# USE OF QUANTITATIVE PCR TECHNIQUE FOR STUDYING RUMEN CELLULOLYTIC BACTERIA

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## INTRODUCTION

Anaerobic rumen fibrolytic bacteria, protozoa and fungi degrade fibrous material, allowing ruminants to utilize plant fiber for nutrition. Bacteria are the most numerous of these microorganisms and play a major role in the biological degradation of dietary fiber. *Fibrobacter succinogenes, Ruminococcus albus* and *Ruminococcus flavefaciens* are presently recognized as the major cellulolytic bacterial species found in the rumen. Competitive PCR (c-PCR) assay development for the cellulolytic rumen bacterial species *R. albus* and *R. flavefaciens* was described in some reports. Variations in population sizes of these species can be accurately detected using this technique. However, real-time PCR is also a relevant and useful tool to study the dynamics of microbial populations in complex ecosystems such as the rumen. Its sensitivity and accuracy allow the differentiation of slight changes in bacterial numbers that cannot be seen using microbial techniques or other molecular approaches (16S probing and competitive PCR). The main aim of the study was applying c-PCR and real-time PCR for enumeration of the rumen cellulolytic bacterial species: *R. albus* and *R. flavefaciens* in our lab conditions.

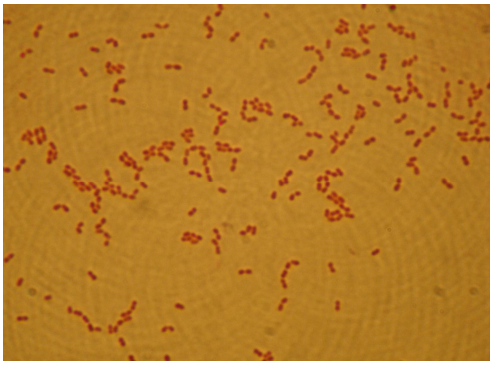
## MATERIALS AND METHODS

- 15 rumen fluid samples were collected from cattle at Ruminant Research Centre.
- Isolation were done by specific process and isolated bacteria were named by specific microbiological tests (Gram staining, Indol, Catalase and Motility test) and sequencing on the region of 16S rDNA.
- Competitive PCR: applied c-PCR procedure and primer sequence of Satoshi and Yasuo (2001)
- Real-time PCR with SYBR probe: protocol of BIO-RAD application guide book with the iCycler iQ Multicolor Realtime PCR Detection System and primer sequence of Satoshi and Yasuo.

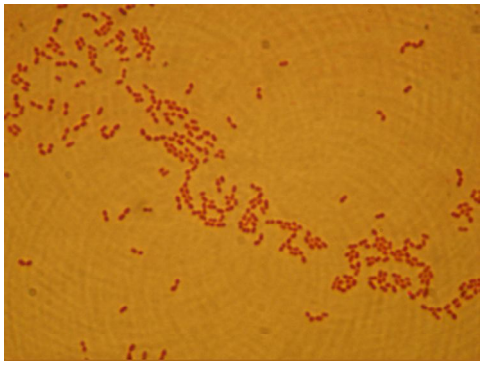
## RESULTS AND DISCUSSION

### Isolate culture of *R. albus* and *R. flavefaciens* from rumen fluid of cattle

Results of specific microbiological tests and sequencing of the region of 16S rDNA proved two isolated bacteria from rumen fluid were *R. albus* and *R. flavefaciens.*



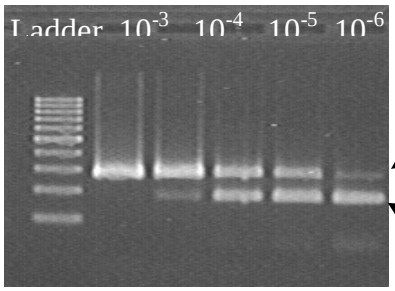
*Ruminococcus albus* - Gram staining



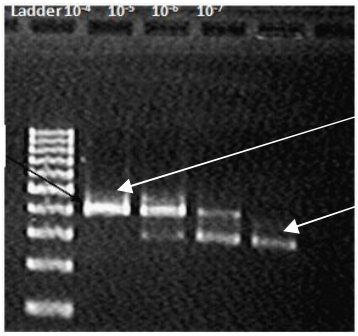
*Ruminococcus flavefacien* - Gram staining

### Competitive PCR

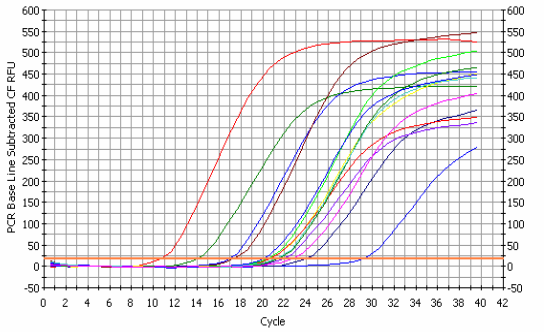
Competitive PCR which had been conducted successfully with two bands of competitor DNA and target DNA showed on gel electrophoresis. However, quantitative results of two bacteria did not get high exact level by ImageJ Window software.



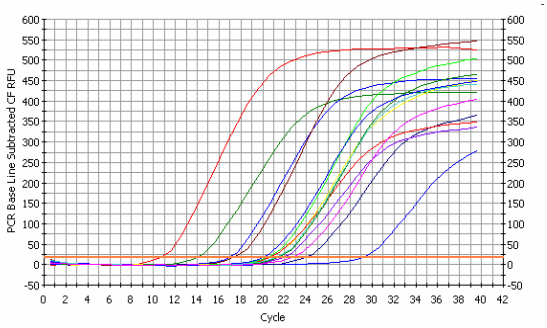

c-PCR - Gel electrophoresis of *R. albus*




c-PCR - Gel electrophoresis of *R. flavefacien*




PCR Amp/Cycle Graph for SYBR-490 *R.albus*




PCR Amp/Cycle Graph for SYBR-490 *R. flave.*

**Real-time PCR with SYBR**

- The number of *R. albus* in collected rumen fluid ranged between 1,25 and $9{,}34 \times 10^6$ copies per ml.
- The number of *R. albus* in collected rumen fluid ranged between 1,31 and $5{,}22 \times 10^7$ copies per ml.

**CONCLUSIONS**

- *R. albus* and *R. flavefaciens* were isolated from collected rumen fluids.
- Competitive PCR had been done successfully but not high exact level yet.
- Real-time PCR with SYBR had quantified *R. albus* and *R. flavefaciens* in rumen fluids within rather wide range.

**SUGGESTIONS**

Real-time PCR with SYBR should be applied and optimized continuously.

Collecting and preservation procedure of rumen fluid samples should be studied continuously.

**REFERENCES**

Satoshi Koike, Yasuo Kobayashi (2001). Development and use of competitive PCR assays for the rumen cellulolytic bacteria: Fibrobacter succinogenes, Ruminococcus albus and Ruminococcus flavefaciens. Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved. PII: S 0 3 7 8 - 1 0 9 7 ( 0 1 ) 0 0 4 2 8 – 1.

Bio-Rad Laboratories, Inc (2005). Real-Time PCR Application Guide Book.