final report

Project code: B.CCH.1005

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Date published: April 2012

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Metagenomic analysis of feed utilization and hydrogen balance in Australian livestock for lower methane emissions (CSIRO)

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

Despite reducing methane emissions per tonne of red meat production, the “clean and green” image of Australia’s livestock industries continues to be eroded away. Our livestock industries also face a future increasingly reliant on fibrous feedstuffs such as native grasses, which require a step-wise change in their digestibility to enhance animal productivity. Rumen microbes govern both these processes, but the vast majority of them are “unknown”; so our ability to productively manipulate these processes has been limited. This project developed and used new approaches in microbiology, referred to as “metagenomics”, to better understand how feed utilization and hydrogen balance might be affected to reduce methane emissions. The outcomes of the project include: i) producing the genetic “blueprint” of rumen microbiology in northern beef cattle; ii) identifying “new” bacteria in these cattle relevant to feed digestion and/or hydrogen utilization *without* methane formation; iii) new resources that can be used to track and/or quantify the abundance of these bacteria and; iv) the isolation of some of these bacteria for the first time. We now have some of the resources needed to monitor how the rumen microbiota might be changed via diet and supplementation to reduce methane emissions and ideally, improve feed utilization.

Executive Summary

Why the work was done and what was achieved: Our livestock industries require transformational advances that improve the utilization of fibrous feeds and redirect hydrogen transactions away from methane production, if herd size and profitability are to be retained in an era dominated by concerns about climate change, water use and global warming. Such advances will also be relevant for our industries' ability to meet the growing demand for high quality red meat products, especially in China and greater Asia, as these economies, and the prosperity of their citizens grow.

We contend that some of the transformational advances required by our livestock industries will need to be microbiological in nature. However, our understanding of the roles played by ruminal microbes in feed utilization and hydrogen transactions is compromised without obtaining insights into the "not yet cultured" fraction of microbial communities. The traditional way of investigating rumen microbes has been to try to isolate and culture as many species as possible. The advent of recombinant DNA technologies during the 1980s and 1990s led to a "reductionist" phase of research, which provided detailed knowledge of select processes. Unfortunately, much of these efforts were without real-world context, because of the myriad of intra- and intercellular interactions at play.

Recent advances in molecular biology and genomics now allow very detailed examination of the structure and function of microbial communities, including those organisms that cannot readily be cultured. These approaches are referred to as "metagenomics". **The principal strategic context of this proposal was to use (meta)genomics approaches to produce new understanding of both the *structure and function* of the rumen microbiota in North Australian beef cattle, with a view to improve fibre utilization and reduce methane emissions.**

1) We constructed the "genetic blueprint" of the rumen microbiota in North Australian cattle.

- Methods for the recovery of rumen microbes tightly adherent to plant biomass were developed (Rosewarne et al. 2010) and used to prepare both DNA samples and cultures from north Australian cattle.
- The project team established both the laboratory and computational resources needed to produce a comprehensive assessment of rumen microbial diversity using "next generation" sequencing technologies.
- A library of metagenomic DNA was constructed from these same samples and the 25,000 clones represents more than 10^9 basepairs of genetic information, roughly equivalent to 250 bacterial genomes.
- Total metagenomic DNA prepared from digesta samples of north Australian cattle has been subjected to "shotgun" pyrosequencing.

2) New concepts and understanding of the enzymatic and ecological inputs central to hydrogen transactions within the microbiome were produced.

- Animals either selected for a "high" or "low" methane phenotype, or treated with compounds known to inhibit methanogens, have different rumen microbiota "phenotypes".
- The meta-analysis also showed the differences in bacterial populations could be further stratified by diet, *supporting the contention there is not a "signature" (singular) population of rumen microbes responsible for a low methane phenotype.*
- The fosmid libraries showed that the repertoire of enzymes involved with plant cell wall deconstruction, and the mechanisms of adhesion and biofilm formation do not conform with the paradigms developed from the study of pure cultures of bacteria.

3) We have isolated and evaluated "novel" microorganisms with potential to reduce enteric methane emissions.

- We designed primers that can be used to monitor 4 specific groups of rumen bacteria that might provide alternative pathways of hydrogen utilization *without* methane production (at least in northern Australian cattle) and showed these tools are sensitive, precise and accurate.
- Using novel combinations of substrates we have isolated some "new" rumen bacteria that may utilize hydrogen *without* methane formation.
- We have also produced a culture of three different bacteria, which appears capable of rapid growth and hydrogen utilization without methane formation.

