

Artisanal cheeses safety is associated with the native microbiota

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Canastra Cheese is one of seven acknowledged artisanal cheese-producing regions in Minas Gerais state, and it was recognized as Brazilian Intangible Cultural Heritage. The production of Serra da Canastra cheese uses a fermentation starter culture, popularly known as "*pingo*", which consists of a portion of the whey recovered from the previous day's production. Studies on this endogenous starter composition are especially focused on lactic acid bacteria (LAB), using traditional microbiological analysis methods. In this study we aimed to isolate a broad range of bacteria present in this traditional starter culture (*pingo*), and to characterize representative strains using traditional culture-based and genomic-based methods. We evaluated and isolated bacterial strains from *pingo* samples obtained from 10 different cheese producers using various culture media. Bacterial isolates were initially screened based on colony morphology and Gram staining. Subsequently, a collection of 113 isolates was screened by sequencing the 16S rDNA gene, to select representative isolates for full genome sequencing. These selected isolates were also characterized for biogenic amine production, hemolytic activity, gelatinase activity, bacteriocin production, mucin degradation and antimicrobial susceptibility. The results revealed a diverse microbial community dominated by LAB such as *Enterococcus faecalis*, *Streptococcus* spp., and *Leuconostoc* spp., alongside nonstarter culture, including *Staphylococcus sciuri*, *Staphylococcus saprophyticus*, and *Rothia kristinae*. The complete genome of 11 strains was sequenced. Pangenome analysis together with ANI demonstrated similarities between the isolated LAB and others deposited in GenBank and also related to cheese production, such as *Lactococcus lactis* and *Streptococcus* sp. However, *Leuconostoc* sp., even though it is associated with cheese production, is still poorly characterized, which makes it difficult to compare to the point of knowing whether or not it is a new species. [Hemolytic activity was observed in all LAB isolates, raising concerns about potential pathogenicity](#). Encouragingly, none of the strains demonstrated. Hemolytic activity was observed in all LAB isolates, raising concerns about potential pathogenicity. Encouragingly, none of the strains demonstrated gelatinase activity, neither biogenic amine production, suggesting a reduced risk of toxic amine accumulation in matured cheeses. Bacteriocin production tests indicated that some Enterobacteriaceae strains exhibited inhibitory effects against *Listeria monocytogenes*. Antimicrobial susceptibility profiling revealed concerning levels of antibiotic resistance among strains historically linked to bovine mastitis (Enterococci), emphasizing the need for stringent hygiene practices and improved farm management strategies. Our study underscores the need for scientifically guided interventions in artisanal cheese production to enhance production practices. One approach is to locally screen for safe starter cultures, or to use standardized microbial consortia made with native strains tested for safety. Such strategies could reduce microbial risks while preserving Brazil's cheese heritage. Our findings advance understanding of microbial dynamics in cheese producing environments and highlight the role of microbiological innovation in ensuring food safety and sustainable production.

## References

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