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Cultivation and community composition analysis of plant-adherent rumen bacteria

A thesis presented in partial fulfilment of the requirements for the
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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. Ruminant 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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TABLE OF CONTENTS

Abstract	iii
Acknowledgements	v
Table of contents	vii
List of tables	xv
List of figures	xviii
Non-standard abbreviations	xxiii
CHAPTER 1 LITERATURE REVIEW.....	1
1.1 Introduction – importance of agriculture to New Zealand.....	1
1.2 Ruminant animals	2
1.3 Ruminant digestive system	2
1.4 Forage fibre	4
1.4.1 Forages	4
1.4.2 Fibre	4
1.5 Rumen microbiota.....	7
1.6 Rumen bacteria.....	8
1.6.1 Rumen bacterial functions.....	8
1.6.2 Bacterial diversity	11
1.6.3 Bacterial attachment to feed.....	19
1.6.4 Diversity and activity of fibre-attached bacteria.....	20
1.7 Techniques to identify rumen bacteria - what makes a species	23
1.8 Molecular ecology techniques to study rumen communities.....	29
1.8.1 Automated ribosomal intergenic spacer analysis.....	29
1.8.2 Denaturing gradient gel electrophoresis.....	30
1.8.3 Real time quantitative PCR.....	31
1.8.4 Terminal restriction fragment length polymorphism analysis	32
1.8.5 16S rRNA gene clone libraries	32
1.8.6 Fluorescence <i>in situ</i> hybridisation.....	33
1.8.7 Pyrosequencing	33
1.9 Tools for analysing molecularly detected communities	34
1.10 Effect of methodology on community detection.....	35

1.11	Techniques for culturing bacteria	37
1.12	Medium design.....	41
1.13	Cultured bacteria from the rumen	42
1.13.1	Why it is difficult to culture	44
1.13.2	Why culture?	45
1.14	Aims of thesis or research gaps.....	46
CHAPTER 2 MATERIALS AND METHODS		47
2.1	Materials.....	47
2.1.1	DAPI stain.....	47
2.1.2	DGGE 2× Gel loading dye.....	47
2.1.3	DNA-free water.....	47
2.1.4	10× Green loading dye.....	47
2.1.5	PCR reagents.....	48
2.1.6	Primers	48
2.1.7	1× PBS	48
2.1.8	4% paraformaldehyde solution	48
2.1.9	Modified Karnovsky's fixative	49
2.1.10	50× TAE buffer.....	49
2.1.11	1× TAE buffer.....	49
2.2	Media	50
2.2.1	Media solutions	50
2.2.1.1	Salt solution A.....	50
2.2.1.2	Mineral solution I (Salt solution 2B)	50
2.2.1.3	Mineral solution II	50
2.2.1.4	Na ₂ CO ₃ solution.....	50
2.2.1.5	Reducing agent.....	51
2.2.1.6	Selenite / Tungstate solution	51
2.2.1.7	Trace element solution SL10	52
2.2.1.8	Vitamin 10 concentrate	53
2.2.2	Media additives	54
2.2.2.1	Anaerobic glycerol solution	54
2.2.2.2	Rumen fluid collection for preparation of media.....	55