4) We supported our RELRP-funded partners by providing instruction in specific methods; as well as examining samples from their studies and projects.

- The primer sets described above were used to enumerate the 4 bacterial groups in rumen fluid samples from a study conducted by Peter Kennedy, Nigel Tomkins and Ed Charmley; with thirteen rumen fistulated Brahman-cross steers fed a variety of tropical forages.
- We have also completed the sample preparation, data production, and data processing for all the samples provided by Dr Peter Moate as part of the dry and wet grape marc feeding trial undertaken at Victoria DPI, supported via RELRP-1.
- Additionally, the CSIRO St Lucia group has supported Dr. Valeria Torok and her technician from SARDI to become more familiar and proficient with pyrosequencing data analysis.

When and how industry can benefit from the work, who can benefit from the results?

In the continuum from laboratory bench to paddock, this project resides closer to the former. However, the project has provided knowledge, tools and biological resources that have immediate, medium and longer term implications for how industry R&D attempts to productively improve fibre utilization and reduce methane emissions:

In the immediate-near term:

- Our findings raise a caveat about the genetic selection of animals for a low methane phenotype. Genetic selection for a low methane phenotype in grazing livestock may be unsuitable for retaining the same phenotype once these animals are feedlotted, or fed total mixed rations; and vice-versa.
- The research supported by B.CCH.1005 has provided tools to quantify and monitor specific populations in response to animal selection and (or) breed, as well as dietary interventions, supplements, or management decisions designed to reduce methane emissions.
- We have integrated these approaches into more "applied" studies via actively seeking new collaborations and providing consel and guidance for others to effectively use the same technologies.

In the medium-long term:

- The project has provided new genomic information and microbial isolates from which new strategies that strive to improve fiber utilization and hydrogen transactions, especially in north Australian beef cattle can be produced.
- these studies still need to identify structural and functional details about "microbial hormones": biomolecules that, in very limited concentrations, elicit large effects
- by doing so, opportunities to utilize both agonistic and antagonistic approaches to modify ruminal function are expected to arise, which should be compatible with available technologies such as slow-release ruminal boluses
- demonstrate to industry that microbiology is the functional interface influencing many G x E x M interactions, and needs to be better understood if any are to be positively impacted.

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Background

Australia's livestock industries, like those in many other Western countries, are being confronted with public concerns and challenges to change; with respect to resource use, environmental impact, and public health. The biology underpinning many of these challenges is microbiological in cause-and-effect. For instance, ionophore use by the beef and dairy industries to manipulate rumen microbiology has been very effective but continues to receive scrutiny. All Australian livestock industries most likely face a future of greater dependence on feedstocks that are drought-resistant and highly fibrous; especially if the demand for cereal grains for human food, fuel ethanol production, or other industrial fermentations continues to increase relative to supply. **However, the most pressing challenge presented to our livestock industries by society right now is to reduce their methane emissions.**

This project has been funded under the Climate Change Research Program's Reducing Emissions from Livestock Research Program (**CCRP-RELRP**). The goal of the RELRP was to create anational collaborative program of research on methane emissions from ruminants, to deliver knowledge and technologies that enable producers to breed and/or manage ruminants to significantly reduce their methane emissions; while maintaining livestock productivity for a viable agriculture industry.

In that context, the principal strategy of this Project was to use (meta)genomics approaches to understand both the *structure and function* of the rumen microbiomes in North Australian cattle as a basis to develop practical ways to reduce enteric methane emissions. The hypotheses for this Project were:

- Ruminal hydrogen transactions are poorly characterized, beyond the biochemical pathways coordinating hydrogen utilization (e.g. methane, acetate, and succinate production). Metagenomics approaches integrated with animal feeding and breeding trials will expand our understanding of these syntrophic associations; as well as potential agonistic and antagonistic approaches to redirect fermentation away from methane formation.
- Heterotrophic (organic acid utilizing) archaea make a significant contribution to livestock methane emissions but many of these prokaryotes are "not-yet-culturable". Metagenomic analyses will support the development of strategies to negatively affect their colonization and persistence in ruminal microbiomes, and support their displacement with alternative hydrogen utilisers.

