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Microbiome analysis of raw milk samples from Rio Paranaíba producers, MG.

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Minas Gerais is one of the states with the highest milk production in the country. Dairy cattle farming represents not only one of the largest economic activities in the state but also a significant source of employment. However, the quality of raw milk is directly related to the hygiene of the utensils used during its collection and handling. Otherwise, it can lead to the spread of pathogenic microorganisms from the environment and the handlers involved in milking, resulting in diseases in cattle. These diseases pose a significant threat to milk production, reducing both the quality and quantity produced and ultimately causing economic losses in the dairy industry. In this regard, the objective of this study was to assess and compare the microbiota of three milk producers from Rio Paranaíba, MG, using Next-Generation Sequencing (NGS). The samples were collected, properly stored under refrigeration, and sent to the Molecular Diagnostics Laboratory (LDM) at the Federal University of Viçosa - Rio Paranaíba Campus. DNA preparation from the samples followed the protocol of purification by casein dissolution and phenol/chloroform and subsequent extraction with magnetic beads (Zymo), followed by PCR amplification to enrich the 16S rDNA segments of the microbiota. After amplification, the PCR product was purified and sent for third-party sequencing services. Bioinformatics tools were used to assemble the microbiomes and identify the reads (approximately 45 thousand per sample) obtained by comparing them with microorganism databases. Taxonomic assignment of the reads was performed using Kraken2 with the Silva and GreenGenes 16S databases, and the composition of the reads was visualized using Pavian for an interactive analysis of metagenomic classification results. As a result, we detected the presence of psychrotrophic bacteria, of particular concern to the dairy industry, as they produce heat-stable enzymes contributing to the deterioration of milk and dairy products during product storage. Genera such as *Pseudomonas, Bacillus*, and *Acinetobacter* were identified. Furthermore, in one of the samples, the presence of *Salmonella enterica* was detected, a bacterium responsible for severe food poisoning, considered one of the main zoonoses involved in salmonellosis outbreaks reported in various countries, due to its high morbidity characteristics, and particularly the difficulty in implementing measures for its control.

Key words: milk; bacterium; microbiomes; bioinformatics.

Análise do microbioma do leite cru em amostras de produtores de Rio Paranaíba, MG.

Minas Gerais, grande produtora de leite, enfrenta desafios na qualidade devido à higienização inadequada de utensílios usados na ordenha. A contaminação por microrganismos pode afetar a produção e gerar perdas econômicas na indústria de laticínios. O estudo realizado em Rio Paranaíba, MG, utilizou a plataforma Illumina para avaliar a microbiota do leite de três produtores. Identificamos a presença de bactérias psicrotróficas como *Pseudomonas, Bacillus e Acinetobacter*, preocupantes pela produção de enzimas estáveis ao calor, contribuindo para a deterioração do leite

e seus produtos ao longo do armazenamento. Além da detecção de *Salmonella enterica*, uma bactéria associada a intoxicações alimentares graves.

Palavras-chave: leite; bactéria; microbiomas; bioinformática.

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