays the most powerful tools available to access the diversity , abundance and distribution of microorganisms in nature and engineered ecosystems.

FISH has become, over the past decade, an appropriate tool to detect and study microorganisms in their natural habitats. The use of 16S and 23SRNA sequences has been especially valuable, since ribosomes are present in high numbers and sequence divergences reflect phylogenetic relationships. FISH has been used in different studies to detect specific groups of bacteria and archea in order to characterize the microbial population located in anaerobic biofilm.

Polymerase chain reaction (PCR) technique based on the natural process of DNA replication inside the bacterium, resulting in big amount of copies of specific sequences for each taxonomic group.

The following reviews elaborate on these themes using examples from pioneer research work on the molecular microbial ecology of the various treatment plants.

History of molecular biology

Though microbial community determination was very ‘old’, techniques used in the past decades had some limitation in determination due to some inavilabilities. Drawbacks in the conventional detection and identification of microbial community shown below:

- A single species may exhibit polymorphism or different species may look the same
- Only indicative quantification is possible, since filaments that reside inside flocs can remain unnoticed
- The methods is subjected to the training level of the personnel

Molecular identification techniques based on new biological molecular tools, rectify the limitations present in traditional techniques and made it possible to:

- Determine the composition of microbial communities
- Detect and quantify the presence of individual microbial species

INFORMATION ABOUT MOLECULAR METHODS

The ability of molecular methods to provide high information not attainable by traditional methods comes about because they directly interrogate the genetic information contained by the microorganisms composting the microbial community. Direct interrogation obviates the need to grow the cells selectively or to identify them by morphological features, approaches then often are inadequate and biased

Genetic informations

Genetics is nothing but the respiratory information of the ‘gene’. Gene is the starting point for genetics and information tranfered. Table 1 identifies the targets for genetic interrogation and what can be learned from them. These targets are illustrated in the Figure 1, which summaries the machinery that cells use to translate and transcribe the genetic code present in DNA into working protein molecules. The information gained is stated in two ways, present in Table 1. The first way uses the technical terms phylogenetic and phenotypic. Phylogenetic refers to the genetic code itself, while phenotype refers to the function

that the cells carry out when a gene expressed to produce a protein product. The second way identifies from the microbial ecology (Rittmann, 2002).

The common target today is ribosomal RNA (rRNA), which is believed to be reliable target for accessing phylogenetic identity of the community, or what are the members are. The phylogenetic identities and abundance of specific microorganisms within a microbial community comprise the community structure. One reason rRNA is such a common target is that it is present in every independently living cell because rRNA is required to translate the genetic information into working proteins. A second reason that rRNA is an excellent target is that living cells contain a large number of ribosomes; hence, rRNA is relatively easy to detect because of its abundance in cells. Finally the small subunit (SSU) rRNA, also known as the 16Sr RNA for bacteria and archea, has approximately 1500 based that can be sequenced. These 1500 bases provide enough evolutionary diversity that selected sequences of 15-20 bases can be used as unique targets for identifying individual strains or coherent groups of relatives.

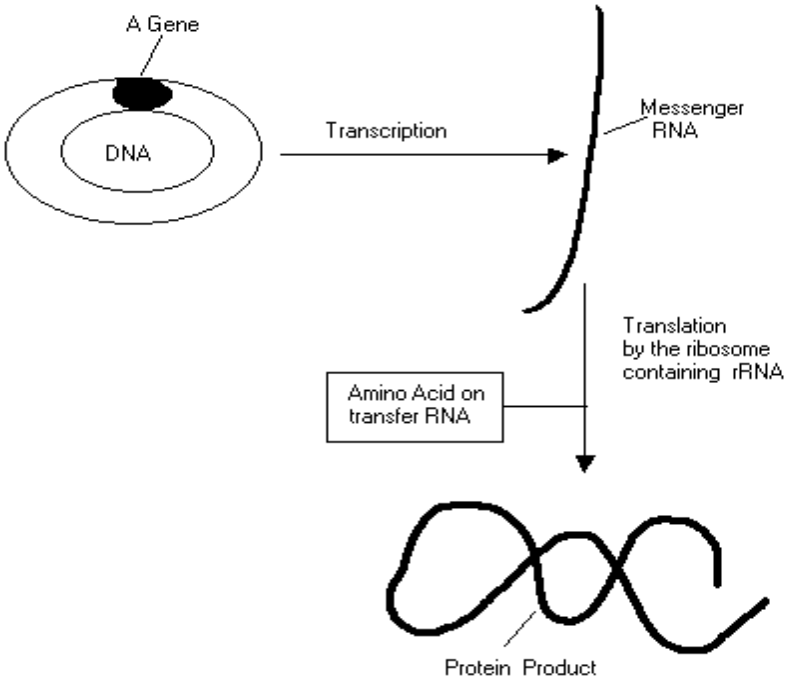

Figure 1-Schematic representation of the machinery that cells used to convert the genetic code present in a gene in the DNA to a working protein molecule. The gene, the mRNA, rRNA or the protein product can be targeted for molecular interrogation.(Rittmann, 2002)

