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Review

Molecular methods for characterizing mixed microbial communities in hydrogen-fermenting systems

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ABSTRACT

Molecular methods, both qualitative and quantitative, have become important in hydrogen-fermenting community analyses to reveal the complexity of the community and to monitor the changes and interactions in the communities during bioprocesses. This review presents a survey of the methods used to characterize hydrogen-fermenting mixed cultures. Each method has its pros and cons and, therefore, several methods are often used in combinations to provide the best results. The methods used are mainly PCR based targeting the 16S rRNA gene, but also other methods and targets, such as the hydrogenase genes or their transcripts, are well represented.

1. Introduction

Mixed microbial communities are efficient factories in producing a wide variety of products. Fermentation industry is best known from edible products and in many cases the conversion of substrates is conducted by a community working in quorum to lead to a product with specific flavor, morphology or texture. To monitor the success of the process rapid methods have been developed, which recently have been applied into other than food sector as useful tools for the understanding of causes and consequences how a bioprocess is working.

There are numerous methods available for characterizing microbial communities, and many of them have been used in analyzing the composition of complex hydrogen-fermenting communities (Table 1). The methods used range from conventional microscopic approaches to highly specific and quantitative PCR based technologies. Conventional approaches mostly rely on culture based techniques and are limited by species specific morphological variation and by the fact that most bacteria have not been successfully cultivated in laboratory conditions [1,2].

Molecular biology approaches mainly relying on PCR technologies are able to provide sequence information on uncultivable strains and to provide visual fingerprints on how communities change in time [2–4]. However, even in the era of rapid molecular tools the demand for traditional cultivation and strain enrichment remains. These strains serve as important fixed points in phylogenetical and physiological analyses and cultivation is also the only way to characterize different strains in detail [2,5].

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Table 1 – Microbial community characterization methods used for hydrogen-fermenting mixed cultures.

Technique	Target	Notes	Reference
Strain isolation		Additional characterization methods required	[6,10,13]
Cloning and sequencing	16S rRNA gene		[6,9,10,14–17,23–25,27,28]
T-RFLP (Terminal restriction fragment length polymorphism)	16S rRNA gene		[10,38]
RISA (ribosomal intergenic spacer analysis)	ribosomal spacer region		[43]
FISH (Fluorescence *in situ* hybridization)	16S rRNA gene	different probes	[8,11,45,50]
	hydrogenase	batch system	[51]
	archaea		[10]
PCR-DGGE (Denaturing gradient gel electrophoresis)	16S rRNA gene	universal primers; for species recognition sequencing required	[6–9,11–13,15,18–21,23,26–30,45,53–69]
	16S rRNA gene	reverse transcription PCR-DGGE	[16]
	hydrogenase	specific primers	[22]
qPCR (Quantitative PCR)	16S rRNA gene	strain specific primers and probes	[7]
	[FeFe]-hydrogenase	strain specific primers and probes	[80]
	[FeFe]-hydrogenase	universal primers	[12,83]
Melting curve analysis	[FeFe]-hydrogenase	universal primers	[12]

This review presents molecular techniques and targets used in community characterization in various hydrogen-fermenting systems. All methods have their positive and negative characteristics, and therefore often more than one method is used for the community analysis [6–12]. In this review we describe different methodologies how microbial communities and ecology have been analyzed in hydrogen-fermenting systems as well as try to evaluate the performance of the methodologies with regard to cost, robustness, and speed. Microbial community characterization methods used for hydrogen-fermenting mixed cultures are presented in Table 1 and the pros and cons for each method in Table 2.

2. Strain isolation, cloning, and sequencing

Although most of the bacteria still cannot be cultivated in laboratory conditions, some of the known strains can serve as important fixed points in phylogenetical analyses. While strain isolation might be labor intensive and occasionally extremely time consuming, it is still the only way to characterize strains in detail and therefore culture methods will remain important tools for studying the bacterial diversity. Strain isolation techniques have been applied in hydrogen-fermenting community studies, but since these methods only give limited amount of qualitative information on the community, they are usually used in combination with other community characterization techniques (Table 1) [6,10,13].

Cloning methods have been used in several hydrogen-fermenting community analyses [6,9,10,14–18]. Usually cloning is used to create libraries of the 16S rRNA genes and clones are then sequenced and analyzed further [4,5]. Although cloning is a good way to gain phylogenetic information especially when large inserts are possible, the cloning step remains time consuming and limits the application of the method when high throughput or fast analysis of samples is required.

When considering hydrogen-fermenting communities the sequencing has so far been limited mostly to clone libraries and DGGE bands [6,9,10,14–17,19–30]. There are, however, sequencing methods that are rapidly gaining popularity among microbiologists. Whole genome sequencing, shotgun sequencing of environmental samples, and meta-genomic approaches are rapidly expanding the knowledge on microbial genomes and community structures [31,32]. In metagenomics the environmental DNA is directly cloned into libraries for sequencing and sequences are then analyzed using bioinformatics tools [33,34]. Due to advances in sequencing technologies it has become more affordable and faster even when whole microbial genomes or mixed microbial communities are in question [31,32]. Sequencing could be beneficial in determining the microbial composition of the bioreactor community, but at the moment it is not suitable for bioreactor monitoring due to time consuming sample and data analyses.

3. T-RFLP and RISA

Terminal restriction fragment length polymorphism (T-RFLP) is a PCR based analysis method targeting the 16S rRNA gene. The PCR products are tagged by using labeled primers and after amplification the amplicons are digested with restriction enzymes. The resulting fragments containing the labels are detected using capillary electrophoresis producing a fingerprint of the microbial community in question [35,36]. This method is sensitive (limit of sensitivity approximately 0.5% of the community), suitable for high throughput analyses, and the specificity can be tuned by selecting primers of suitable taxonomic resolution amplifying groups or individual species, but the biggest drawback is the need for restriction digestion and the problems arising from incomplete digestions [35,37]. T-RFLP has gained popularity among scientists studying microbial communities since it provides a high throughput community profiling method, and it has been used in a few studies dealing with hydrogen-fermentation [10,38].

Ribosomal intergenic spacer analysis (RISA) is also a PCR based method but instead of targeting the 16S rRNA gene it targets the spacer region between 16S and 23S genes [39,40]. This method can provide a community fingerprint and does not require denaturing gradients or restriction digestions [41]. The database for intergenic spacer regions is not, however, as large as