2.2.2.3	Clarified rumen fluid for preparation of media	55
2.2.2.4	GenRFV – General Rumen Fluid-Vitamin mix	56
2.2.2.5	2xS GenRFV - 2× Sugars General Rumen Fluid - Vitamin mix	57
2.2.2.6	GenV mix	57
2.2.2.7	2xS GenV mix	58
2.2.2.8	Titanium (III) NTA solution	58
2.2.3	Preparation of isolation media	59
2.2.4	Sub-culturing media	59
2.2.5	Storage media	60
2.2.6	RM02 medium	60
2.2.7	Solid RM02 medium	61
2.2.8	Grass-containing RM02 medium (RM02 + grass)	61
2.2.9	Sisal-containing RM02 medium (RM02 + sisal)	61
2.2.10	Pectin-containing RM02 medium (RM02 + pectin)	61
2.2.11	Titanium-containing RM02 medium (RM02 + titanium)	61
2.2.12	BY medium	62
2.2.13	CRF medium	62
2.2.14	Sisal-containing CRF medium (CRF + sisal)	63
2.2.15	Medium 98-5	63
2.2.16	LB (Luria-Bertani) liquid and solid medium	64
2.3	Methods	64
2.3.1	Agarose gel electrophoresis	64
2.3.2	Animals and ethics approval	65
2.3.3	DAPI staining and cell counting	65
2.3.4	DNA quantification	66
2.3.5	General laboratory equipment	66
2.3.6	Feed analysis	67
2.3.7	Live/dead stain	68
2.3.8	Most Probable Number of viable cells	68
2.3.9	Purification of PCR products	68
2.3.10	Scanning Electron Microscopy (SEM)	69
2.3.11	SEM sample preparation	69

2.3.12	Rumen sample collection	69
2.3.13	Volatile fatty acids analysis	70
2.4	Isolation experiments	70
2.4.1	Rumen content fractionation trial	70
2.4.1.1	Rumen content fractionation test - Cow (723).....	70
2.4.1.2	Rumen contents fractionation test – Sheep (4572)	71
2.4.2	Optimisation of rumen content fractionation	73
2.4.2.1	Wash experiment 1	73
2.4.2.2	Wash experiment 2.....	73
2.4.3	Trial cultivation.....	74
2.4.4	Cultivation experiment.....	76
2.4.4.1	Preparation and storage of rumen samples for DNA extraction	78
2.4.4.2	Samples for cell counts	78
2.5	Identification of cultures	78
2.5.1	Phase contrast microscopy	78
2.5.2	DNA extraction from cultures.....	79
2.5.3	Purification of DNA from cultures	79
2.5.4	16S rRNA gene sequencing for culture identification	80
2.5.5	Primers for culture identification	82
2.5.6	Sanger sequencing.....	82
2.6	Culture-independent community analysis.....	83
2.6.1	DNA extraction from rumen samples	83
2.6.1.1	DNA extraction buffers.....	84
2.6.2	Denaturing gradient gel electrophoresis (DGGE).....	84
2.6.2.1	PCR for DGGE analysis	85
2.6.2.2	Analysis of DGGE gels.....	86
2.6.3	Clone library construction.....	87
2.6.3.1	Colony PCR	88
2.6.3.2	Analysis of clones	89
2.6.4	Pyrosequencing	90
2.6.4.1	Pyrosequencing sample preparation.....	90
2.7	Phylogenetic analysis	93

2.7.1	Computer software used in phylogenetic analysis	93
2.7.2	Checking and correcting isolate sequences.....	94
2.7.3	Elimination of PCR-mediated chimeras.....	94
2.7.4	Phylogenetic analysis of partial (V1-V3) isolate sequences	94
2.7.5	Clean-up of pyro-sequence reads	95
2.7.6	QIIME analysis	95
2.7.7	Pyrosequence analysis, CD-HIT-OTU pipeline.....	96
2.7.8	Choosing representative isolate sequences	96
2.7.9	Phylogenetic analysis of full-length isolate sequences	97
2.7.1	Statistical analysis	98
2.7.2	Isolate sequence and pyrosequence comparisons	98
CHAPTER 3 CULTIVATION METHOD DEVELOPMENT		99
3.1	Introduction	99
3.2	Rumen content fractionation test	100
3.3	Optimisation of rumen content fractionation	102
3.3.1	Wash experiment 1	102
3.3.2	Wash experiment 2.....	108
3.4	Trial cultivation.....	111
3.4.1	Estimating the inoculum size	111
3.4.2	Estimating percentage of single cells.....	113
3.4.3	Trial cultivation test	114
3.4.4	Identification of isolates	115
3.5	Discussion	117
CHAPTER 4 ISOLATION.....		125
4.1	Introduction	125
4.2	Experimental design.....	127
4.2.1	Cultivation.....	127
4.3	Animal selection for the main sampling experiment	127
4.3.1	Sampling and media.....	130
4.3.2	Cultivation dilution range	131
4.4	Results	132

