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**Identifying and improving the fibre-degrading  
activity of rumen microbe-derived fibrolytic  
bacteria**

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

Master of Science

in

Genetics

at Massey University, Manawatū, New Zealand.

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2017

## Abstract

The dairy industry is extremely important to the New Zealand (NZ) economy, and it accounts for approximately \$16.6 billion in exports each year (1). In NZ, traditional feedstock for cattle, such as grass and hay, consists of cellulose-based fibrous material that have limited nutritional value due to their inherent resistance to degradation. As commercially available feed treatments that use fibrolytic enzymes (FEs) from aerobic fungi are not currently available in NZ, it is possible that pre-treatment of such foodstuffs with rumen microbe-derived FEs could enhance fibre degradation, boosting animal performance and productivity as such enzymes should be better suited to the anaerobic conditions of the rumen.

The main aim of this thesis was to identify effective fibre-degrading rumen bacteria and attempt to improve their fibre-degrading, or fibrolytic, activity using non-genetically modified methods. This was carried out by culturing 15 different rumen bacterial isolates on five separate fibrous substrates, which resulted in 46 strain/substrate combinations that were screened for fibrolytic activity.

The fibrolytic activity of each strain/substrate combination was assessed using two distinct biochemical assays: 1) degradation of oat spelt xylan (OSX) or filter paper (FP), and 2) degradation of *para*-nitrophenol-conjugated substrates that represent major biochemical linkages in the plant cell wall. Three candidate strains were chosen based on these results to improve fibrolytic activity further using mutagenesis and positive selection, and of these, two strains showed a statistically significant increase in fibrolytic activity after 31 subcultures on ryegrass (RG). The secretomes of these two strains was then investigated using proteomic methods, which included 1D SDS PAGE, in-gel trypsin digest and mass spectrometry.

The overall results from this research serve as a foundation for the development of a feed treatment to be used in NZ, which could provide financial benefit not only to dairy farmers, but the NZ economy as well.

## Acknowledgements

First and foremost, I would like to sincerely thank the contribution from each of my supervisors, Dr Graeme Attwood, Dr Christina Moon, A/Prof Gill Norris and Dr Mark Patchett, towards my postgraduate study. Without their direction, recommendations, support and corrections, this research and thesis would not have been possible. This project has been a tremendous learning curve for me, and I am truly appreciative of having excellent supervisors with an infinite supply of invaluable advice to guide me through it.

I would also like to thank Dr Bill Kelly, Dr Dragana Gagic and Mr Trevor Loo for their input into the research component of this project. I am extremely privileged that each of them was willing to offer me their expertise in their respective fields, and this helped immensely with my research.

To Ann Truter and Sharron Smith, I would like to say thank you for your administrative support, which was paramount in ensuring that this thesis was completed on time and in the correct format, and for answering the numerous questions I had pertaining to such issues as the submission of internal assignments and the external release of information from AgResearch.

Lastly, I would like to acknowledge Massey University and AgResearch, particularly the Rumen Microbiology team, for the opportunity to complete my Masters in such an interesting field. The research component of my studies was partly funded by AgResearch Core funding contract A20561, and I was also extremely fortunate to receive scholarships from Pūrehuroa Awards, Massey University, and Paraninihi Trust, Taranaki, which contributed directly to my study fees. Thank you kindly!

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**List of abbreviations**

AmBic	Ammonium bicarbonate
AP	Apple pectin
APS	Ammonium persulphate
BSA	Bovine serum albumin
BTP	Bis-tris propane
CFUs	Colony forming units
CP	Cell pellet
dH <sub>2</sub> O	Distilled water
DNA	Deoxyribonucleic acid
DNS	3,5 dinitrosalicylic acid
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
EMS	Ethyl methanesulfonate
FDR	False discovery rate
FE	Fibrolytic enzymes
Fig	Figure
FP	Filter paper
g	Gram
GenRFV	General rumen fluid with vitamins
GH	Glycoside hydrolase
GM	Genetic modification
GMO	Genetically modified organism
hr	Hour
ICRF	Incubated clarified rumen fluid
ID	Identification
kb	Kilobase
kD	Kilodalton
L	Litre
LOD	Limit of detection
LOQ	Limit of quantification
MES	2-ethanesulfonic acid
μL	Microlitre

Preface

mg	Milligram
mL	Millilitre
MQ	MilliQ water
MW	Molecular weight
NDF	Neutral detergent fibre
nm	Nanometres
NZ	New Zealand
OD	Optical density
OSX	Oat spelt xylan
PCR	Polymerase chain reaction
<i>p</i> NP	<i>Para</i> -nitrophenol
RG	Perennial ryegrass
RM02	Rumen Microbiology media series number 2
RPM	Revolutions per minute
rRNA	ribosomal ribonucleic acid
RT	Room temperature
sec	Seconds
SDS	Sodium dodecyl sulphate
SDS PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SE	Standard error
SN	Supernatant
TAE	Tris-acetate EDTA buffer
TEMED	Tetramethylethylenediamine
UV	Ultraviolet
2SGenRFV	2x sugar and general rumen fluid with vitamin