

TITLE: BIODIVERSITY AND GENETIC DIVERSITY OF *Lactococcus lactis* subsp. *lactis* STRAINS OBTAINED FROM DAIRY ENVIRONMENT

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ABSTRACT: *Lactococcus lactis* subsp. *lactis* is a member of the lactic acid bacteria (LAB) used worldwide in the dairy industry. This study aimed to identify and evaluate the genetic diversity of *L. lactis* subsp. *lactis* isolated from different dairy environments, including dairy source as raw milk from cow, goat and buffalo, artisanal cheese from the Amazon region and Marajó Island, and non-dairy source as grass and peanut silages. From a bacteria culture collection belonging to InovaLeite (Universidade Federal de Viçosa), 90 isolates were select based on the morphological characteristics compatible with the genus *Lactococcus*. The isolates were submitted to 16S rRNA sequencing and 23 isolates were identified as *L. lactis*. The species were confirmed by specie-specific PCR and the subspecies were identified by subspecie-specific PCR, revealing that all strains belong to the subspecie *lactis*. Subsequently, the 23 strains were submitted to rep-PCR and PFGE analyzes to verify the presence of clones in the collection and rep-PCR had greater discriminatory power. For rep-PCR analysis, the dendrogram of the strains presented 18 clusters, considering 90% of similarity. The groups formed had low homology to each other, indicating high diversity among the strains. In the PFGE analysis, the existence of four profiles with 100% of similarity was found. In general, the cluster obtained in the PFGE confirmed the result of rep-PCR when the clustering tendency between dairy and non-dairy strains was observed. The typing of the *L. lactis* subsp. *lactis* strains was performed by MLST and revealed the existence of 11 STs, of which 9 had not yet been identified in MLST database, indicating a high level of heterogeneity among the strains. In the analysis of the ST grouping, the formation of 2CC was made and CC1 was formed by 3 isolates and CC2 by two isolates. All other STs were distributed as *singletons*. The study of the ST distribution carried out in the e-BURST program did not indicate a common ancestor among the organisms in the studied collection. These results show that this collection has an interesting diversity of *L. lactis* subsp. *lactis* to be explored and can contribute to the supply of new strains with potential to be used as starter culture in the dairy industry.

Keywords: dairy ecosystem, lactic acid bacteria, *Lactococcus*, MLST, PFGE,