Table 1 Targets for molecular methods (Rittmann and McCarty, 2001)

Target	Information Gained
*rRNA	Phylogenetic identity
*Genes for rRNA on DNA	Phylogenetic identity
**Others gene on DNA	Phenotypic potential
#mRNA	Expressed phenotypic potential
##Protein product	Expressed phenotypic potential

*One of the first steps for using molecular tools to address the evolutionary relationships (i.e. Phylogeny) is to sequence the SSUrRNA of the microorganism. Figure2 shows the phylogenetic tree that they developed for all available pure culture of nitrifying bacteria. The phylogenetic tree is a map of the evolutionary relationships among organisms. The length of the horizontal lines connecting the two species indicates the phylogenetic difference between two species (Mobbarry et al.1996, Purkhold et al. 2001, Rittmann and McCarty. 2001). For example, the phylogenetic tree shown in figure 2,Nitrosporia briensis is most closely related to nitrosovibrio tenuis than it is to Nitrobacter hamburgensis. Figure shows that the ammonia oxidizers (Nitroso prefix) are relatively closely related, but clearly distinct from the nitrite oxidizer (Nitro prefix), while the two nitrosomonas species are closely related to each other, they are phylogenetically distinguishable from some other ammonia oxidizers, such as Nitrosospira briensis. Oligonucleotide probing exploits these unique sequences in the rRNA by creating small DNA molecule (the probes) that are perfectly complement to the target sequence and not complementary to other sequences. Figure2 shows the set of oligonucleotide probes that developed for the nitrifiers for which they had sequence. The genes that code for rRNA (ie.rDNA) also can be a target for hybridization. While the genetic information in the rDNA is essentially same as in rRNA, the primary difference between them as targets is that the rDNA is present in such a small quantity in the cell that it cannot be detected directly. Thus the rDNA must be amplified to make many copies.

