METHODS IN GUT MICROBIAL ECOLOGY FOR RUMINANTS

Methods in Gut Microbial Ecology for Ruminants

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Contents

Foreword vii
Introduction ix
Editorial Note xi

Part 1. Designing in-vivo Microbial Ecology Studies

1.1. Experimental designs for rumen microbiology 03
Adrian R. Egan

Part 2. Classical Methods for Isolation, Enumeration, Cultivation and Functional Assays of Rumen Microbes

2.1. Rumen bacteria 23
Christopher S. McSweeney, Stuart E. Denman and Roderick I. Mackie

2.2. Bacteriophages 39
Athol V. Klieve

2.3. Methanogenic archaea 47
Keith N. Joblin

2.4. Anaerobic fungi 55
Michael K. Theodorou, Jayne Brookman and Anthony P.J. Trinci

2.5. Ciliate protozoa 67
Burk A. Dehority

Part 3. PCR-Based Methods for Analysis of Populations and Gene Expression

3.1. Nucleic acid extraction, oligonucleotide probes and PCR methods 81
Zhongtang Yu and Robert J. Forster

3.2. Quantitative (real-time) PCR 105
Stuart E. Denman and Christopher S. McSweeney

Part 4. Molecular Fingerprinting Techniques for Genotypic Analysis of Pure Cultures and Microbial Communities

4.1. Denaturing gradient gel electrophoresis 119
Svetlana A. Kocherginskaya, Isaac K.O. Cann and Roderick I. Mackie

4.2. Bacteriophage populations 129
Athol V. Klieve and Rosalind A. Gilbert

4.3. Anaerobic fungal populations 139
Jayne L. Brookman and Matthew J. Nicholson

4.4. RAPD, RFLP, T-RFLP, AFLP, RISA 151
Stuart E. Denman, Makoto Mitsumori and Christopher S. McSweeney

Part 5. DNA Clone Libraries of Microbial Communities

5.1. 16S/18S ribosomal DNA clone library analysis of rumen microbial diversity 163
André-Denis G. Wright, Kiyoshi Tajima and Rustam I. Aminov

Part 6. Use of Small Subunit Ribosomal RNA Directed Oligonucleotide Probes for Microbial Population Studies

6.1. Northern blot analysis to investigate the abundance of micro-organisms 177
Denis O. Krause

6.2. Whole cell probing with fluorescently labelled probes for *in situ* analysis of microbial populations 191
Linda L. Blackall

6.3. Combined fluorescence in situ hybridization and microautoradiography (FISH-MAR) 201
Maneesha P. Ginige

Part 7. Genomic Analysis of Microbial Ecosystems

7.1. Metagenomic analysis of the microbiomes in ruminants and other herbivores 209
Mark Morrison, Sarah E. Adams, Karen E. Nelson and Graeme T. Attwood

Chapterwise Keywords 221

Keyword Index 223

Foreword

As a result of various human activities, such as increase in human population, decrease in arable land due to soil degradation, urbanization, industrialization and associated increase in the demand for livestock products, dramatic changes are occurring in the global ruminant livestock sector. These changes include shift in the size of regional livestock populations and in the types of management and feeding systems under which ruminant livestock are held, and increased demand of a wider range of quality attributes from animal agriculture, not just of the products themselves but also of the methods used in their production. The livestock sector will need to respond to new challenges of increasing livestock productivity while protecting environment and human health and conserving biodiversity and natural resources.

The micro-organisms in the digestive tracts of ruminant livestock have a profound influence on the conversion of feed into end products, which can impact on the animal and the environment. As the livestock sector grows particularly in developing countries, there will be an increasing need to understand these processes for better management and use of both feed and other natural resources that underpin the development of sustainable feeding systems.

Until recently, knowledge of ruminant gut microbiology was primarily obtained using classical culture-based techniques, such as isolation, enumeration and nutritional characterization, which probably only account for 10–20% of the rumen microbial population. New gene-based technologies can now be employed to examine microbial diversity through the use of small sub-unit ribosomal DNA analysis (e.g. 16S rDNA) and to understand the function of complex microbial ecosystems in the rumen through metagenomic analysis. These technologies have the potential to revolutionize the understanding of rumen function and will overcome the limitations of classical-based techniques, including isolation and taxonomic identification of strains important to efficient rumen function and better understanding of the roles of micro-organisms in relation to achieving high productivity and decreasing environmental pollutants.

