

TITLE: CELLULOLYTIC BACTERIA OCURRING IN THE RUMEN OF BOVINES CONDITIONED TO DIFFERENT DIETS

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Abstract:

The rumen is a complex ecosystem colonized by a diversity of organisms, including bacteria, protozoa, fungi, archaea and viruses, that establish a symbiotic relationship with the animal host. The ruminant animals do not secrete digestive enzymes in their forestomach, but the microbial communities that colonize the rumen are able to produce hydrolytic enzymes required for fiber digestion and utilization of soluble and insoluble substrates. The present study aimed to isolate cellulolytic bacteria occurring in bovine rumen conditioned to two different diets: Diet 1, star grass hay (8.6% crude protein), and Diet 2, star grass hay (7 kg) and soy protein (0.27kg). The ruminal fluid was collected four hours after the first feeding and taken to the Laboratory of Rumen Microbiology, Embrapa Dairy Cattle. Aliquots of 1 ml of rumen fluid were transferred to Hungate tubes containing GSM broth (Growth Study Medium) and filter paper strips as cellulose source. The tubes were incubated at 39°C, in anaerobiosis, until the filter paper stay partially or totally degraded. After 48-72h of growth, two transfers were performed using the same growth medium, followed by plating on selective growth media containing 1% carboxymethylcellulose (CMC). Eighty colonies were recovered, and the screening of cellulase production was performed using the enzymatic assay of Congo Red. Twenty three isolates showed clearing zones ≥ 11 mm of diameter, indicating CMC hydrolysis, and for these isolates the enzymatic assay was determined. Gram stain, conventional biochemical tests and the ability for carbohydrate fermentation were performed to identify the selected isolates. The enzymatic index ranged from 1.0 to 2.4mm (average of 1.12mm). All the 23 isolates selected were facultative anaerobe, and six isolates were Gram-negative cocci, seven Gram-positive cocci, five Gram-positive bacilli and five Gram-negative bacilli. The biochemical tests performed for Gram-positive cocci suggest the presence of five *Staphylococcus aureus*, one *Staphylococcus* spp., and one *Enterococcus* spp. Four Gram-negative bacilli were identified as *Escherichia coli*. A greater diversity of cellulose-degrading bacterial species can be found in the rumen and the bacteria isolated in this study have shown varied cellulolytic activity. Subsequent studies are needed to optimize the cellulase production and to confirm the putative phenotypical identification by molecular approaches.

Keywords: cellulolytic bacteria, bovine rumen, hydrolytic enzymes

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