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

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## 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.

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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 |
| $dH_2O$ | Distilled water |
| DNA | Deoxyribonucleic acid |
| DNS | 3,5 dinitrosalicyclic 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 |

| | |
|---|---|
| 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 |
| *p*NP | *Para*-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 |