The specific outcomes of this Project were expected to include the biological knowledge and resources necessary to help develop diagnostic/monitoring approaches of methane output in livestock; and assist breeding programs that seek to establish a heritable "low methane phenotype" in ruminants. The ultimate goal is to contribute to the development of products and technologies compatible with the industries' management strategies and breeding programs, which will positively alter ruminal function in a "clean and green" way.

Project Objectives:

1) To construct the metagenomic blueprint of the rumen microbiome in North Australian cattle
2) To identify the enzymatic and (or) ecological step(s) central to hydrogen transactions within the microbiome.
3) In collaboration with MLA- and DAFF-funded collaborators establish how supplementation strategies in North Australia (cattle) impact ruminal microbiology, hydrogen transactions and methane producing microbes.
4) Formulate agonistic and antagonistic approaches to alter ruminal microbiology and redirect ruminal fermentation, which can include the isolation and evaluation of novel microorganisms with potential to reduce enteric methane emissions

Methodology

1) **Construct the metagenomic blueprint of the rumen microbiome in North Australian cattle.** A detailed "gene-centric" assessment of the targeted microbiomes in North Australian cattle will be produced and used to help identify steps potentially rate-limiting to hydrogen transactions and fermentative schemes other than methanogenesis. Similar studies will be initiated in collaboration with other projects that seek to deliver a low methane phenotype. Specific methodologies include:
    - Bacterial separation and DNA preparation from North Australian cattle
    - Metagenomic library construction, storage, and quality control
    - Development and use of key bioinformatic analyses
2) **Identify the enzymatic and (or) ecological step(s) central to hydrogen transactions and carbon capture.** The selected microbiomes were to be analyzed without the necessity or dependency of first growing microorganisms in the laboratory. The experiments should provide novel insight and understanding of the microbiology, biochemistry and genetics underpinning alternative hydrogen transactions in North Australian cattle. From this, new strategies to productively alter these schemes should be possible including DNA marker genes, proteins or microbes useful to monitor and (or) support selection of animals with a low methane phenotype. Specific methodologies were to include:
    - Bioinformatic analyses continued
    - Microarray based screens
    - Functional and hybridization based screens
    - Analysis of positive clones
3) **Integration and use of the knowledge arising from this program.** The objective was to produce and use tools to track and monitor the persistence of select bacteria in rumen microbiomes that might support reductions in methane emissions; along with genomics-based strategies designed to isolate some of these microbes. From this foundation, it is expected that gene-based, bioactive compounds, and organisms that positively impact (agonistically and (or) antagonistically) rumen methane emissions will be identified. Specific methodologies included:
    - *Functional analyses of metagenomics resources*
    - *Microbe isolation*
    - *Ecological studies*
4) **Collaboration with other RELRP support projects and scientists.** This Project will collaborate with other projects to support the achievement of the Project Objectives including B.CCH.1008, rumen profiling; B.CCH.1006 genetic phenotyping; and B.CCH.1009 Methane Abatement Strategies, B.CCH.1004 and B.CCH.1012 feed variations in relation to enteric methane emissions from animals.

Results

In the progress report for milestone 3, we were able to validate that our methods of microbe desorption remove all types and kinds of microbes tightly adherent to rumen digesta; so that our more detailed analyses will provide a precise and accurate inventory of the genetic potential resident within this previously uncaptured part of the "bovine genome". In addition, the CLI group at St Lucia initiated the creation of a more streamlined and faster bioinformatics platform that can be used to support this and these activities expedited our ability to capture the metagenomic blueprints of Northern Australian cattle under various feeding regimes.

These results were presented in detail as part of the progress report for milestone 4. We had developed the collaborative linkages with animal scientists in northern Australia, and worked with samples collected from their previous and current experiments. In brief detail we: i) begun the metagenomic "genotyping" the rumen microbiomes of beef cattle maintained on tropical pastures with different levels of methane emissions (per unit of feed intake); ii) completed the first round of sampling and enrichment experiments designed to recover the "uncultured" archaeabacteria and bacteria relevant to hydrogen transactions and methane emissions and; iii) attempted to develop a method for the subtractive enrichment of rumen archaebacteria that provides access to the metagenome (and proteome) of these populations in northern beef cattle. The samples were also used to construct the metagenomic libraries needed for the functional screens. **At this point, we were especially pleased to have established a good working relationship with our colleagues in northern Australia, and we expect this to continue and grow; so we expect the microbiological studies to be placed within the context of overall animal performance, rather than "stand alone".**

The milestone 6 and 7 reports focused on our new results to produce a metagenomic "genotype" of the rumen microbiomes of beef cattle maintained on tropical pastures; and we also completed the first round of sampling and enrichment experiments designed to recover the "uncultured" heterotrophic archaea and bacteria from North Australian cattle. We also initiated and described the construction of the metagenomic libraries needed. Our analyses of these enrichments showed we have successfully recovered "new" ruminal microbes that might provide routes of hydrogen disposal independent of methane formation. The team also succeeded in producing pure cultures for some of these bacteria; and also designed PCR primers to track and quantify these (and other) bacteria in rumen samples.

