Adaptation Mechanisms of Rumen Bacteria to Niches in the Rumen

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SUMMARY

The rumen houses a wide variety of microorganisms that are responsable of feed digestion. Bacteria, which are the predominant domain in the rumen, play a central role in fiber digestion. The quality and quantity of rumen fermentation products could affect the quality and quantity of milk and meat. In ruminants, the optimization of feed efficiency leads to improved animal performance. Many studies based on 16S rRNA and metagenomic methods showed compositions of the rumen microbiota. Throughout the lifetime of the ruminant, the rumen microbiota changes dramatically according to many factors. To improve fiber digestion, a profound knowledge of the dynamics that governs the changes of rumen microbiota is needed. In ecological systems, dynamics in bacterial communities are regulated by interaction among community members as well as by the fluctuation of environmental conditions. Therefore, this study attempted to investigate mechanisms that rumen bacteria might use to regulate their dynamics.

In this study, we used metagenomic and metatranscriptomic ruminal datasets and complete genomes of rumen bacteria to mine two genes belonging to two quorum sensing (QS) systems. Firstly, *luxS* gene sequences, coding for autoinducer 2 (AI-2), belonging to *Bacteroidetes* (66.75%), *Firmicutes* (29.80%), *Fusobacteria* (2.64%) and *Actinobacteria* (0.74%) were collected from metagenomic datasets. In the *Firmicutes*, the vast majority of *luxS* gene sequences belonged to the order *Clostridiales* such as a cellulolytic bacterium *Ruminococcus albus*. In *Bacteroidetes*, the majority of *luxS* genes sequences belonged to the genus *Prevotella*. The metatranscriptome dataset *luxS* gene sequences belonged to *Bacteroidetes* (75.5%), *Firmicutes* (15.54%) and *Spirochaetes* (8.88%). In *Bacteroidetes*, 80% of *luxS* gene sequences belonged to the *Prevotella* spp. The *luxS* genes of *Spirochates* was represented by the genus *Treponema*. The conservation of key amino acids in metagenomic and metatranscriptomic LuxS proteins indicates that they might be functional in the rumen.

Secondely, *luxR* gene sequences, coding for *N*-acylhomoserine lactones (AHLs), collected from metagenomic dataset belonged to *Proteobacteria* (9.06%), *Bacteroidetes* (73.57%), *Firmicutes* (7.05%), *Actinobacteria* (4%), *Chloroflexi* (1.47%), *Verrucomicrobia* (2.16%), *Cyanobacteria* (2%), Deinococcus (0.35%) and *Spirochaetes* (0.26%). In *Proteobacteria*, 35% of *luxR* genes belonged to *Alphaproteobacteria*, 34% of them belonged to *Betaprteobacteria* and 32% of them belonged to the class *Gammaproteobacteria*. Many *luxR* gene sequences belonged to plant associated bacteria like *Rhizobium* in the class *Alphaproteobacteria*. *Betaprteobacteria* was dominated by *Burkholderiaceae* family. The *luxR* gene sequences belonging to *Pseudomonadaceae* and *Vibrionaceae* families in *Gammaproteobacteria* accounted for 57% of them. In *Bacteroidetes*, 96.30% of the collected

luxR gene sequences belonged to *Bacteroidales*. In *Firmicutes*, the vast majority of *luxR* gene sequences belonged to *Clostridiales*. *luxR* gene sequences from metatranscriptomic dataset belonged to 12 phyla: *Proteobacteria* (10.41%), *Bacteroidetes* (54.16%), *Firmicutes* (7.29%), *Actinobacteria* (9.37%), Chloroflexi (5.20%), *Spirochaetes* (3.12%), *Verrucomicrobia* (3.12%), *Elusimicrobia* (2.08%), *Acidobacteria* (1.04%), *Cyanobacteria* (1.04%), *Deinococcus* (1.04%), *Fusobacteria* (1.04%). Multiple alignment of LuxR proteins collected from the rumen showed that while three amino acids in their DNA binding domain was conserved, a WYDPWG-motif for AHL-binding domain was lacked.

Moreover, we investigated the adaptation of a major cellulolytic bacterium, *Fibrobacter succinogenes* S85, to carbon sources. Additionally, we showed that *F. succinogenes* S85 was able to grow very slowly on 2.5% of lactose. Furthermore, the addition of cellobiose (0.2%, 0.1%, 0.05% and 0.01%) to the media containing lactose impacted usage of lactose by *F. succinogenes* S85. The microorganism quickly metabolized glucose yielded from lactose, while galactose accumulated in the culture medium. Cellular β-galactosidase activity attained a maximum during the exponential phase of *F. succinogenes* S85 grown on lactose only or with association of 0.01% of cellobiose. Theses findings might explain the existence of the bacterium in the rumen of pre-weaned calves.

In conclusion, many *luxS* and *luxR* genes sequences were retrieved from the rumen. It was presumed that rumen bacteria might use AI-2- and AHL-based QS systems to regulate biofilm formation on feed particles. Additionally, it was revealed that *F. succinogenes* S85 might use lactose in the early colonization of the preruminant rumen.