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Comparative genomics of
*Butyrivibrio* and *Pseudobutyrvibrio*
from the rumen

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

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Abstract

Determining the role of rumen microbes in plant polysaccharide breakdown is fundamental to understanding digestion, and maximising productivity, in ruminant animals. Rumen bacterial species belonging to the genera *Butyrivibrio* and *Pseudobutyryrivibrio* are important degraders of plant hemicellulose, an abundant heterogeneous, branched polymer, involved in crosslinking cellulose microfibrils to lignin. To investigate their genes required for hemicellulose degradation, the genomes of 40 *Butyrivibrio* and 6 *Pseudobutyryrivibrio* strains isolated from the plant-adherent microbiome of New Zealand bovine ruminants, were sequenced, and their CAZyme-encoding genes compared. Within the *Butyrivibrio* and *Pseudobutyryrivibrio* pan-genomes, respectively, there were a total of 4,421 and 441 glycoside hydrolases, as well as 1,283 and 122 carbohydrate esterases with predicted activities involved in the degradation of the insoluble plant polysaccharides such as xylan and pectin. To examine species differences, the genes of the previously characterised bacterium *B. proteoclasticus* B316 were compared in detail with those from the newly sequenced *B. hungatei* MB2003. B316 was found to encode a much more developed polysaccharide-degrading repertoire and it was thus hypothesised that B316 would out-compete MB2003 when grown in co-culture on the insoluble hemicellulose substrate, xylan. To test this hypothesis, the two strains were grown on xylan and pectin, either alone in mono-cultures, or in direct competition in a co-culture. The results showed that MB2003 had little ability to utilise xylan or pectin alone, but was capable of significant growth when co-cultured with B316. This indicates a commensalistic interaction between these species, in which B316 initiates the primary attack on the insoluble substrate, while MB2003 has a secondary role, competing for the released soluble sugars. This work provides the first systematic phenotypic, comparative genomic and functional analysis of ruminal *Butyrivibrio* and *Pseudobutyryrivibrio* species, which not only defines their conserved features involved in hemicellulose degradation, but is also beginning to differentiate their unique gene complements and growth characteristics that separate them as discrete species.
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Dedication

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<tr>
<td>AA</td>
<td>Auxiliary Activities</td>
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<td>aa</td>
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<td>ABC</td>
<td>ATP-binding cassette</td>
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<tr>
<td>ACS</td>
<td>American Chemical Society</td>
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<tr>
<td>AF</td>
<td>Alignment fraction</td>
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<tr>
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<td>Average nucleotide identity</td>
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<td>ANOVA</td>
<td>One-way analysis of variance</td>
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<td>BCVFA</td>
<td>Branched chain volatile fatty acids</td>
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<td>BLAST</td>
<td>Basic Local Alignment Sequence Tool</td>
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<td>Base pair(s)</td>
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<td>CA</td>
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<td>CAZY</td>
<td>Carbohydrate-Active enZYmes</td>
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