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Scope of common DNA based methods for the study of rumen bacterial population

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Abstract

Innovative methods in relation to rumen microbiology are mainly focused on the study of rumen microbial population. Rumen ecosystem is highly responsive to changes in diet, age, antibiotic use, health of the host animal, which varies according to geographical location, season, and feeding scheme. Until recently, knowledge of rumen fermentation was primarily studied using classical culture-based techniques, such as isolation, enumeration and nutritional characterization, which probably only account for 10 to 20% of the rumen microbial biomass. An increase in bacterial numbers recovered from the rumen is the most reproducible effect of dietary yeast supplementation, and it has been recognized that the increased bacterial population especially cellulolytic bacteria is central to the action of the yeast in improving ruminant productivity . Many DNA based methods have described the diet dependent shift in the diversity of rumen bacterial population. This paper is primarily aimed to see different DNA based methods for study rumen bacterial population.

Key words: ecology, rumen analysis, microbial, molecular methods

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Introduction

Innovative methods in relation to rumen microbiology are mainly focus on the study of microbial population as they directly involves in the rumen fermentation process to provide nutrients for the ruminant (Harinder and McSweeney, 2005). Ruminants can eat different types of feed sources that are digested by microbial biomass which helps for better metabolism. The efficiency of ruminants to utilize such a wide variety of feeds is due to highly diversified rumen microbial ecosystem consisting of bacteria (10 10–10 11 cells/ml, representing more than 50 genera) (Kamara 2005).

Until recently, knowledge of rumen microbiology was primarily obtained using classical culture-based techniques, such as isolation, enumeration and nutritional characterization. It is well studied now that only 10 to 20% of the microbial population of rumen can be studied by these methods (Duan et al. 2006). These traditional methods are time consuming and having many disadvantage, (Orpin and Joblin 1997). These methods have identified more than 200 species of bacteria and at least 100 species of protozoa and fungi inhabiting the rumen (Stewart et al. 1998). These numbers might even be larger as majority of them are non-culturable and cannot study completely (White et al. 1999). New DNA-based technologies can now be employed to examine microbial diversity primarily through the use of small subunit (SSU) rDNA analysis (e.g. 16S and 18S rDNA) and to understand the function of complex microbial ecosystems in the rumen. These methods can be used for identify species in rumne samples using each species molecular signature, without having to isolate the species in pure culture (Williams and Coleman 1998). Recent reviews and technical manuals written on the subject of molecular microbial ecology of animals provide good ideas of various techniques available and their potential application in the field of ruminant microbiology (Zoetendal et al. 2004; Makkar and McSweeney 2005). This paper is primarily aimed to focus on DNA/RNA based molecular methods which have recently been developed for describe the diversity of rumen bacterial population.

The bacteria

The rumen bacteria are 99.5% obligately anaerobic. In rumen 200 species with many subspecies of bacteria are present. Rumen bacteria mainly involved in the fermentation of fiber, starch and sugar in the feed and converted into volatile fatty acid, H_2 and CO_2. Microbial protein in the rumen was mainly produced by that fermentation process (Leedle et al. 1982). Much research which has working molecular analysis of

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rumen bacteria has hub on the role of the three main fibre degrading bacteria *Fibrobacter succinogenes, Ruminococcus albus and Ruminococcus flavefaciens*. Many studies have published different sets of PCR primers for study of the rumen bacteria. The use of some of these primer sets and the relative advantages of each have been evaluated and discussed by Mosoni et al. (2007). Many primer pairs have now been published for exposure of other rumen bacterial population (*Anaerovibrio lipolytica, Butyrivibrio fibrisolvens, Eubacterium ruminantium, Prevotella albensis, P. brevis, P. bryantii, P. ruminicola, Ruminobacter amylophilus, Genus Prevotella*) using different qPCR assays (Stevenson and Weimer 2007; Tajima et al. 2001). Specific primers for *Megasphaera elsdenii* have also been available for studies on rumen acidosis which is a common disease (Ouwerkerk et al. 2002).

Extraction of DNA from rumen fluid for molecular studies

All molecular techniques mostly involves in the study of genomic community DNA directly extracted from rumen samples. Genomic DNA is important as, it representing the complete diversity of rumen microbial communities. Many DNA extraction methods, including commercial kits, have been tried on rumen samples (Forster et al. 1997; Whitford et al. 1998; Krause et al. 2001; Sharma et al. 2003). But problems are seen in terms of comparatively low DNA yields and/or recovering inhibitory substances free DNA. A new repeated bead beating plus column (RBB+C) method was found to be superior to other methods because it improved DNA yields more than 5-fold (Yu and Morrison, 2004).

