Title: CONSTRUCTION AND PROSPECTION OF A METAGENOMIC DNA LIBRARY FROM BOVINE RUMEN FOCUSING ON CELLULOLITYC ACTIVITIES.

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Abstract:
The rumen is an ecosystem where ingested food is converted though fermentation to volatile fatty acids and microbial biomass, which serve as energy and protein sources for ruminants. Microbial species into bovine rumen have coevolved and established complex interactions making this environment one of the most important landscape of symbiosis among microorganisms in nature. Rumen microbiome is able to convert cellulose, lignin and other substrates into organic acids, amino acids, and vitamins. Due the great microbial diversity in rumen, including the majority of uncultivable and unidentified microorganisms, their actual representation becomes unsupportable by means of limitations on traditional microbiological techniques applied to extremophiles in laboratories (hyperthermophilic, psychrophilic, and barophilic, for example). In this case, the application of new and wider approaches to understand that microbiome turns necessary. Molecular techniques independent of microorganisms cultivation, as the case of metagenomics, consist in the obtainment of total nucleic acids form environmental samples and their association with hybridization, PCR, DNA cloning and sequencing techniques. The main aim of this work was the exploitation of genetic potential of Nellore bovine ruminal microbiome through the construction of a metagenomic DNA library and its further prospection. For this purpose, cattle were fed with high concentrations of fiber in their diet viewing the prospection of glycosidases on clones of total DNA libraries. The high molecular weight DNA was used for library construction according to *CopyControl™ HTP Fosmid Library Production Kit with pCC2FOS™ Vector* (Epicenter). The validation of the metagenomic library was performed by fosmidial DNA restriction analysis using *Acc*I e *Puv*II endonucleases on ten random clones. Cellulolityc activities were assessed through the screening of clones growing on Luria-Bertani broth supplement with carboxymethylcellulose (CMC) and arabinose. A total of 9120 clones were screened and 380 positive ones were found expressing cellulolityc activities. This microbiome has shown a promising source for search of genes responsible for cellulose degradation.

Key-words: Cellulase, plant biomass deconstruction, Nellore cattle, Carboxymethylcellulose.

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