**Characterization of rumen associated bacteria using fractionation of ruminal fluid**

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Bovines are mammals that obtain their energy by fermenting the grasses they consume in their diet. This process occurs in the rumen and is carried out by a community of anaerobic microorganisms such as bacteria, fungi, archaea, protozoa, and bacteriophages. The members of this community are poorly identified and characterized. An essential step in this direction is the functional and phylogenetic characterization of the microbial species composing the ruminal microbiota. To address this challenge, we separated a ruminal fluid sample by size and density using a sucrose density gradient to assemble Metagenome-assembled Genomes (MAGs). We used different binning programs such as Concoct, MaxBin2 and Metabat2, and refining tools such as Anvi'o and DASTool. The combination of all these bioinformatic tools contributed to reconstruct the genomes of 16 MAGs from Colombian creole breeds that live in tropical environments. Fifteen of these MAGs were enriched in the smallest fraction of the gradient (5%), belonging to the phyla Bacteroidota, Firmicutes_A, Firmicutes, Proteobacteria and Spirochaetota. Fifteen MAGs were novel at the species level, and four at the genus level. The functional characterization of these MAGs suggests differences to what is currently known from the genomic potential of abundant members from this complex environment. On one hand, species of the phyla Bacteroidota and Spirochaeotota show the potential for

hydrolysis of complex polysaccharides in the plant cell wall and towards the production of B-complex vitamins and protein degradation in the rumen. Furthermore, the MAGs belonging to Firmicutes and Alpha-proteobacteria, showed a reduction in several metabolic pathways, however they have genes for lactate fermentation and the presence of hydrolases and esterases related with chitin degradation. Only between 10 to 19 % of the proteins of each MAG could be annotated by RAST, therefore there is still an enormous functional potential that needs to be further explored. Our results demonstrate that the separation of the rumen microbial community by size and density and the simultaneous use of different binning programs enhanced the capacity to characterize ruminal bacteria.