

TITLE: ISOLATION AND PARTIAL CHARACTERIZATION OF CELLULOLYTIC BACTERIA FROM BOVINE RUMEN

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Abstract:

The development of technologies for ethanol production from lignocellulosic biomass is promising, but the high cost of the enzymatic hydrolysis is one of the challenges facing second-generation ethanol production. Thus, the prospection of ruminal microorganisms is an alternative to be explored, since the diet of these animals is rich in lignocellulosic material. The aim of this study was to isolate and characterize cellulolytic bacteria from ruminal content of cannulated cows, fed two different diets: Diet 1, composed by star grass hay (7.0kg), soy protein (0.150kg) and urea ((0.135kg); Diet 2, star grass hay (7.0kg) and urea (0.270kg). 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 as cellulose source. The tubes were incubated at 39°C, in anaerobiosis, until the filter paper stay softened or degraded, which meant use of cellulose. After initial growth, two transfers were performed using the same growth medium, and the last one was plated in 1% carboxymethylcellulose (CMC)-containing media for isolation of cellulolytic bacteria. Eighty colonies were recovered, and the preliminary qualitative analysis of cellulase production was performed using the Congo red enzymatic assay. The enzymatic index was determined for the isolates that showed the largest clearing zones (indicating CMC hydrolysis) in Congo red assay. Gram stain, biochemical tests and the ability for carbohydrate fermentation were performed to identify the selected isolates. Twenty three isolates showed clear zones around the colonies ≥ 11 mm of diameter, and the average of enzymatic index was 1,2mm (ranging from 1,0 mm to 1,95mm). All isolates were facultative anaerobe, and five isolates were Gram-negative cocci, eight Gram-positive cocci, four Gram-positive bacilli and six Gram-negative bacilli. The biochemical tests performed for Gram-positive cocci suggest the presence of five *Staphylococcus aureus*, two *Staphylococcus* sp., and one *Enterococcus* sp.. All the six Gram-negative bacilli were identified as *Escherichia coli*. According to the data presented, the bacteria isolated have shown to be suitable for cellulose production, and they may be further investigated to confirm the identification by molecular analysis and to improve the cellulase production.

Keywords: rúmen, cellulolytic bacteria, cellulase.

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