

TITLE: DIVERSITY OF PROKARYOTES PRESENT IN THE SOLID AND IN THE FLUID PHASE OF THE BOVINE RUMEN.

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ABSTRACT:

Ruminants are herbivorous animals that do not have the ability to degrade cellulose and other plant polysaccharide complexes. To obtain energy from these compounds a symbiotic relationship with anaerobic microorganisms was developed. The study of ruminal microbial diversity is important to increase the nutritional efficiency of the animal's diet. The main limitation for this study is the fact that most of these microorganisms are not cultivable. Molecular biology techniques are important tools to aid in the study of ruminal microbiota diversity as these techniques are independent of culture. The objective of this work was to evaluate the diversity of prokaryotes present in the cattle's rumen consuming corn silage. Samples of rumen (solid and fluid phase) were obtained from two animals (1 and 2) consuming corn silage. The samples were collected every six hours (6, 12 and 18h) from the first morning feeding (6:00 a.m.). The total DNA was extracted from the samples using the PSP Stool DNA Spin Kit (Invitac ®) following the manufacturer's instructions. The amplification of the region V3 of the 16S ribosomal DNA was performed using the primers 338F (clamp GC) and 518R. For PCR was used the Master Mix kit (Quiagen) and 4 µL of DNA template. The amplicons were separated by Denaturing Gradient Gel Electrophoresis (BioRad Universal Dcode Mutation Detection System, USA) using 8% polyacrylamide gel with a denaturing gradient of 30 to 60%. Electrophoresis was performed at a constant voltage of 85V for 16h with a constant temperature of 60°C. The large number of amplicons observed reinforces the great diversity of prokaryotes in the ruminal environment. The profile of amplicons observed in corn silage was different from ruminal profiles. Greater diversity was observed between the different animals and sampling times in the same animal. In addition, it was possible to observe that there are differences among microbiota in solid and fluid phase of the ruminal content.

Keywords: DGGE, Corn silage, Molecular analysis, Rumen.

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