Monitoring the cellulolytic bacteria of the rumen

Most research in this field has centred on the role of the three predominant fibre degrading bacteria as *Fibrobacter succinogenes, Ruminococcus albus and Ruminococcus flavefaciens* (McSweeney et al. 2009).

16S rRNA probing and PCR

The first quantitative molecular methods to appear for monitoring specific populations within the rumen were performed employing 16S rRNA probing techniques and then later competitive PCR (Briescaher et al. 1992). These studies specify bacterial populations from animals fed on various diets and at various times per day which indicated F. succinogenes as the predominant cellulolytic bacteria (Dore et al. 1993). With the advancement of molecular methods, in particular 16S rRNA probing techniques, researchers were able to monitor bacterial species within the rumen (Stahl et al. 1988).

PCR Primers and rumen bacteria

Different authors have published different sets of PCR primers that have been designed to amplify each of fibre degrading bacteria of the rumen. The use of some of these primer sets have been discussed in table 1. It appears that primer design for these bacteria has been sufficiently developed and can be worked with assurance in the quantitative specificity of the results. The quantification of the three species is highly dependent on the quantity and quality of the DNA extracted from rumen samples. The DNA extraction method used in different studies for PCR running is optimize by Yu and Morrison (2004) to fulfil desired conditions and to get the most representative DNA of the whole microbial community (Mosoni et al. 2007). Primer pairs have now been published for detection of other rumen bacteria (*Anaerovibrio lipolytica, Butyrivibrio fibrisolvens, Eubacterium ruminantium, Prevotella albensis, P. brevis, P. bryantii, P. ruminicola, Ruminobacter amylophilus, Selenomonas ruminantium, Streptococcus bovis, Succinivibrio dextrinisolvens, Treponema bryantii, Genus Prevotella*) by using qPCR methods (Stevenson and Weimer 2007; Weimer et al. 2008). Primers for *Megasphaera elsdenii* have also been developed for studies on rumen acidosis (Ouwerkerk et al. 2002).

Table 1. Oligonucleotide primers for fiber degrading bacteria (Mosoni et al. 2007)

Reference	Forward primer	Reverse primer	Bacteria
Koike and Kobayashi (2001)	5 ¢ -CCCTAAAAGCAGTCTTAGTTCG-3	5 ¢ -CCTCCTTGCGGTTAGAACA-3 ¢	*Ruminococcus albus*
Tajima et al. (2001)	5 ¢ -GGTATGGGATGAGCTTGC-3 ¢	5 ¢ -GCCTGCCCCTGAACTATC-3 ¢	*Fibrobacter succinogenes*
Tajima et al. (2001)	5 ¢ -GGACGATAATGACGGTACTT-3 ¢	5 ¢ -GCAATC(CT)GAACTGGGACAAT-3	*Ruminococcus flavefaciens*

Real-time PCR

It is a powerful tool that allows for the rapid quantification of a target DNA sequence (Freeman et al. 1999). Design of specific primer sets targeted against the 16S rDNA and the use of standard curves generated from known cell numbers allow for absolute quantification (Krause et al. 1999). Researchers have shown that this technique can be used successfully on nucleic acids extracted from rumen contents to monitor microbial populations in the rumen (Tajima et al. 2001; Ouwerkerk et al. 2002; Klieve et al. 2003).

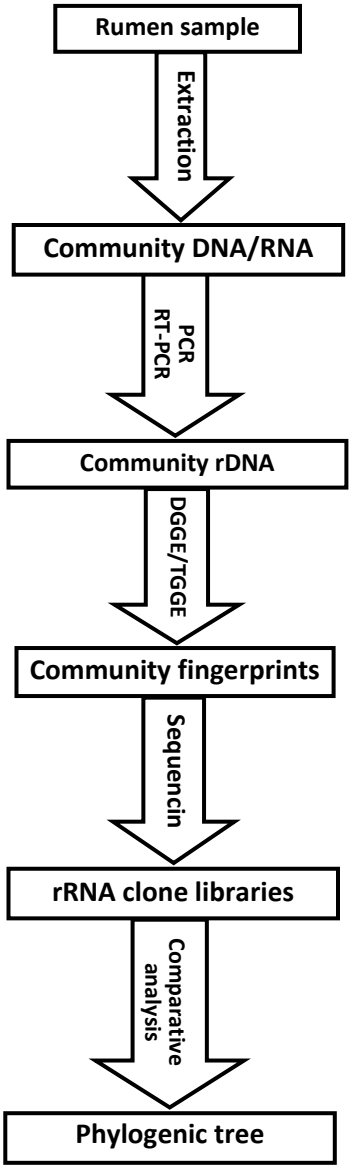

Figure 1*. Current molecular schematics used to analyze rumen microbial ecosystem (Modified from Zoetendal et al. 2004)*

However, care must be taken when designing primers, as Tajima et al. (2001) were able to demonstrate that the 16S rDNA gene from different rumen bacteria exhibited varying rates of amplification. Standard curves generated from pure cultures of the target species must also be carefully investigated. Ouwerkerk et al. (2002) were able to demonstrate that when the target cells were added to fresh rumen fluid prior to DNA extraction they were able to obtain more accurate values.