4.4.1	Sequencing controls	132
4.4.2	Most probable number (MPN)	133
4.4.3	Culturability	138
4.4.4	Isolates	138
4.4.5	Isolates that were omitted from further analysis	141
4.4.6	Phylogenetic placement of isolates	144
4.4.6.1	BLASTN analysis	145
4.4.6.2	QIIME analysis	148
4.4.6.3	Phylogenetic trees	153
4.4.7	Selection of representative isolates for further study	161
4.5	Discussion	163
4.5.1	Culturability	163
4.5.1.1	Dilution to extinction	164
4.5.1.2	Quantitative effects of medium choice on culturability	165
4.5.2	Diversity of bacteria cultured	168
4.5.2.1	Taxonomic placement of new isolates	170
4.5.2.2	The effect of medium on diversity	170
4.5.3	What was missed?	171

CHAPTER 5 PLANT-ADHERENT RUMEN COMMUNITY

	COMPOSITION	173
5.1	Introduction	173
5.2	Results	174
5.3	Rumen and forage nutritive properties	174
5.4	Analysis of plant-adherent bacterial communities	178
5.4.1	Culture independent assessment of community structure	186
5.5	Pyrosequencing	188
5.5.1	Sequencing controls	190
5.5.2	OTU analysis	192
5.5.3	Plant-adherent rumen community composition	194
5.5.4	Comparing sequences from different animals or sample months	199
5.6	Discussion	203
5.6.1	Discussion of methods	203

5.6.1.1	Bacterial communities associated with rumen fractions	204
5.6.1.2	Bacterial communities associated with different animals	205
5.6.1.3	Bacterial communities associated with different sample months	206
5.6.2	Bacterial diversity	206

CHAPTER 6 COMPARISON OF ISOLATES WITH THE PLANT-ADHERENT BACTERIAL COMMUNITY.....209

6.1	Introduction	209
6.2	Results and discussion	210
6.2.1	Phylogenetic placement of isolates with near full-length 16S rRNA gene sequence	210
6.2.2	Description of isolates within Firmicutes	213
6.2.3	Firmicutes, class Clostridia (order Clostridiales).....	213
6.2.3.1	Lachnospiraceae	215
6.2.3.1.1	Butyrivibrio and Pseudobutyrvibrio	217
6.2.3.2	Ruminococcaceae	230
6.2.3.3	Clostridiales Family XIII <i>Incertae Sedis</i>	235
6.2.3.4	R-7 group	235
6.2.3.5	Veillonellaceae	236
6.2.4	Firmicutes, Class Bacilli	242
6.2.5	Firmicutes, Class Erysipelotrichia	246
6.2.6	Bacteroidetes	250
6.2.6.1	RC9 gut group	250
6.2.6.2	Prevotellaceae	251
6.2.7	Spirochaetes	256
6.2.8	Fibrobacteres	257
6.2.9	Actinobacteria	262
6.2.9.1	Coriobacteriaceae	262
6.2.9.2	Propionibacteriaceae	263
6.3	Discussion of tree-based comparisons	267
6.4	Comparison of isolate sequences and pyrosequences by OTU clustering	273

CHAPTER 7	SUMMARY, CONCLUSIONS AND FUTURE	
	DIRECTIONS	283
7.1	Rationale	283
7.2	Summary of results	284
7.3	Conclusions.....	287
7.4	Future perspectives	289
APPENDIX 1		292
APPENDIX 2		363
References.....		369