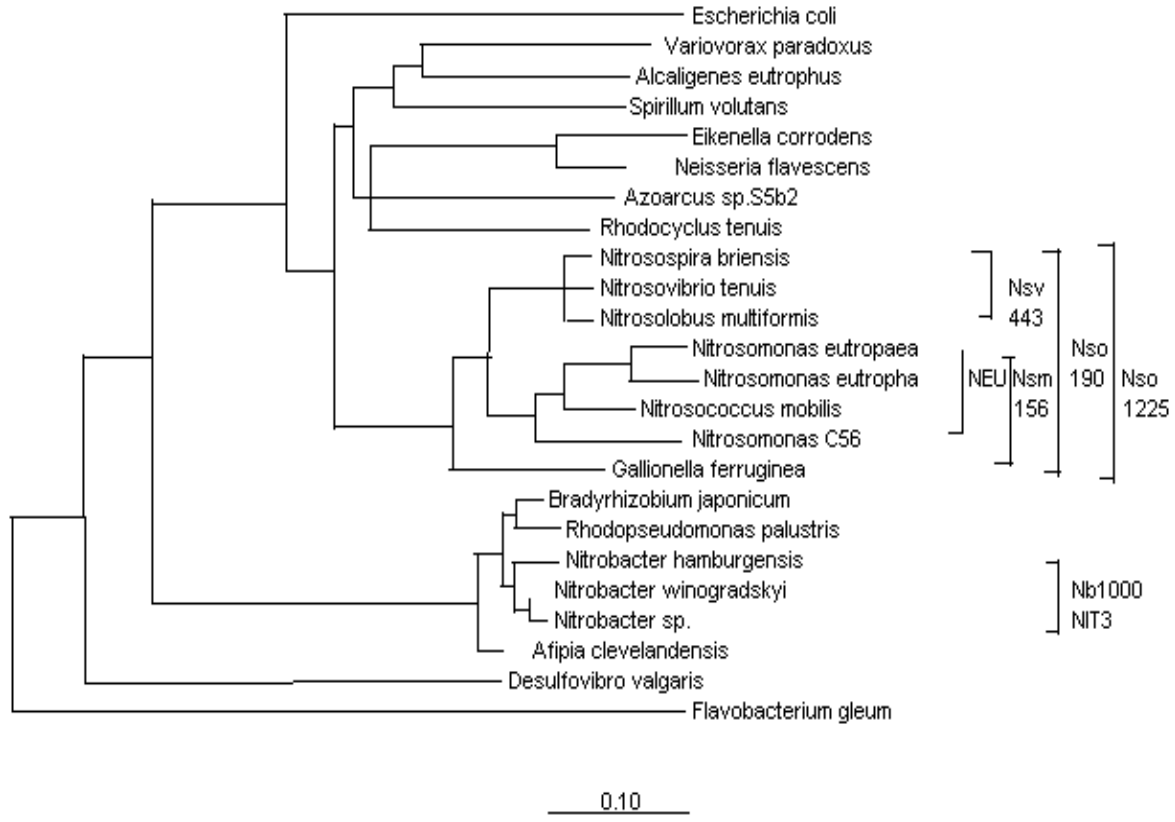

Figure 2 The phylogenetic diversity of ammonia-oxidizing and nitrite- oxidizing bacteria based on the base sequences of the SSU rRNA. Shown to the right of the tree are the nested probes that target the rRNA.

**The second kind of target addresses the phenotypic potential of a community, or "what they can do". Phenotypic potential involves interrogating the DNA to determine whether or not it contains genes for proteins that catalyze the reactions of interest. By seeing what genes are present, the functions (or phenotypes) that are contained within the microbial community can be

ascertained. In some cases, a community's phenotypic potential is partly or totally independent of the community's phylogenetic structure (Purkhold et al. 2001, Rittmann and McCarty. 2001). One of the circumstances in which community structure does not determine its phenotypic potential is when critical gene is carried on a plasmid, a small piece of DNA that is separate from the chromosomal DNA and replicated independently from chromosome representation.

#The third kind of target assesses phenotypic activity, or "what they are doing". The most direct way to use genetic interrogation and assay for the messenger RNA (mRNA). The mRNA is produced by transcription of the genes to form an RNA product that faithfully carries the genetic code from the DNA to the ribosome, where it is translated into protein (Figure1). Finding mRNA means that the microorganisms are expressing, or "turning on", their genetic potential. Generally, transcription of mRNA corresponds to full expression, but it is possible that translation to the protein product does not occur.

##The fourth kind of target is a product of gene expression and subsequent protein synthesis .A example to detect an enzyme product is the soluble methane monooxygenase (sMMO) protein, which, like AMO (Ammonia monooxygenase), can catalyze co metabolic dechlorination of TCE. However sMMO is produced by methane oxidizing bacteria, not ammonia oxidizers.

MOLECULAR TOOLS

Numerous molecular tools are being developed to overcome the biases of traditional enrichment culturing. Instead of enriching for cells based on some phenotypic trait, molecular tools directly interrogate the community's genetic information. Specifically, the molecular techniques assay the base sequence of the cell's DNA or RNA. Molecular tools applicable in the wastewater treatment described in this section:

We need to identify and enumerate the different microorganisms according to their inheritable genetic content. SSUrRNA is most commonly detected by Oligonucleotide hybridization. Oligonucleotide hybridization can be carried out in two basic formats. The more traditional format is called slot blotting, and it requires that RNA be extracted from the sample.

The second format for Oligonucleotide probing is fluorescence hybridization, or FISH. FISH and slot blotting differ in key ways. First Oligonucleotide probe in FISH is labeled with a molecule that fluorescence when excited by light of a given wavelength. Therefore, detection is through fluorescent microscopy, in which the hybridized sample is illuminated with the exciting light, and the emitted light is observed through the microscope. Second the RNA is not extracted with FISH but remains inside the cells (insitu), which are fixed and made porous to the probe. Because the cells are not destroyed. FISH is able to provide information on the spatial relationships among the different types of cells.

Similarly information on community structure can be obtained from the chromosome DNA. The DNA is first extracted from the sample in a manner similar to that for the RNA. It is then amplified selectively using the polymerase chain reaction (PCR) and primers specific for the gene that code for the SSUrRNA. The amplified DNA is fixed to a membrane and hybridized with the Oligonucleotide probes of interest. Figure 5 illustrates the different Oligonucleotide probes relevant to the methanogens and all archea. One drawback of Oligonucleotide probing is that it can be used confidently only for strains that have been isolated and sequenced. It is very useful to have a molecular technique that provides a fingerprint of the community's diversity whether or not the key strains have been isolated and sequenced.

