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Popular Article

Concepts of Metagenomics in Rumen Manipulation

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Abstract

The genetic and biological variety of microbes is an important topic of scientific study. The ability of ruminants to convert locally available feedstock to animal products should be enhanced given the significance of ruminants in livestock strategy. The structure and function of rumen communities may now be studied more comprehensively as a result of recent developments in molecular biology and genomics. Today's rumen microbiologists face a significant difficulty in trying to comprehend how complex microbial communities' function and how organisms interact within their niches. In order to identify the complex community of bacteria, protozoa, archae, and fungi, among others, and to understand how they interact, metagenomics is helpful. Understanding how microbial communities function is one of the main objectives of rumen microbiology. It aids in both the immunomodulation of both animals and poultry as well as the formulation of feed ingredients containing probiotics.

INTRODUCTION

Microbial populations are essential to life on Earth and have huge practical implications in medicine, engineering, and agriculture (Sloan *et al*., 2006). Rumen microbial diversity is constituted of bacteria, archaea, fungus, protozoa, and virus in varying proportions depending on the rumen habitat. The rumen's stability and dynamics are critical in maintaining balance within the rumen ecosystem, where there is equilibrium between the microbial community and their metabolism. When new feed types or new organism enter the rumen ecosystem, the balance is disrupted. The change is possible only if the entering new microbes fit into the rumen environment, if not the microbes are eliminated. Rumen is anaerobic in nature, the feed particles in rumen trap small air pockets containing oxygen which are used by the facultative anaerobes ensuring perfect anaerobic condition. Rumen ecosystem diversity, anaerobiosis, pH and various other factors make it difficult to culture the

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organism. The synergetic and antagonistic effect on account of feeding different type of feeds makes it difficult to quantify the role played by any particular group of microbes among the consortia inside the rumen. The conventional technique of culture-dependent cloning gives a limited and biased knowledge about the prevailing rumen microbial population. The global microbial diversity presents an enormous, largely untapped genetic and biological pool that could be exploited for the recovery of novel genes, biomolecules for metabolic pathways and various valuable products (Cowan, 2000). However, current research indicates that more than 99% of microorganisms in the environment are not easily cultivable (Hanada, 2003; Kamagata and Tamaki, 2005; Sekiguchi, 2006). The knowledge about the prevailing organism in the rumen and an insight of the rumen microbial changes during different conditions can be studied with the help of next-generation sequencing (NGS) technique – metagenomics. Metagenomics has recently emerged as a highly strong approach for analysing microbial communities regardless of individual microbial culturing conditions. Metagenomics isolates DNA from the whole community, to sequence and to analyze the obtained data to provide intervention, microbial understanding, therapeutic and biotechnological applications.

The term ‘‘metagenomics’’ was first coined by Handelsman *et al*., 1998 to study the genomes from all microbes in a particular environment as opposed to the genome from one organism isolated from the environment and cultured in vitro. Metagenomics is a rapidly expending field of study that aims at studying uncultured organisms to understand the true diversity of microbes, their functions, cooperation and evolution, in environments such as soil, water, ancient remains of animals, or the digestive system of animals and humans (Huson *et al*., 2009).

Application of rumen metagenomics:

The invention and application of metagenomics has provided access to the uncultivated ecosystem as well as insight into the metabolic capacities of microbial communities that have yet to be grown. Here are some examples of metagenomics applications:

Rumen Lipolysis and Biohydrogenation:

The metagenomics in this field have to access the microbial ecology of lipolysis and biohydrogenation, as well as develop techniques to manipulate ruminal microbes to increase the flow of PUFA (Polyunsaturated Fatty Acids) and CLA (Conjugated Linoleic Acid) from the rumen into meat and milk. Because of the fatty acids that escape ruminal metabolism, the quantity and composition of dietary lipids have a significant impact. Fatty acids, on the other hand, may have a direct modifying effect by inhibiting biohydrogenation. Biohydrogenation is affected indirectly too when other activities are changed, because fatty acid metabolism is inextricably linked to other areas of ruminal metabolism, through a common reliance on H_2 metabolism or the microbial species that are involved in multiple metabolic processes (Lourenço *et al*. 2010).