LIST OF TABLES

Table 1-1. The characteristics of the main well characterised ruminal bacteria.....	10
Table 1-2. Phylum-level composition of rumen bacterial communities in various ruminants revealed by analysis of 16S rRNA gene libraries.....	16
Table 1-3. Similarity of rumen bacterial 16S rRNA genes to those of already cultured organisms.	18
Table 2-1. Trace element solution SL10 components.....	52
Table 2-2. Vitamin 10 concentrate components.	53
Table 2-3. Anaerobic glycerol solution components.	54
Table 2-4. GenRFV solution components.....	56
Table 2-5. 2xS GenRFV solution components.	57
Table 2-6. Suppliments added to isolation media.	59
Table 2-7. Suppliments added to sub-culturing media.....	59
Table 2-8. RM02 medium components.....	60
Table 2-9. BY medium components.	62
Table 2-10. Medium 98-5 components.	64
Table 2-11. Centrifuge specifications.	67
Table 2-12. Cows sampled from DairyNZ, Hamilton.....	77
Table 2-13. Media types used at each sampling time.	77
Table 2-14. Components and their volumes in a typical 16S rRNA gene amplification PCR reaction for unknown rumen cultures.....	81
Table 2-15. Primers used on unidentified rumen cultures.	82
Table 2-16. Clone library PCR components.	88
Table 2-17. Colony PCR components.....	89
Table 2-18. PCR components for DGGE analysis.....	86
Table 2-19. Pyrosequencing PCR components.	91
Table 2-20. Barcoded primers for pyrosequencing PCR.	92
Table 2-21. Software programs.....	93
Table 3-1. Comparison of microbial counts from blended rumen fractions.....	114
Table 3-2. Growth of cultures in trial cultivation experiment on rumen contents from cow 723.	115

Table 3-3. Descriptions of 24 isolates obtained from the trial cultivation experiment.....	116
Table 4-1. Milk production values of the nine animals under consideration for sampling.	128
Table 4-2. Effect of medium type on most probable number (MPN).....	134
Table 4-3. Percentage of growth-positive tubes in different experiments.	135
Table 4-4. Estimates of culturability and adherent cells in rumen content.	138
Table 4-5. Similarities of sequences from new isolates with those of previously-cultured bacteria.	146
Table 4-6. Identity of groups of isolates with identical V1-V3 region 16S rRNA gene sequences.	146
Table 5-1. Nutritive value and components of pasture silage (silage) and fresh pasture (pasture) used as feed for the animals in this study.	176
Table 5-2. Characteristics of rumen contents of cows sampled over the course of a year.....	177
Table 5-3. Number of sequences ^a and taxa obtained from all animals and sampling times ^b	189
Table 5-4. Percentage of unique OTUs and percentage of sequences in the processed dataset represented by those OTUs in each animal at each sampling ^a	193
Table 5-5. The percentage of major bacterial taxa found plant-adherent rumen samples.	197
Table 5-6. Minor bacterial taxa found in plant-adherent rumen samples.	198
Table 6-1. Identity of isolates within Lachnospiraceae.	219
Table 6-2. Identity of isolates within Ruminococcaceae.	232
Table 6-3. Identity of isolates within Clostridia Family XIII <i>incertae sedis</i>	238
Table 6-4. Identity of isolates within R-7 group.....	238
Table 6-5. Identity of isolates within Veillonellaceae.	239
Table 6-6. Identity of isolates within Bacilli.....	243
Table 6-7. Identity of isolates within Erysipelotrichia.....	248
Table 6-8. Identity of isolates within Bacteroidetes.	253
Table 6-9. Identity of isolates within Spirochaetes.....	259

Table 6-10. Identity of isolates within Fibrobacteres.....	260
Table 6-11. Identity of isolates within Actinobacteria.....	265
Table 6-12. Number of uncharacterised species-level clusters and isolates at different taxonomic levels.....	271
Table 6-13. The effect cleanup steps of pyrosequences have on the number of OTUs.	278
Table A-1. Description and phylogenetic placement of isolates obtained in this study.	292