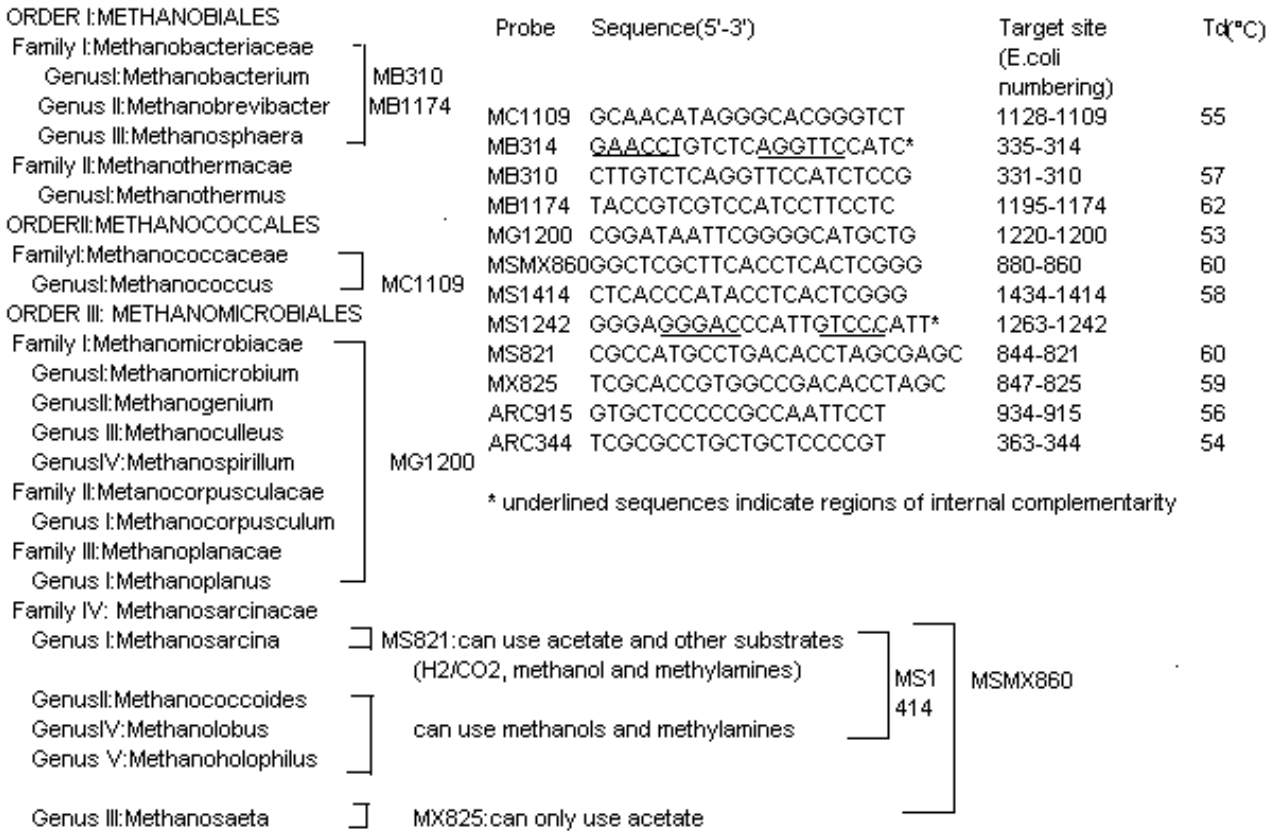

Figure 5 Oligonucleotide probes designed for the Methanogens and all Archea

New fingerprinting techniques are being developed. The basic principle underlying them is that the DNA coding for a specified and universal function of the microorganism of interest is selectively amplified by PCR. The PCR amplifies only DNA associated with the function. Limitation of using PCR as the first step for obtaining fingerprints of community structure discussed below. Biases can be introduced if the extraction efficiency varies among strains. If some DNA is more sensitive to shear breakage during handling, or if the primer does not work equally well for all gene of same type. Although less problematic than traditional enrichment methods, PCR amplification can introduce its own biases, particularly if minor components in the community have a gene that is amplified much more efficiency than genes of other related strains.

CONCLUSIONS

- Molecular methods provide a powerful complement to traditional and quantitative measures used to analyze biological treatment processes.
- Molecular methods can track critical group of microorganisms, such as ammonia oxidizers, that comprise a small fraction of the total biomass.
- They also can track specific metabolic reactions or other functions that are key to the satisfactory performance of a system.
- Molecular methods should not be used alone. The aggregated measures remain necessary to establish mass balance, to quantify the function of microbial community and to connect the results of molecular assays to practice.
- When used in comparison with traditional measures and quantitative analysis, molecular methods can serve as excellent early –warning devices of possible process upset and as mean to diagnose the causes for changes in process performance.

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