FABÍOLA FARIA DA CRUZ RODRIGUES

BIODIVERSIDADE E CARACTERIZAÇÃO TECNOLÓGICA DE BACTÉRIAS LÁTICAS ISOLADAS DE QUEIJOS MINAS ARTESANAL DA REGIÃO ENTRE SERRAS DA PIEDADE AO CARAÇA

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, para obtenção do título de *Magister Scientiae*.

Orientador: Antônio Fernandes de Carvalho

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Antônio Fernandes de Carvalho
Orientador

A minha família.

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“Não é o mais forte que sobrevive, nem o mais inteligente.
Quem sobrevive é o mais disposto a mudança”.

(Charles Darwin)

RESUMO

RODRIGUES, Fabíola F. C., M.Sc., Universidade Federal de Viçosa, setembro de 2024. **Biodiversidade e caracterização tecnológica de bactérias láticas isoladas de queijos Minas artesanal da região Entre Serras da Piedade ao Caraça**. Orientador: Antonio Fernandes de Carvalho. Coorientadora: Andressa Fusieger.

Bactérias ácido-láticas (BAL) são um grupo diverso de bactérias encontradas em alimentos, especialmente derivados lácteos, produzindo ácido lático na fermentação de carboidratos. São gram-positivas, formadoras de esporos, predominantemente anaeróbicas facultativas e desempenham um papel crucial na produção de queijos, influenciando sabor, aroma e textura, além de possuírem propriedades antimicrobianas. O estudo focou na diversidade de BAL em queijo Minas artesanal (QMA) da região Entre Serras da Piedade ao Caraça, utilizando métodos genotípicos para identificação de isolados e fenotípicos para avaliação do potencial tecnológico na indústria de laticínios. Inicialmente foi realizado uma análise microbiológica geral dos queijos de 4 fazendas da região de estudo com contagem de patógenos (*Escherichia coli, Staphylococcus aureus, Salmonella e Listeria monocytogenes*) nos tempos 0,30 e 60 e também contagem de BAL nos tempos 0, 7, 21, 30, 45 e 60. Com isso, vimos que, após 30 dias de maturação os queijos se encontravam seguros para consumo, com redução das contagens de patógenos a níveis aceitáveis e, as contagens de BAL tiveram aumento ao longo do tempo de maturação, demonstrando sua forte influência na redução das contagens dos patógenos, além de revelar a importância da etapa de maturação para esse tipo de queijo. Depois disso, foi selecionado uma fazenda específica e foram obtidos 956 isolados da coleção de culturas do Inovaleite (Laboratório de Leite e Derivados, Universidade Federal de Viçosa), provenientes de QMA da região de estudo, em diferentes tempos de maturação. Os isolados foram purificados e testados quanto a produção de catalase e coloração de Gram. O DNA foi extraído e realizado o sequenciamento do gene 16S rRNA, e os isolados identificados foram submetidos à REP-PCR para avaliar a diversidade genética da espécie *Lacticaseibacillus paracasei*. Os isolados de *L. paracasei* foram testados para características tecnológicas. Os resultados do sequenciamento identificaram seis gêneros: *Lactiplantibacillus, Lacticaseibacillus, Enterococcus, Pediococcus, Weissella* e *Lactococcus*, com *Lactiplantibacillus* sendo o mais frequente. Os isolados de *L. paracasei* (n=197) foram agrupados em 31

clusters e 31 perfis únicos após a REP-PCR, indicando alta diversidade entre os isolados. Em relação aos testes tecnológicos, na produção de diacetil, quatro isolados (770, 500, 38 e 743) apresentaram alta produção de diacetil. A atividade proteolítica foi positiva para todos os isolados, com destaque para o isolado 743. Todos os isolados demonstraram capacidade fermentativa, com padrões de coagulação uniformes na maioria. Quanto à capacidade de acidificação, todos os isolados foram eficazes, com variação na redução do pH, destacando-se dois isolados que reduziram o pH entre 1,5 e 2,0 unidades em 24 horas (703 e 401), enquanto outros reduziram mais de 2,0 unidades. Esses resultados sugerem a viabilidade de uso desses isolados na produção de derivados lácteos fermentados, devido às suas capacidades tecnológicas distintas. Conclui-se que, a diversidade de BAL na região é rica e ainda pouco explorada, indicando potencial para encontrar outros isolados com variadas características. A fazenda estudada apresentou dominância de um gênero específico de BAL pelo método analisado, que pode ser melhor explorado para oferecer benefícios à indústria de alimentos. Dos 55 isolados analisados nos testes tecnológicos, a maioria demonstrou bom potencial tecnológico, indicando viabilidade para uso na indústria de laticínios. No entanto, sua aplicação depende das funções desejadas no produto final, exigindo estudos mais avançados e detalhados para melhor caracterizar o potencial tecnológico das BAL.