LIST OF FIGURES

Figure 1-1. The first four compartments of the ruminant digestive system.....	2
Figure 1-2. Generalized structure of the primary plant cell wall (not lignified).....	6
Figure 1-3. General scheme of plant polysaccharide fermentation in the rumen.....	9
Figure 1-4. Schematic representation of the number of phylotypes of rumen bacteria grouped at various taxonomic levels	26
Figure 1-5. The effect of inoculum size on the number of cultures derived from single cells..	39
Figure 2-1. Procedure for separating plant-adherent rumen bacteria from rumen contents.	72
Figure 2-2. Optimised protocol for separating and cultivating plant-adherent bacteria from rumen contents.....	75
Figure 2-3. Thermal cycler conditions for DGGE PCR.....	85
Figure 3-1. Estimated distribution of bacterial cells in rumen fractions.....	101
Figure 3-2. DAPI-stained micro-organisms attached to plant material in rumen contents from cow 723.....	102
Figure 3-3. Microbial cell numbers during fractionation of rumen contents.....	104
Figure 3-4. PCR amplification using DGGE primers targeting bacterial 16S rRNA genes (V3 region) in DNA extracted from rumen fractions:.....	105
Figure 3-5. DGGE fingerprints of bacterial 16S rRNA genes (V3 region) from successively washed rumen fractions.....	106
Figure 3-6. Comparison of DGGE profiles of bacterial 16S rRNA genes (V3 region) generated from DNA extracted from rumen fractions with successive washes..	107
Figure 3-7. Microbial cell numbers during fractionation of the rumen contents.	109
Figure 3-8. Comparison of the numbers of micro-organisms (per g of digesta) counted in each fraction when using either 200 mL of medium for 50 g or 400 mL of medium for 100 g.....	110

Figure 3-9. The number of microscopically counted micro-organisms in rumen digesta (per g of digesta), before and after blending of otherwise identical subsamples.	111
Figure 3-10. Estimated numbers of cells that will be inoculated into each tube from serially diluted blended, washed rumen digesta.	112
Figure 3-11. Number of cells observed as singletons, in pairs or in clumps with three, four or more cells in washed rumen digesta treated by blending.....	113
Figure 4-1. Comparison of DGGE profiles of bacterial 16S rRNA genes (V3 region) generated from DNA extracted from rumen contents of nine animals under consideration for sampling.....	129
Figure 4-2. Most probable numbers (MPNs) of viable bacteria in the plant-adherent fraction per g of rumen content.	137
Figure 4-3. Summary of the numbers of cultures from inoculation through to identification by comparative gene sequence analysis.....	139
Figure 4-4. Examples of different cell morphologies found in the isolates.	140
Figure 4-5. Scanning electron micrograph images of a strand of sisal string.	143
Figure 4-6. The percentage of isolates with usable 16S rRNA gene sequence reads for each sampling experiment.....	144
Figure 4-7. Comparison of new plant-adherent rumen isolates to previously known bacteria by level of 16S rRNA gene sequence identity.....	147
Figure 4-8. The effect of season, animal and medium on the proportions of different phylogenetic groups cultured from the plant-adherent fraction of rumen contents.....	150
Figure 4-9. Proportions of bacteria belonging to different taxa cultured from the plant-adherent rumen fractions on different types of media.	152
Figure 4-10. Rarefaction curve for different bacterial OTUs (grouped at 96% similarity)	153
Figure 4-11. Overview of the phylogenetic relationships of rumen plant-adherent bacterial OTUs based on partial (V1-V3) 16S rRNA gene sequences.	155

