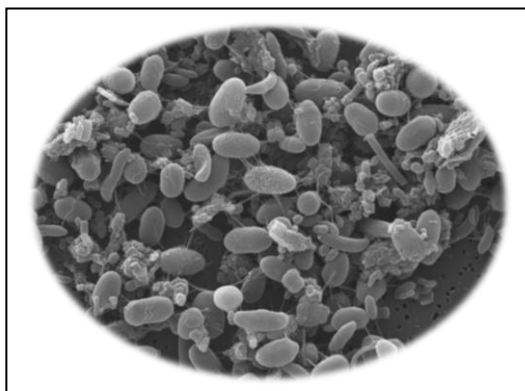


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# Physiology of rumen bacteria associated with low methane emitting sheep



## Key external stakeholders:

Livestock industry, Universities, Animal Nutrition companies, DAFM, Greenhouse gas monitoring agencies.

## Practical implications for stakeholders:

- Methane (CH<sub>4</sub>) is a by-product of feed digestion that contributes to anthropogenic greenhouse gases.
- There is a natural variation in CH<sub>4</sub> emissions among sheep, and this variation is heritable.
- Breeding for sheep that naturally produce less CH<sub>4</sub> is a viable strategy to reduce anthropogenic greenhouse gas emissions.
- Rumen bacterial community compositions in low CH<sub>4</sub> emitting sheep differ to those in high CH<sub>4</sub> emitting sheep.
- An increase in the abundance of a bacterium, called *Quinella* (see image) in the rumens of sheep would lead to lower CH<sub>4</sub> emissions.
- This project developed molecular probes to detect and quantify *Quinella*, which may be of further use for the rapid identification and estimation of *Quinella* population from rumen samples.

## Main results:

- Rumen bacterial community compositions in low CH<sub>4</sub> emitting sheep differ to those in high CH<sub>4</sub> emitting sheep.
- Rumen bacteria, *Sharpea* and *Kandleria* are associated with low CH<sub>4</sub> emissions in sheep and produce mainly lactate and some acetate, ethanol and formate as their end product.
- *Quinella* was found in large numbers in low CH<sub>4</sub> emitting sheep.
- The genus *Quinella* is more diverse than previously suspected, and may contain at least eight potential species, although to date none have been maintained in laboratory culture. *Sharpea* and *Kandleria* contain two and one species respectively.
- Analysis of four different *Quinella* genomes indicates that *Quinella* has all the genes needed to produce propionate and acetate as well as lactate.
- *Quinella* has an uptake hydrogenase gene suggesting that this bacteria may use free H<sub>2</sub> in the rumen, explaining why the increased abundance of *Quinella* would lead to lower CH<sub>4</sub> emissions in those sheep.

## Opportunity / Benefit:

- This project confirmed the physiology of low CH<sub>4</sub> associated bacterial group *Sharpea* and *Kandleria*
- This project provided first insight into the physiology of *Quinella*, an abundant bacterium found in low CH<sub>4</sub> emitting sheep rumen.
- This project developed molecular probes to detect and quantify *Quinella*, which may be of further use for quick identification and estimation of *Quinella* population from rumen samples.

## Collaborating Institutions:

AgResearch, Hamilton, New Zealand. Aberystwyth University, UK.

Teagasc project team: Dr Sinéad Waters

External collaborators  
Dr Peter Janssen (AgResearch)  
Dr Mark Patchett (Massey University)  
Dr Chris Creevey (Aberystwyth University)  
Dr Sandeep Kumar (AgResearch)

### 1. Project background:

Feed is digested in the rumen of cattle and sheep by a diverse population of microbes in a process called rumen fermentation. Methane (CH<sub>4</sub>) gas is produced as a by-product of feed digestion that contributes to anthropogenic greenhouse gases. Most CH<sub>4</sub> formed in the rumen is generated using H<sub>2</sub> produced during the fermentation of feed with H<sub>2</sub> being incorporated stoichiometrically into CH<sub>4</sub> (4 H<sub>2</sub> + CO<sub>2</sub> → CH<sub>4</sub> + 2 H<sub>2</sub>O). There is natural variation in CH<sub>4</sub> yields among sheep, expressed as the amount of CH<sub>4</sub> formed per unit of feed consumed. Some sheep are naturally high CH<sub>4</sub> emitters while others are low CH<sub>4</sub> emitters, and these are characterised by different rumen bacterial populations. It can therefore be hypothesised that bacteria associated with low CH<sub>4</sub> emitting animals should ferment the feed in such a way that less H<sub>2</sub> is formed, which would ultimately lead to less CH<sub>4</sub> emission. This project aimed to provide insights into bacteria that were differentially-associated with CH<sub>4</sub> yields in sheep, using culture dependent and culture independent approaches to study their fermentation products and understand how they contribute to differences in CH<sub>4</sub> yields.

### 2. Questions addressed by the project:

- What is the identity of bacteria associated with high and low CH<sub>4</sub> yields in sheep?
- Can we identify these bacteria at a species level?
- What is the physiology of differentially-associated bacteria with respect to their fermentation products and the implications for CH<sub>4</sub> formation in the rumen?

### 3. The experimental studies:

#### A. Identification and deeper analysis of low methane associated bacteria taxa

- OTU level analysis of rumen bacterial taxa associated with high and low methane emitting sheep was conducted.
- Taxonomy of *Quinella*, *Sharpea* and *Kandleria* that were found to be differentially-associated with low CH<sub>4</sub> emitting sheep rumen were refined using clone library sequences of these bacteria.