In this milestone period, we also described the fosmid library from metagenomic DNA extracted from the Rhodes grass enrichments. The library weas confirmed to be comprised of ~20,000 clones with inserts of approximately 40 kbp (i.e. representing 800 Mbp sequence, equivalent to ~200 bacterial genomes). It had was screened for clones encoding endo-β-1,4-glucanase activity using carboxymethyl cellulose. These screens identified 140 clones with measurable hydrolytic activity against the substrate, and these were prepared and subjected to shotgun DNA sequencing to reveal their genetic potential, using facilities available from Macrogen and the Australian Centre for Ecogenomics.

Third, we described in detail a more comprehensive analysis of the microbial profiling data produced as part of B.CCH.1005 and other RELRP and CLI-led projects, which revealed a strong influence from diet on the profiles of rumen bacteria. Despite this "diet effect" there are still resolvable differences among animals that are fed similar diets but treated with an anti-methanogen compound (BCM), as well as among animals considered to be low or high methane emitters. However, there is no discernable "convergence" of these microbiomes from low emitting animals, with those animals treated with BCM to reduce methanogens and methane emissions. Our current interpretation of these data is that there are measurable shifts in the structure of rumen microbiomes caused by dietary interventions or selection of animals for low methane emissions; but there is not a "signature profile" that is reflective of a microbiome with "low" methane emissions. Instead, there are likely to multiple "signature profiles" reflective of the form of diet the animal consumes.

The reports submitted in relation to milestones 9 and 10 described our use successful recovery of "new" microbes that might provide routes of hydrogen disposal independent of methane formation. Four of these bacteria were cultured axenically and partially characterized. Oligonucelotide primers were also designed and used to monitor their abundance in some of the digesta samples obtained from our collaborators in northen Australia; and the results of these assays were described in detail as part of the milestone 9 report. Genomic DNA has since been prepared from three of these strains for genome sequencing, and has provided a more holistic representation of their metabolic potential and strategies to enhance their persistence.

Nucelotide sequence data for the 140 fosmid clones recovered during the milestone 7 reporting period was returned and annotated; these results are described in detail as part of the milestone 10 report. The analyses produced new information about the genetics underpinning the hydrolytic enzymes, adhesion, and biofilm formation mechanisms present in the rumen bacteria in northern Australian beef cattle. Although it is reasonable to argue the functional screens used here are quite targeted, it is notable that our efforts perpetuate a growing enigma in the field: that the "classic" GH families required for cellulose solubilisation (GH6, GH7, processive GH9's, and GH48) are NOT readily abundant in the microbiomes from ANY herbivore. Instead, we continue to produce evidence for the role of gene clusters encoding polysaccharide-utilization-loci (PUL), which most likely coordinate the adhesion of the bacterium to the structural matrix, in addition to its hydrolysis.

Two other important activities since the last milestone report centre on new collaborative studies with other RELRP project teams. First, we are in the final stages of producing 454 pyrosequencing data for the rumen and faecal samples collected as part of the grape marc trial conducted by Richard Eckard and Peter Moate. Second, we supported Valeria Torok and her technician in becoming familiar with pyrosequence data analysis, by visiting the St Lucia group during late September. Finally, we are close to finalising the sequencing contracts with external vendors, and by doing so, should see the project underspend substantially reduced.