Target cells that were diluted in buffer without the addition of rumen fluid resulted in a standard curve that would have underestimated the true rumen population by almost 10 times (Ouwerkerk et al. 2002). This is most likely due to potential PCR inhibitory compounds that were present within the rumen fluid. The exact nature of these inhibitory compounds is not known, some studies indicate that it may be a carbohydrates (water-soluble polysaccharides) (Reilly and Attwood, 1998) or polyphenolic compound similar in nature to the humic acids (Leser 1998). The use of a SYBR Green real-time PCR assay to study two fibrolytic bacterial species, F. succinogenes and R. flavefaciens and recently describes the total anaerobic fungal population (Denman and McSweeney, 2005).

Metagenomics

Metagenomics is the genomic analysis of microbial communities without culturing (Handelsman, 2004). The word is comes from the statistical concept of meta-analysis, which means the process of statistically combining separate analyses, and genomics, means the comprehensive analysis of an organism's genetic material (Rondon et al. 2000). Metagenomics mostly used to studying prokaryotes in the environment that are not culturable and which represent more than 99% of the organisms in some environments (Amann et al. 1995). This method is build on recent advances in microbial genomics and in the polymerase chain reaction amplification and gene cloning that have similarity in sequence (McSweeney et al. 2009)

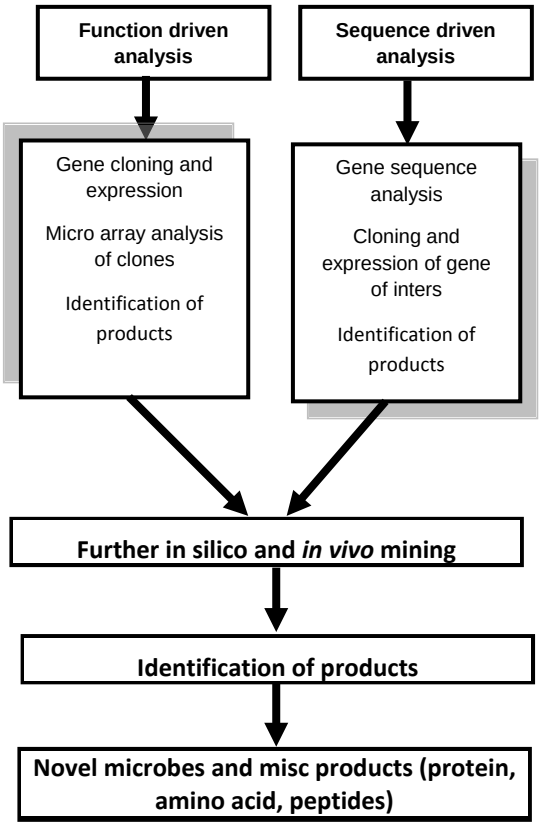

Figure 2. *Omic based approaches to identified novel rumen ecosystem*

Metagenome technologies, DNA extraction, library construction, screening

The basic steps in metagenomics are the extraction of DNA from the microbial community of rumen then cloning of the DNA fragments in the host using a vector which results in a library of many thousands of clones (Fig 2). The clone library then screened using DNA sequences in a PCR. Ultimately metagenomic analysis creates a list of genomic information which can be cross-examined to see close information related to main genes that how they are regulated and their role to ecosystem environment. By using this approach, a list of the genetic capability of the ecosystem can be resolute as well as given that a road to identify novel genes or genes of unknown function from the environment. This approach has been employed to screen rumen-DNA metagenomic libraries for enzymes involved in degrading of lignocelluloses bond in the fibrous feed of ruminates (Beloqui et al. 2006). Many new enzymes have been discovered by metagenomics (Ferrer et al. 2005; 2007). A bacterial artificial chromosome (BAC) library has also been constructed from the rumen of a dairy cow and is being used to screen for novel enzyme activities (Zhu et al. 2007).

Conclusions

DNA-based techniques will allow the genomic level study of individual bacterial cell and rumen samples from different ruminant. These methods are processed rapidly and also not very much expensive. This will help study the structure and function of complex microbial ecosystem and detailed analysis of important rumen microbes. These technologies have the ability to revise the understanding of rumen fermentation. However, an improved knowledge of microbial function and ecology will need to be incorporated with nutritional principles for the improvement of ruminant productivity.

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