#### B. Understanding physiology of *Sharpea* and *Kandleria*

- Pure culture of *Sharpea* and *Kandleria* were grown on different substrate to perform substrate utilization test.
- Co-culture experiment of *Sharpea*, and *Kandleria* with *Methanobrevibacter olleyae* was conducted to test fermentation pattern of these bacteria. End product analysis was conducted using gas chromatography.

#### C. Identification of *Quinella* in rumen samples

- Fluorescence in-situ hybridisation (FISH) probes for *Quinella* were designed and evaluated. Enriched *Quinella* cells from low CH<sub>4</sub> yield sheep were used for this experiment.

#### D. Understanding physiology *Quinella*

- Concentrated *Quinella* cells were used for metagenome sequencing and *Quinella* genome were used to study key genes coding for enzymes involve in *Quinella* metabolism were studied in details to deduce the fermentation pathway of *Quinella*.

### 4. Main results:

- Rumen bacterial community compositions in low CH<sub>4</sub> emitting sheep differ to those in high CH<sub>4</sub> emitting sheep.
- Rumen bacteria, *Sharpea* and *Kandleria* are associated with low CH<sub>4</sub> emissions in sheep and produce mainly lactate and some acetate, ethanol and formate as their end product.
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explaining why the increased abundance of *Quinella* would lead to lower CH<sub>4</sub> emissions in those sheep.

#### 5. Opportunity/Benefit:

- This project confirmed the physiology of low CH<sub>4</sub> associated bacterial group *Sharpea* and *Kandleria*.
- This project provided first insight into the physiology of *Quinella*, an abundant bacterium found in low CH<sub>4</sub> emitting sheep rumen.
- This project developed molecular probes to detect and quantify *Quinella*, which may be of further use for quick identification and estimation of *Quinella* population from rumen samples.

#### 6. Dissemination:

##### PhD thesis:

**Kumar, S.** 2017. Physiology of rumen bacteria associated with low methane emitting sheep. Massey University, Palmerston North, New Zealand.

##### Main Publication:

- **Kumar, S.**, Bryan T., Koon H. T., Henderson G., Attwood, G. T., Waters S. M., Patchett, M. L., Peter H. J. 2018. *Sharpea* and *Kandleria* are lactic acid producing rumen bacteria that do not change their fermentation products when co-cultured with a methanogen. *Anaerobe*. 54:31-38.
- **Kumar, S.**, Henderson G., Kittelmann S., Leahy, S. C., Altermann E., Patchett, M. L., Attwood, G. T., Jonker A., Waters S. M., Peter H. J. Metagenomic sequencing yields insights into the physiology of *Quinella*, an iconic uncultured rumen bacterium. (In preparation).
- **Kumar, S.**, Henderson G., Kittelmann S., Patchett, M.L., Jonker A., Waters S. M., Peter H. J. Deeper examination of rumen bacterial taxa associated with high and low methane emitting sheep. (In preparation).

##### Conference Proceedings:

- **Kumar, S.**, Henderson, G., Kittelmann, S., Patchett, M. L., Jonker, A., Leahy S., Attwood, G.T., Waters, S.M., Janssen, P. H (2016). *Quinella*: A diagnostic rumen bacterium in low methane emitting sheep. New Zealand Microbiological Society/New Zealand Society for Biochemistry and Molecular Biology Conference (NZMS-NZSBMB), Christchurch, New Zealand. Finalist in Student oral competition.
- Soni, P., Kamke J., **Kumar, S.**, Janssen, P. H., Attwood G. T (2016). Development of single cell sorting methods for rumen microbes. New Zealand Microbiological Society/New Zealand Society for Biochemistry and Molecular Biology Conference (NZMS-NZSBMB), Christchurch, New Zealand. Poster.
- **Kumar, S.**, Kittelmann, S., Henderson, G., Siva G., Patchett M. L., Waters S. M., Janssen, P. H (2016). Diagnostic rumen bacteria associated with low-methane emitting sheep. 6th Greenhouse Gas and Animal Agricultural Conference, Melbourne, Australia. Poster.
- **Kumar, S.**, Henderson, G., Kittelmann, S., Siva G., Patchett M. L., Waters S. M., Janssen P. H. (2016). *Quinella*: low-methane associated bacteria in the sheep rumen. 16th International Symposium on Microbial Ecology, Montreal, Canada. Poster.
- **Kumar, S.**, Kittelmann, S., Henderson, G., Siva G., Lindy G., Patchett M. L., Waters S. M., Janssen P. H. (2014). A closer look at rumen bacterial species associated with low-methane emitting sheep. New Zealand Microbiological Society/New Zealand Society for Biochemistry and Molecular Biology Conference (NZMS-NZSBMB), Wellington, New Zealand. Poster.

##### Publicly available news articles:

- Getting to the bottom of a beneficial bacterium. <https://www.nzagrc.org.nz/agresearch/listing,477,Getting+to+the+bottom+of+a+beneficial+bacterium.html>
- The secrets of low-methane emitting sheep. [http://www.massey.ac.nz/massey/about-massey/news/article.cfm?mnarticle\\_uuid=82CCFC8D-3043-4D8E-BB96-CE6BB5EEF7B8](http://www.massey.ac.nz/massey/about-massey/news/article.cfm?mnarticle_uuid=82CCFC8D-3043-4D8E-BB96-CE6BB5EEF7B8)

#### 7. Compiled by: Dr. Sinéad Waters and Dr. Sandeep Kumar