Cultivation and community composition analysis of plant-adherent rumen bacteria

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ABSTRACT

Ruminants have a symbiotic relationship with the complex community of microbes that reside within their rumen. These microbes are able to break down recalcitrant plant material that would otherwise be indigestible by the host. Ruminal bacteria that attach to the ingested plant material are important for the degradation of plant fibre. The number of bacteria cultured from the rumen is estimated to represent only some 10% of the total diversity. This has led to the belief that a large proportion of bacteria in the rumen are unculturable. In this study, liquid media that mimic the physico-chemical composition of the rumen, were used in combination with dilution to a single cell, to obtain >1000 cultures of anaerobic bacteria from the plant-adherent fraction of bovine rumen contents from 20 rumen samples. The phylogenetic affiliation of 828 of these cultures was assessed by comparative analysis of partial 16S rRNA gene sequences. There were 626 unique sequence types (V1-V3 of the 16S rRNA gene), and 200 of these isolates were novel (<96% similarity to a previously-cultured bacterium). The near full-length 16S rRNA gene sequences from 186 selected isolates (representing 801 of the total sequenced isolates) were classified into 14 families including two potentially new families, 77 genera including 59 potentially new genera, and 122 species including 103 potentially new species.

The total bacterial communities in the same rumen samples were characterised using FLX-titanium 16S rRNA gene amplicon pyrosequencing of the V1-V3 region of the 16S rRNA gene. These data were then compared with the isolates that had been cultured. The majority of the isolates and amplicon sequences were associated with the phyla Firmicutes and Bacteroidetes. Sequences were grouped into operational taxonomic units (OTUs) at 96% sequence similarity. At this level, 32% of the plant adherent community (i.e., total pyrosequences) and 7.7% of the observed diversity (i.e., unique OTUs) were in OTUs that contained a newly-cultivated isolate. More OTUs (169) contained a sequence from an isolate cultured for the first time in this study compared to the number of OTUs (103) that contained sequences from previously-isolated bacteria. The isolates gained in this study can begin to bridge the gap between the cultured and the uncultured.

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NON-STANDARD ABBREVIATIONS

BLASTN	basic local alignment sequence tool (necleotide)
bp	base pairs
DGGE	denaturing gradient gel electrophoresis
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
EDTA	ethylenediaminetetraacetic acid
h	hour
Ltd	Limited
M	molar
min	minutes
m/s	meters per second
ML	maximum likelihood
MPN	most probable number
NJ	Neighbor Joining
OTU	operational taxonomic unit
PCR	polymerase chain reaction
QIIME	quantitative insight into microbial ecology
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid
U	unit
UV	ultraviolet
VFA	volatile fatty acid
s	seconds
SDS	sodium dodecyl sulfate
L	litre
mL	millilitre
v/v	volume per volume
w/v	weight per volume
X-gal	5-bromo-4-chloro-indolyl-β-D-galactopyranoside
nt	nucleotide(s)