Discussion and Conclusions

We feel that our project has produced a series of important research findings and achievements relevant not only to reducing methane emissions in livestock, but also for future improvements in fibre utilization in north Australian cattle. The project has provided new genomic information and microbial isolates from which new strategies that strive to improve fiber utilization and hydrogen transactions, especially in north Australian beef cattle can be produced. However, more progress still needs to be made, to support the transformation from invention to innovation and ultimately impact:

- these types of studies still need to provide coverage of the genetic potential resident within select rumen microbiomes, and from which comparative studies reveal candidate diagnostic or prognostic strategies to assist management decisions
- these studies need to identify structural and functional details about "microbial hormones": biomolecules that, in very limited concentrations, elicit large effects
- by doing so, opportunities to utilize both agonistic and antagonistic approaches to modify ruminal function are expected to arise, which should be compatible with available technologies such as slow-release ruminal boluses
- we need to build partnerships beyond RELRP to accelerate progress
- we need to more case-studies demonstrating to industry that rumen microbiology is a key functional interface influencing many GxExM interactions.
- There is unlikely to be a silver bullet approach that serves all livestock sectors equally well, we need to approach this with more of a "personalised medicine" ambit: which will better take into account the diversity of genotypes, environments and management practices employed throughout our livestock industries.

I also feel there are synergistic opportunities for our collective Australian activities supported via MLA-DAFF to acquire, if we work more closely with our New Zealand colleagues. They have taken the leadership and intend to utilize local funds from the Global Research Alliance to advance some key international initiatives (the Rumen Microbial Genomics Network, and a "community sequencing project" application to the US Department of Energy, to produce metagenomic data for high and low methane emitting sheep). We have much to gain in Australia through effective and strategic partnership with our New Zealand colleagues, given the complementarities that exist in the livestock production systems between the two countries; coupled with our mutually shared need to effectively reduce livestock methane emissions as soon as possible.

Relevant Appendices

Rosewarne et al. 2010

Microb Ecol
DOI 10.1007/s00248-010-9745-z

METHODS

High-Yield and Phylogenetically Robust Methods of DNA Recovery for Analysis of Microbial Biofilms Adherent to Plant Biomass in the Herbivore Gut

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Received: 11 July 2010 / Accepted: 1 September 2010

Abstract Recent studies have shown the microbial biofilms adherent to plant biomass in the gastrointestinal tracts of humans and other herbivores are quite different to planktonic populations. If these biofilm communities are to be properly characterized by metagenomics methods, then the microbial desorption methods used must ensure the phylogenetic diversity and genetic potential recovered is biologically valid. To that end, we describe here two different methods for desorbing microbes tightly adherent to plant biomass; and used PCR-DGGE analyses of the *Bacteria* and *Archaea* *rrs* genes to show both these desorption methods were effective in recovering the adherent microbial biofilm with no apparent biases in microbe recovery. We also present a derivation of the "repeated bead beating and column (RBB+C) purification" method of DNA extraction that results in the recovery of high molecular weight DNA. These DNA samples can be fragmented and size fractionated by sucrose density gradient centrifugation, bypassing the use of gel-plug lysis and pulsed-field gel electrophoresis separation of DNA for metagenomic library constructions.

Electronic supplementary material The online version of this article (doi:10.1007/s00248-010-9745-z) contains supplementary material, which is available to authorized users.

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Introduction

It is well recognized that plant biomass is rapidly colonized by microbes once it enters the gastrointestinal tracts of animals and humans; and that these microbes subsequently form specialized biofilms which facilitate hydrolysis and fermentation of the ingested material [6, 7, 10]. With specific reference to cellulose hydrolysis, the studies undertaken with pure cultures of specialist cellulolytic microorganisms has provided an in-depth understanding of the broad variety of glycoside hydrolases (GH), carbohydrate binding modules (CBM), fimbrial-like structures and multiprotein complexes (e.g., cellulosomes) that these microbes produce to support this process (e.g., [2, 3, 11, 12, 17, 19, 20]). In addition to these microscopic and culture-based studies, cultivation-independent approaches have further revealed that the cohort of microorganisms resident in these biofilms are phylogenetically distinct from those "free-living" microbes, and few have previously been cultured [5, 13, 15, 25]. Recent metagenomic studies with digesta and/or fecal samples from humans, mammalian and invertebrate herbivores have all shown that the functional diversity of carbohydrate active enzymes in these microbial communities may be driven by both diet- and host-specific adaptations [4, 18, 23, 24, 26]. However and with respect to cellulose hydrolysis specifically, a uniform observation from these studies is that the GH families shown by pure culture studies to coordinate cellulose solubilisation (e.g., the GH48 β-1,4-exoglucanases) are virtually absent from these datasets. Similarly, the recovery of

Published online: 14 September 2010

Rosewarne et al. 2012 (draft)

Draft genome sequence of *Treponema sp*. JC4, a novel spirochaete isolated from the bovine rumen

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Running title: Genome sequence of *Treponema sp*. JC4
Subject category: Genome Announcement
Keywords: *Treponema*/genome/bovine/rumen/fermentation

Morphologically and biochemically diverse members of the *Treponema* genus are present in the gastrointestinal tract of ruminants, yet very little is understood about their functional importance to this microbiome. Here we describe the annotated draft genome sequence of *Treponema sp*. JC4, a novel spirochete isolated from a bovine rumen sample.