Figure 4-12. Expanded view A: The phylogenetic relationships of plant-adherent bacteria isolated from rumen contents based on partial (V1-V3) 16S rRNA gene sequences.	158
Figure 4-13. Expanded view B: The phylogenetic relationships of plant-adherent bacteria isolated from rumen contents based on partial (V1-V3) 16S rRNA gene sequences.	159
Figure 4-14. Expanded view C: The phylogenetic relationships of plant-adherent bacteria isolated from rumen contents based on partial (V1-V3) 16S rRNA gene sequences.	160
Figure 5-1. Comparison of DGGE profiles of bacterial 16S rRNA genes (V3 region) from combined wash fraction (TW) from five animals sampled in five seasons	180
Figure 5-2. Comparison of DGGE profiles of bacterial 16S rRNA genes (V3 region) from blended digesta fraction (BD) from five animals sampled in five seasons.	181
Figure 5-3. Comparison of DGGE profiles of bacterial 16S rRNA genes (V3 region) from blended total (BT) and unblended total (UT) rumen contents from five animals sampled in five seasons.	182
Figure 5-4. Comparison of DGGE profiles of bacterial 16S rRNA genes (V3 region) from (A) cow B and (B) Cow C from five animals sampled in five seasons.	184
Figure 5-5. Comparison of DGGE profiles of bacterial 16S rRNA genes (V3 region) from (A) cow D from five animals sampled in five seasons, and (B) Cow E (sampled in four seasons) and Cow A (sampled in one season).	185
Figure 5-6. Identity of 16S rRNA genes amplified from plant-adherent fraction of rumen contents from cow A in May 2009.....	187
Figure 5-7. Amplification of V1-V3 region of the 16S rRNA gene with two different barcoded forward primers paired with the same reverse primer from the same DNA aliquot extracted from sample FD.....	191

Figure 5-8. The number of OTUs generated when different clustering thresholds were applied to the processed dataset of 110,585 sequence reads.....	192
Figure 5-9. Phylum-level assignment of sequences obtained from the plant-adherent fraction of rumen contents, summarised from all 21 samples.....	195
Figure 5-10. Bacterial taxa from the plant-adherent fraction of rumen contents.....	196
Figure 5-11. The effect of (a) animal and (b) sample month on the bacterial families identified from the plant-adherent fraction of rumen contents.....	200
Figure 5-12. Principal coordinate analysis (PCoA) showing the similarity of sequenced plant-adherent rumen bacterial communities.	201
Figure 5-13. Principal coordinate analysis (PCoA) showing the similarity of sequenced plant-adherent rumen bacterial communities.	202
Figure 6-1. Overview of the phylogenetic relationships of new isolates cultured in this study.....	212
Figure 6-2. Overview of the phylogenetic relationships of the families within the phylum Firmicutes.....	214
Figure 6-3. Clusters formed at various similarity levels.	224
Figure 6-4. Expanded view of Lachnospiraceae.	227
Figure 6-5. Expanded view of <i>Butyrivibrio</i> from Figure 6-4.....	228
Figure 6-6. Expanded view of <i>Pseudobutyrvibrio</i> from Figure 6-4.	229
Figure 6-7. Expanded view of Ruminococcus.	234
Figure 6-8. Expanded view of Clostridia family XIII <i>incertae sedis</i>	240
Figure 6-9. Expanded view of the R-7 group.....	240
Figure 6-10. Expanded view of Veillonellaceae.	241
Figure 6-11. Expanded view of Bacilli.	244
Figure 6-12. Expanded view of Streptococcaceae..	245
Figure 6-13. Expanded view of Erysipelotrichia.	249
Figure 6-14. Expanded view of Bacteroidetes.	254
Figure 6-15. Expanded view of Prevotellaceae.....	255

Figure 6-16. Expanded view of Spirochaetes.	261
Figure 6-17. Expanded view of Fibrobacteres.	261
Figure 6-18. Expanded view of Actinobacteria.	266
Figure 6-19. Summary of isolate identification.	270
Figure 6-20. Proportions of uncharacterised family-, genus-, and species-level clusters and isolates at different taxonomic levels. Family-, genus- , and species-level clusters were defined by a combination of 16S rRNA gene sequence similarity groupings and two different tree methods.	272
Figure 6-21. Major taxa identified from pyrosequence data after different quality and cleanup procedures.	279
Figure 6-22. Shared OTUs between (A) isolate sequences and pyrosequences from plant-adherent rumen bacteria and (B) isolate sequences, pyrosequences from plant-adherent rumen bacteria all near full- length isolate sequences of good quality from RDP.	280
Figure 6-23. Cultivation success. The OTUs were ranked from largest to smallest.	281

NON-STANDARD ABBREVIATIONS

BLASTN	basic local alignment sequence tool (nucleotide)
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)