# Genome Sequencing of Rumen Bacteria Involved in Lignocellulose Digestion

A thesis presented in fulfilment of the requirements for the degree of

Master of Science

in the

Institute of Molecular Biosciences

At Massey University, Palmerston North,

New Zealand.

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA

Nikola Palevich

2011

# Abstract

Determining the role of rumen microbes and their enzymes in plant polysaccharide breakdown is fundamental to understanding digestion and maximising productivity in ruminant animals. In order to learn more about lignocellulose degradation in pasture-grazed dairy cows under NZ conditions, twenty representative strains from five major phylotype clusters (*Butyrivibrio fibrisolvens/hungatei* cluster 383, *Pseudobutyrivibrio xylanivorans* clusters 247 and 245, *Selenomonas ruminantium* cluster 212, and *Lachnospiraceae* cluster 121), cultivated directly from the fibre-adherent rumen microbial fraction of dairy cows were selected. Genotypic and phenotypic analysis of these strains led to identification of *Butyrivibrio* sp. MB2003 that adheres to and efficiently degrades the plant fibre. The 3.3 Mb MB2003 genome was sequenced and annotated and found to consist of four replicons: a chromosome (7 contigs, in 1 super scaffold), a chromid (Bhu II), a megaplasmid (pNP144) and a small plasmid (pNP6). A novel feature of the MB2003 genome is the presence of a chromid (Bhu II) which is now the smallest chromid reported for all bacteria. The MB2003 polysaccharide-degrading enzymes, surface structures and predicted strategy for attachment to, and degradation of, complex polysaccharides was found to be comparable to that of the fibrolytic bacterium *Butyrivibrio proteoclasticus* B316. Both MB2003 and B316 are non-motile, despite the presence of flagellar gene clusters, and utilise a range of insoluble plant polysaccharides, but not cellulose. Xylan is the preferred insoluble substrate of MB2003 and its genome encodes a large repertoire of enzymes predicted to metabolise this complex polysaccharide. The MB2003 draft genome produced in this work is the first opportunity to conduct comparative analysis of two rumen bacteria belonging to the same genus. Although both MB2003 and B316 have similar phenotypic characteristics and occupy the same habitat, the genome of MB2003 is much smaller and contains fewer extracellular polysaccharide degrading enzymes. From this comparison it can be concluded that MB2003 is a secondary hemicellulose degrader, offering an alternate view of the genes required for a xylanolytic lifestyle in the rumen, and posing an interesting question about the purpose of the wider range of polysaccharide degrading enzymes found in B316.

## Acknowledgements

To my supervisors Dr Graeme Attwood, Dr William (Bill) Kelly and Dr Jasna Rakonjac, thank you for taking me on as a Masters student and for your encouragement throughout this journey. I trust that my work has met your expectations and hope that you have enjoyed teaching me as I have enjoyed learning from all of you.

To the great people of AgResearch for their financial support and making this project possible.

I wish to thank the AgR and FRST for NERF funding for the "Accessing the uncultured rumen microbiome" programme (contract number C10X0803) and financial support for this MSc scholarship.

To the Rumen Nutrition and Microbiology Team particularly Dr Sinead Leahy and Dr Eric Altermann for your guidance with the assembly, annotation and all things computational, Dr Adrian Cookson, Dr Christina Moon and Dr Peter Janssen for your input throughout this project.

To the Rumen microbiology lab, namely Dong Li (aka "the machine"), Dr Gemma Henderson and Samantha Noel thank you for showing me the ropes. I've learned many things from you.

To the students at AgResearch thank you for all the laughs and conversations, in the end it's what gets you through the day. I wish you all the best in what I'm sure will be bright futures.

To my love Faith Cox, thank you for keeping me sane and being my anchor throughout this testing time, even when I was at my most "unappetising" self. I hope you know that the work I do is for our future.

To my mum Desanka Palevich and nana Ruzica Radulovic words cannot express the love and strength that you give me to carry on each and every day. It seems as though there is nothing I can give you in return for your gifts, but I hope you know that…

"Everything I do. I do it for you!" (Bryan Adams)

# Table of Contents

## Figures

## Tables

## Abbreviations

| | |
|---|---|
| bp, kb, Mbp | base pair(s), kilo base(s), mega base pair(s) |
| CDS | Coding Sequences |
| COGs | Clusters of Orthologous Groups |
| DAPI | 4', 6-diamidino-2-phenylindole |
| EC | enzyme classification |
| EDTA | ethylenediamine tetraacetic acid |
| EPS | Extracellular Polysaccharides |
| g | gram(s) |
| hr | hour(s) |
| mg | milligram(s) |
| min | minute(s) |
| ml | millilitre |
| mM, M | milliMolar, Molar |
| mV | milliVolts |
| NDF | Neutral Detergent Fibre |
| nt | nucleotide(s) |
| ORFs | Open Reading Frames |
| sec | second(s) |
| TE | Tris-EDTA buffer |
| v/v | volume : volume ratio |
| w/v | weight : volume ratio |