The genus *Treponema* is comprised of microaerophilic or anaerobic spiral shaped bacteria residing within the phylum *Spirochaetes.* Members of this genus existing as both pathogens and commensals have been cultivated from a variety of host-associated microbiomes {Baseman, 1976 #337;Paster, 2006 #328}. This paper describes the draft genome sequence of *Treponema* sp. JC4, isolated as part of a larger project to characterise mechanisms of lignocellulose degradation in cattle from the Australian semi-arid tropics. Based on comparison of NAST-aligned 16S rRNA genes, the closest cultured representative to strain JC4 is *Treponema bryantii* RUS-1 (91.4% sequence similarity), a saccharolytic spirochaete also isolated from the bovine rumen {Stanton, 1980 #319}.

Treponema sp. JC4 was isolated from pooled rumen contents collected from six fistulated *Bos indicus* steers consuming Rhodes grass (*Chloris gayana*). Samples were initially enriched in liquid anaerobic medium 1 {Balch, 1979 #330} supplemented with 10% (w/v) powdered Rhodes grass. Glycerol stocks from the initial enrichments were plated on medium 10 agar {McSweeney, 2005 #331} containing starch, cellobiose and glucose as the carbohydrate sources. Genomic DNA from an anexic culture was extracted using the NML method {Rosewarne, 2011 #250} and sequenced from a shotgun library using the 454 Life Sciences GS FLX Titanium system, generating 3,033,760 bp of sequence data at 15x coverage. Sequence reads were assembled into 147 contigs (>200 bp) using Newbler v. 2.6. The contig N50 was approximately 71.9 kb and the largest assembled contig was 325.5 kb. The DNA sequence was analysed and annotated using the Integrated Microbial Genomes Expert Review (IMG ER) system {Markowitz, 2009 #338}. Carbohydrate active enzymes were further annotated using dbcan (http://csbl.bmb.uga.edu/dbCAN) to identify functional motifs using hidden Markov models.

The G+C content of the draft genome is 40% and contains 2,614 complete ORFs (2,570 protein coding genes and 44 structural RNAs). Consistent with the central function of rumen bacteria in co-ordinating carbohydrate metabolism, we identified an assortment of cellulases, endohemicellulases and debranching enzymes (glycoside hydrolase (GH) families 5, 10, 51, 53 and 64); oligosaccharide-degrading enzymes (GH families 1, 2, 3, 35, 36, 37, 42, 43, 65 and 94) in addition to members of GH families 13, 16, 18, 23, 46, 57, 73, 77, 109 and 114. Furthermore, we identified a suite of carbohydrate esterases (CE families 1, 2, 4, 7 and 12) responsible for deacteylation of xylans and xylo-oligosaccharides. Prediction of a variety of carbohydrate active enzymes potentially involved in breakdown of cellulose, xylan, arabinogalactan, starch and a broad range of oligosaccharides suggests that *Treponema* sp. JC4 has a broader substrate range than *T. bryantii*, which is only capable of fermenting a restricted subset of the soluble sugars released from cellulose by the action of cellulolytic bacteria {Stanton, 1980 #319}. Additional sequencing and comparative analysis of

genomes from diverse *Treponema* isolates will provide a greater understanding of the contribution of these bacteria to the function of gastrointestinal microbiomes, with particular emphasis on lignocellulose degradation and hydrogen transactions.

Nucleotide sequence accession number

This Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under accession X. The version described here is version 1, X. The near-complete 16S rRNA sequence (derived using Sanger dideoxy sequencing of a PCR product) has been deposited at DDBJ/EMBL/GenBank under accession JQ783348. Genome project data is available at GenBank under Genome Project ID 78730.

Acknowledgements

The current research was supported by funds provided to Meat and Livestock Australia project number B.CCH.1005, as part of the Reducing Emissions from Livestock Research Program.