Palavras-chave: Bactérias ácido láticas. Queijo Minas artesanal. Potencial tecnológico.

ABSTRACT

RODRIGUES, Fabíola F. C., M.Sc., Universidade Federal de Viçosa, July, 2024. **Biodiversity and technological characterization of lactic acid bacteria isolated from Minas artisanal cheeses from the region Entre Serras da Piedade ao Caraça**. Adviser: Antonio Fernandes de Carvalho. Co-adviser: Andressa Fusieger.

Lactic acid bacteria (LAB) are a diverse group of bacteria found in foods, especially dairy products, producing lactic acid in the fermentation of carbohydrates. They are gram-positive, spore-forming, predominantly facultative anaerobic and play a crucial role in cheese production, influencing flavor, aroma and texture, in addition to having antimicrobial properties. The study focused on the diversity of LAB in artisanal Minas cheese (QMA) from the Entre Serras da Piedade to Caraça region, using genotypic methods to identify isolates and phenotypic methods to evaluate technological potential in the dairy industry. Initially, a general microbiological analysis was carried out on cheeses from 4 farms in the study region with pathogen counts (Escherichia coli, Staphylococcus aureus, Salmonella and Listeria monocytogenes) at times 0, 30 and 60 and also BAL counts at times 0, 7, 21, 30, 45 and 60. With this, we saw that, after 30 days of maturation, the cheeses were safe for consumption, with a reduction in pathogen counts to acceptable levels and, BAL counts increased over the maturation time, demonstrating their strong influence in reducing pathogen counts, in addition to revealing the importance of the maturation stage for this type of cheese. After that, a specific farm was selected and 956 isolates were obtained from the Inovaleite culture collection (Milk and Derivatives Laboratory, Federal University of Viçosa), from QMA in the study region, at different maturation times. The isolates were purified and tested for catalase production and Gram staining. The DNA was extracted and the 16S rRNA gene was sequenced, and the identified isolates were subjected to Rep-PCR to evaluate the genetic diversity of the species Lacticaseibacillus paracasei. L. paracasei isolates were tested for technological characteristics. The sequencing results identified six genera: Lactiplantibacillus, Lacticaseibacillus, Enterococcus, Pediococcus, Weissella and Lactococcus, with Lactiplantibacillus being the most common. L. paracasei isolates (n=197) were grouped into 31 clusters and 31 unique profiles after Rep-PCR, indicating high diversity among isolates. Regarding technological tests, in the production of diacetyl, four isolates (770, 500, 38 and 743) showed high production of diacetyl. Proteolytic activity was positive for all isolates,

especially isolate 743. All isolates demonstrated fermentative capacity, with uniform coagulation patterns in the majority. Regarding acidification capacity, all isolates were effective, with variation in reducing pH, highlighting two isolates that reduced pH between 1.5 and 2.0 units in 24 hours (703 and 401), while others reduced it more of 2.0 units. These results suggest the feasibility of using these isolates in the production of fermented dairy products, due to their distinct technological capabilities. It is concluded that the diversity of BAL in the region is rich and still little explored, indicating the potential to find other isolates with varied characteristics. The farm studied showed dominance of a specific genus of LAB using the analyzed method, which can be better explored to offer benefits to the food industry. Of the 55 isolates analyzed in technological tests, the majority demonstrated good technological potential, indicating viability for use in the dairy industry. However, its application depends on the desired functions in the final product, requiring more advanced and detailed studies to better characterize the technological potential of BAL.

Keywords: Lactic Acid Bacteria. Artisanal Minas Cheese. Techonological potential.

SUMÁRIO

1. INTRODUÇÃO GERAL

Bactérias ácido láticas, conhecidas como BAL, formam um grupo diversos de bactérias que são amplamente encontradas na natureza, principalmente em alimentos, como os derivados lácteos. Elas possuem como característica essencial a capacidade de produzir ácido lático através da fermentação de carboidratos. Essas bactérias são classificadas como gram positivas, não formadoras esporos, são negativas para catalase e oxidases, podendo apresentar-se na forma de cocos ou bacilos e são predominantemente anaeróbicas facultativas (Liu *et al*., 2014).