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Identification of novel enzymes/microbes from rumen:

Metagenomics must be used to screen and detect novel microorganisms and biomolecules from the GI tracts of the livestock ruminants adapted to the forages or diets enriched with high fiber and an array of antinutritional Plant Secondary Metabolites (PSMs) such as tannin-polyphenols.

A metagenome expression library of bulk DNA extracted from the rumen content of a dairy cow was established in a phage lambda vector and activity-based screening was used to investigate the functional diversity of the microbial flora.

The investigations have shown that a metagenomic method can be used to obtain novel debranching enzymes, which are vital for the bread/food industries, from microbial habitats with a high rate of plant polymer turnover, such as the cow rumen.

In another study, RL5 (EMBL/DDBJ/GenBankTM accession number AM269758 [GenBank]), a gene coding for a novel Laccases (polyphenol oxidase) was found through activity screening of a metagenome expression library from the bovine rumen micro flora. Laccases in the rumen may be significant in ryegrass lignin breakdown, suggesting that the RL5 enzyme has biotechnological potential for use in pasture-fed animals and pasture grasses. Finally, the study highlights the ability and utility of activity-based metagenomics for exploring functional diversity space and discovering novel enzymes with laccase activity in a protein that has no relationship to any previously reported polyphenol oxidase (Beloqui *et al*., 2006).

Identification of uncultured methanogens:

Methanogens belong to the domain Archaea and are part of the kingdom Euyarchaeota. They are obligate anaerobes and produced methane as a major catabolic product (Bergey, 1994). Interest in ruminal methanogen is on account of the role of methane in global warming and from the fact that enteric methane emission is a key source of greenhouse gas in agriculture sector.

Molecular approaches have been used to identify methanogens in the rumen. A temporal Temperature Gradient Gel Electrophoresis (TGGE) method evaluated to determine the diversity of methanogens in cattle and sheep rumens showed that uncultured methanogens account for the majority of methanogenic archaea in the rumen (Nicholson *et al*., 2007).

Elaborating the molecular mechanisms of association patterns between archaea and rumen protozoa would be helpful in developing strategies to reduce methane emissions by dietary or genetic manipulation of the rumen ecosystem.

Determination and Quantification of rumen biomass:

The quantitative assessment of total rumen microbial biomass and the differentiation of bacterial and protozoal biomass are important applications of microbial metagenomics in animal nutrition. Rapid profiling techniques, such as the real-time PCR assay, can be used to infer likely variations in the community structure of bacteria and archaea present in animals and at different

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periods after feeding diets.

Rumen nitrogen metabolism:

A better understanding of mechanistic process altering the production and uptake of amino nitrogen will help the livestock nutritionists to improve the overall conversion of dietary nitrogen into microbial protein. It will give critical information for further improving the mechanistic models defining rumen function and analysing dietary circumstances that influence the efficiency of dietary nitrogen conversion into milk protein (Firkins *et al*. 2007).

CONCLUSION:

The use of metagenomic libraries derived from distinct rumen habitats as a strategy for successfully exploiting the mainly "untapped" resources found in varied rumen ecosystems. The rumen microbial community is distinguished by its high population density, vast diversity, and interaction complexity. These diverse ecosystems are potentially very useful sources for novel enzymes with distinct characteristics and great biotechnological potential. Only a small percentage of rumen microbial bioresources have been studied, and an even smaller portion has been used. The inability to culture the vast majority of microbes from this ecosystem the metagenomic approaches the sole method currently available to access these unique and useful bioresources. There is an ongoing need for a wide range of novel genes and enzymes which are required to improve fiber digestion, increasing digestibility of low quality forage, by the selected elite rumen flora and explore the nutrients-host tissue interaction. Rumen metagenomics, in conjunction with biotechnology, has the potential to contribute to all these pressing needs. These technologies have the potential to revolutionize the understanding of rumen function and will overcome the limitations of traditional techniques, including isolation and taxonomic identification of strains important to efficient rumen function and better understanding of the roles of microorganisms in relation to achieving high productivity and reducing environmental pollutants.

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