Identification and functional characterization of adhesins involved in attachment of methanogens to rumen protozoa

A thesis presented in partial fulfilment of the requirements for the degree of
Doctor of Philosophy in Microbiology
at Massey University, Palmerston North, New Zealand

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2016

Abstract

Symbiotic interactions are frequently observed amongst members of the complex microbial community inhabiting the fermentative forestomach (rumen) of ruminant animals. In this ecosystem, hydrogen (H_2)-using methanogens can be found as ecto- and endo-symbionts of H_2-producing protozoa, and this interaction contributes to ruminant methane emissions. Rumen symbionts must have the ability to attach to protozoal hosts, presumably *via* protozoa-binding cell surface proteins, however the identity and specificity of these proteins are not known.

A protein of the methanogenic archaeon *Methanobrevibacter ruminantium* M1 that binds to rumen protozoa was identified using phage display technology. A large shot-gun phage display library was constructed from M1 DNA, and affinity screened by biopanning using rumen protozoa as bait. After two rounds of biopanning, a recombinant clone encoding part of a previously annotated putative adhesin, Mru_1499, was identified as a protozoa-binding protein. The protozoal binding region of the affinity selected protein was mapped, and a "reverse panning" procedure was developed to identify protozoal species that bind to the affinity selected protein.

Next, the protozoa-associated methanogen and bacterial communities were characterized, and several taxa of archaea and bacteria were found to be over-represented in the protozoa-associated community relative to their abundance in the rumen contents. Adhesins from this protozoa-associated community were identified by affinity screening of a community-scale phage display library using rumen protozoa as bait, combined with high-throughput single molecule amplicon sequencing. The comparison between pre- and post-panning sequence datasets showed seven highly enriched candidate adhesin-encoding ORFs after affinity-panning of the library on protozoa as bait.

In conclusion, several adhesins mediating interactions between methanogenic archaea, bacteria and protozoa were identified using phage display at both single-organism and metagenome scales. Further assays are required to verify the function of these candidate adhesins as "molecular bridges" in interactions involving rumen protozoa. This is the first report for characterization of the protozoa-associated symbiont community by next generation sequencing of the 16S rRNA gene.

Acknowledgments

I am grateful to my primary supervisor, Dr. Dragana Gagic, for introducing me to the world of rumen microbial symbioses, the autonomy that she trusted me to work with, and her kindness and support during the time we have known each other. Her enthusiasm for research and openness to exploring new ideas have been motivating influences for me in the last four years.

I feel very fortunate for the supportive community that I was surrounded by during the course of my studies. Many thanks to my co-supervisors, Drs. Jasna Rakonjac, Graeme Attwood, and Mark Patchett, who have contributed valuable time and unique perspectives to the project. I would also like to thank Dr. Sandra Kittelmann for her insight into rumen protozoa and her guidance with community analyses, Drs. Peter Janssen and Gemma Henderson for helpful discussions about the rumen microbial community, Michelle Kirk and Sarah Lewis for technical assistance with sample processing for pyrosequencing, Greg Skelton for assistance with rumen sampling, Dr. Ruy Jauregui for writing scripts to process datasets acquired from PacBio sequencing, and Dr. Eric Altermann for running sequences through GAMOLA. It has been a privilege to work with the technicians and scientists in the Rumen Micro lab. It truly feels like a team.

To fellow inhabitants of the FeedTech student office, it has been a pleasure to share an office with thirteen lovely people. I will miss the lively lunchtime discussions.

Thank you to my family, and friends who have become family, for their love and support.

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Abbreviations

ALP	Adhesin-like protein
BSA	Bovine serum albumin
cfu	Colony forming units
DGGE	Denaturing gradient gel electrophoresis
EDS	Energy dispersive X-ray spectroscopy
FISH	Fluorescence *in situ* hybridization
GAPDH	Glycerladehyde-3-phosphate dehydrogenase
GlcNac	*N*-acetylglucosamine
Ig-like	Immunoglobulin-like
IQR	Interquartile range
m.o.i.	Multiplicity of infection
nr	Non-redundant
OD	Optical density
ORF	Open reading frame
OTU	Operational taxonomic unit
PAM1	Shot-gun phage display primary library created from metagenomic DNA
PAM2	Shot-gun phage display primary library after affinity selection against c-myc
PAM_FINAL	PAM2 library after two rounds of biopanning against protozoal bait, followed by affinity selection against c-myc
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PFA	Paraformaldehyde
pfu	Plaque forming units
PMB	Pseudomurein binding
pMru_1499^{A}	Phagemid vector encoding Mru_1499^{A}
PP	Phagemid particle
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM	Scanning electron microscopy
TBS-T	Tris buffered saline containing Tween 20