This book has been produced by the Joint FAO/IAEA Division of Nuclear Technique in Food and Agriculture, IAEA Vienna, Austria in collaboration with the CSIRO Livestock Industries, Brisbane, Australia. It gives a comprehensive up-to-date account of the methodologies and the protocols for conventional and modern molecular

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techniques that are currently in use for studying the gut microbial ecology of ruminants. Each chapter has been contributed by experts in the field. The techniques and procedures described are also relevant and adaptable to other gastrointestinal ecosystems and the microbiology of anaerobic environments in general. The future of ruminant gut microbiology research is dependent upon the adoption of these molecular-based research technologies, and the challenge at present is the use of these technologies to improve ruminant production and decrease environment pollutants through a better understanding of microbial function and ecology. It is hoped that this book will equip the readers better in order to meet this unprecedented challenge.

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Introduction

Current approaches to the evaluation of digestibility and the nutritive value of feed resources using conventional *in vitro* feed evaluation and animal studies have resulted in a large body of information about nutrient composition, digestion kinetics and digestibility. However, these techniques are unable to describe the microbial mechanisms involved in digestion by ruminants and other herbivores, and are unlikely to result in the development of new feeding strategies. Conventional culture-based methods of enumerating and identifying rumen bacteria are being rapidly replaced by the development of nucleic acid-based techniques that can be used to characterise complex microbial communities. Ruminant nutritionists and microbiologists have recognized the importance of molecular microbial ecology, but many have found it difficult to employ the most appropriate techniques because they are not familiar with the methods. In addition, this field is developing very rapidly and even researchers with experience in molecular microbial ecology find it difficult to keep abreast with the increasing number of techniques and alternatives.

This manual is written by an expert group of scientists interested in ruminant digestion and gut microbiology. The most recent and up-to-date methods in molecular microbial ecology with special emphasis on ruminants are collated and interpreted in this book. The methods will provide the readers an easy access to molecular techniques that are most relevant and useful to their area of interest. The authors have attempted to write in a recipe-like format designed for direct practical use in the laboratory and also to provide insight into the most appropriate techniques, their applications and the type of information that could be expected. These aspects have been supported by inclusion of the relevant literature.

The contents of the manual are presented in a sequence that recognizes the key elements in studying gut microbial ecology. The first chapter provides a perspective on how to design animal trials in which microbial ecology is studied. Often the power of the new molecular techniques is diminished by an inappropriate design in terms of animal number, sampling frequency, location and replication. The second chapter describes the classical culture-based methods for studying rumen microbes, as these methods are often a pre-requisite to employing molecular techniques. Chapters 3–6 provide information on the basic underpinning techniques and the protocols in

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molecular ecology, such as DNA extraction from environmental samples, the polymerase chain reaction (PCR), oligonucleotide probe and primer design and DNA fingerprinting amongst others. The application of these techniques to microbial detection and identification are discussed. Specialized techniques such as denaturing gradient gel electrophoresis (DGGE) and 16S/18S ribosomal DNA libraries for studying complex communities that contain unculturable organisms are also described. Many of these techniques are used to identify and enumerate the population of organisms that are present in a sample. However, the field is rapidly moving to a functional analysis of the microbes in an ecosystem, and some of the methods being employed to measure genes expression are described in Chapter 3. In Chapter 6, knowledge about location and spatial relationships of micro-organisms in their natural environment that are often essential for understanding the function of these organisms are discussed. The final chapter deals with metagenomic technologies, which provide the potential to capture and study the entire microbiome (the predominant genomes) from a complex microbial community, such as the rumen. The rapid high-throughput technologies developed in mapping the human genome are now being deployed to study microbial ecosystems. An explosion of knowledge in the field of microbial ecology is now expected.

The editors wish to acknowledge the contributions made by all the authors who participated in the publication of this manual. They have spent considerable time gathering information from many sources into a focussed document that enables the reader to understand how techniques have evolved and the context in which the methods should be applied to address specific issues relating to gut microbial ecology. We believe that this manual will 'demystify' the methods in molecular microbial ecology for readers, who are novice in the field but are excited by the prospects of the technology. It would also be invaluable for the experienced workers striving for giving new dimension to their research – expanding the work in other fields and initiating cross-cutting activities. This manual is seen as the first step towards understanding and manipulating gut micro-organisms as it is expected that the techniques and the methodologies associated with the study of molecular microbial ecology will continue to grow and evolve. A key challenge for the future will be the simplification of these techniques, so that these become tools of routine use in nutritional, environmental and ecological laboratories.

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