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# Rumen methanogens display species-specific methane production kinetics

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The objective of this work was to improve our knowledge of methanogens' metabolism at the intra-cellular level in order to design effective strategies for mitigating methane emission in ruminants



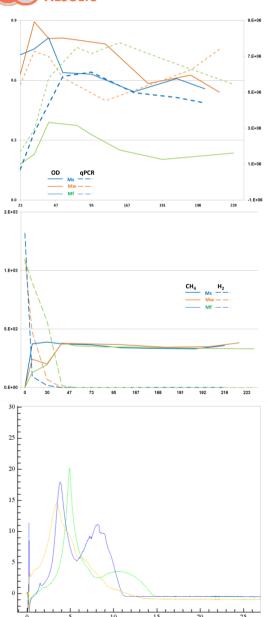
#### **MATERIALS & METHODS**

3 species of methanogenic Archaea

#### Methanobrevibacter smithii (Ms), Methanobacterium formicium (Mf), Methanobrevibacter wolinii (Mw)

- modified Balch medium containing 30% clarified rumen fluid with H<sub>2</sub>/CO<sub>2</sub> over-pressure: 6 ml of media innoculated with 1ml actively growing culture, incubated at 39°C
- daily measures: optical density (OD), H<sub>2</sub> and CH<sub>4</sub> concentrations, qPCR targeting 16S RNA gene
- isothermal microcalorimetry at 39°C; 1.8 ml of media and 0.2 ml of just thawed microbial cultures.

### RESULTS



- According to OD measures, all three species reached the exponential phase in less than 24h and maximum OD values within 2 days after the inoculation. However the maximum OD value for Mf was twice as small as those for Ms and Mw.
- According to 16S rRNA gene copy numbers quantification, the maximum population density was reached within one day for Mw, 2 days for Ms and 3 days for Mf. Differences between species were less pronounced.
- There was no correlation between OD values and 16S rRNA for none of the species.
- All the hydrogen was consumed over a period of two days
- There was no difference in the total amount of methane produced between species
- Isothermal microcalorimetry revealed that methanogenesis is not a simple process. This pathway rather cumulates quite a few simultaneous catabolic and anabolic reactions represented by the two main peaks and the numerous shoulders on the thermal power signal.
- The first peak's form and kinetics are rather comparable but the second one marks the differences between species.
- Though 16S rRNA gene counts and the amount of methane produced were similar between species, total heat was similar for Ms and Mw (6.7 and 6.6 J) but lower for Mf (5.6 J).



#### **IMPORTANCE & PERSPECTIVES**

Species-specific metabolism kinetics revealed by this study suggest differentiation within methanogenesis niche- specialists that may be important to understand. Methanogens physiology will be further studied using transcriptomic and metabolomics approaches to identify the underlying metabolic processes.

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