Essas bactérias são essencialmente mesófilas, embora existam algumas linhagens termófilas, capazes de crescer em temperaturas de 5 a 45 ºC. Elas podem se desenvolver em pH de 3,8, produzem um grande número de enzimas glicolíticas, lipolíticas e proteolíticas, que transformam os nutrientes fundamentais do leite e do queijo em compostos com propriedades sensoriais desejáveis. Além disso, elas produzem diversos fatores antimicrobianos, incluindo ácidos orgânicos, peróxido de hidrogênio, bacteriocinas, diacetil e acetaldeído que exercem efeitos benéficos em produtos alimentícios. Algumas BAL também podem ser usadas por secretarem exopolissacarídeos, influenciando a textura dos alimentos quando desejável (Holzapfel e Wood, 2014; Lahtinen *et al.*, 2011).

A fermentação pode ocorrer por duas vias: a homofermentativa, onde o ácido lático é o único produto formado, e a heterofermentativa, que resulta na formação de outros produtos além do ácido láctico, como ácido acético, etanol e CO_2. Além disso, as BAL podem produzir, em menores quantidades, outros compostos orgânicos que contribuem para o aroma e sabor do produto fermentado (Liu *et al*., 2014).

Em relação aos alimentos, BAL *starter* são responsáveis pela produção de ácidos durante a elaboração do queijo por exemplo, além de contribuírem para o processo de maturação e produzir substâncias antagônicas que inibem o crescimento de bactérias indesejáveis (Macori e Cotter, 2018; Wilkinson e LaPointe, 2020).

Em queijos artesanais, a presença de BAL pode ser favorecida também devido a utilização do leite cru, sendo sua caracterização diferenciada de acordo com a região produtora (Kamimura *et al.,* 2019; Erhardt *et al*., 2022; Hosken *et al*., 2023). Os gêneros de BAL mais encontrados nesses produtos são: *Enterococcus, Lactococcus, Streptococcus, Leuconostoc e Lactobacillus.* Devido a essa diversidade microbiana

dos queijos, cada vez mais tem-se ampliado os estudos de identificação e caracterização da microbiota desse produto (Erhardt *et al*., 2022; Ferreira *et al*., 2023). Durante o processo de fabricação de queijos, essas bactérias podem ser utilizadas como fermento láctico devido à sua capacidade de conferir características sensoriais únicas, como sabor, aroma e textura, que agregam um valor excepcional ao produto final (Hosken *et al*., 2023).

Portanto, esse trabalho teve como finalidade estudar a diversidade de BAL presentes em queijo Minas artesanal (QMA) produzidos em uma fazenda na região Entre Serras da Piedade ao Caraça - MG, por meio de métodos fenotípicos e genotípicos e caracterizar tecnologicamente algumas das cepas encontradas, avaliando seu potencial para uso na indústria de lácteos.

2. IMPORTÂNCIA DO ESTUDO

O estudo de QMA tem grande relevância, pois permite preservar e promover a qualidade e autenticidade do produto, garantindo sua continuidade e valorizando a tradição da região, sendo esse, um produto de alto valor gastronômico para o país. Além disso existe a possibilidade da descoberta de novas cepas com potencial tecnológico para uso na indústria. Nesse sentido, a região das Entre Serras da Piedade ao Caraça - MG, que foi reconhecida como produtora de QMA em 2022, se destaca como foco do trabalho, visando aprimorar os estudos nesta região, com a caracterização e identificação de cepas de BAL.

Ademais, a identificação das características fenotípicas e genotípicas trará diversas informações a respeito dos isolados de queijos da fazenda em estudo, visando assim, o enriquecimento do banco de dados da região, com informações importantes sobre quais microrganismos estão presentes nesses queijos, permitindo também, selecionar cepas específicas que conferem características desejáveis ao queijo, como textura, sabor e aroma. Portanto, a caracterização e identificação de BAL do QMA contribui para o avanço científico no campo da microbiologia e da tecnologia de alimentos. Promovendo a geração de conhecimento sobre as características dessas bactérias, seu metabolismo, interações e comportamento em diferentes condições.

3. OBJETIVOS

3.1 Objetivo geral

Identificar e caracterizar a diversidade de BAL de QMA na região Entre Serras da Piedade ao Caraça - MG, bem como avaliar o seu possível potencial tecnológico para uso industrial.

3.2 Objetivos específicos

- Caracterização fenotípica dos isolados de BAL do QMA de uma fazenda da região Entre Serras da Piedade ao Caraça - MG;

- Identificação dos isolados com características fenotípicas de BAL, por meio do sequenciamento da região do gene 16S rRNA;

- Seleção das cepas de uma espécie específica para realizar caracterização genotípica com Rep-PCR e determinação do potencial tecnológico para uso industrial.

4. REFERENCIAL TEÓRICO

4.1 Queijo Minas artesanal

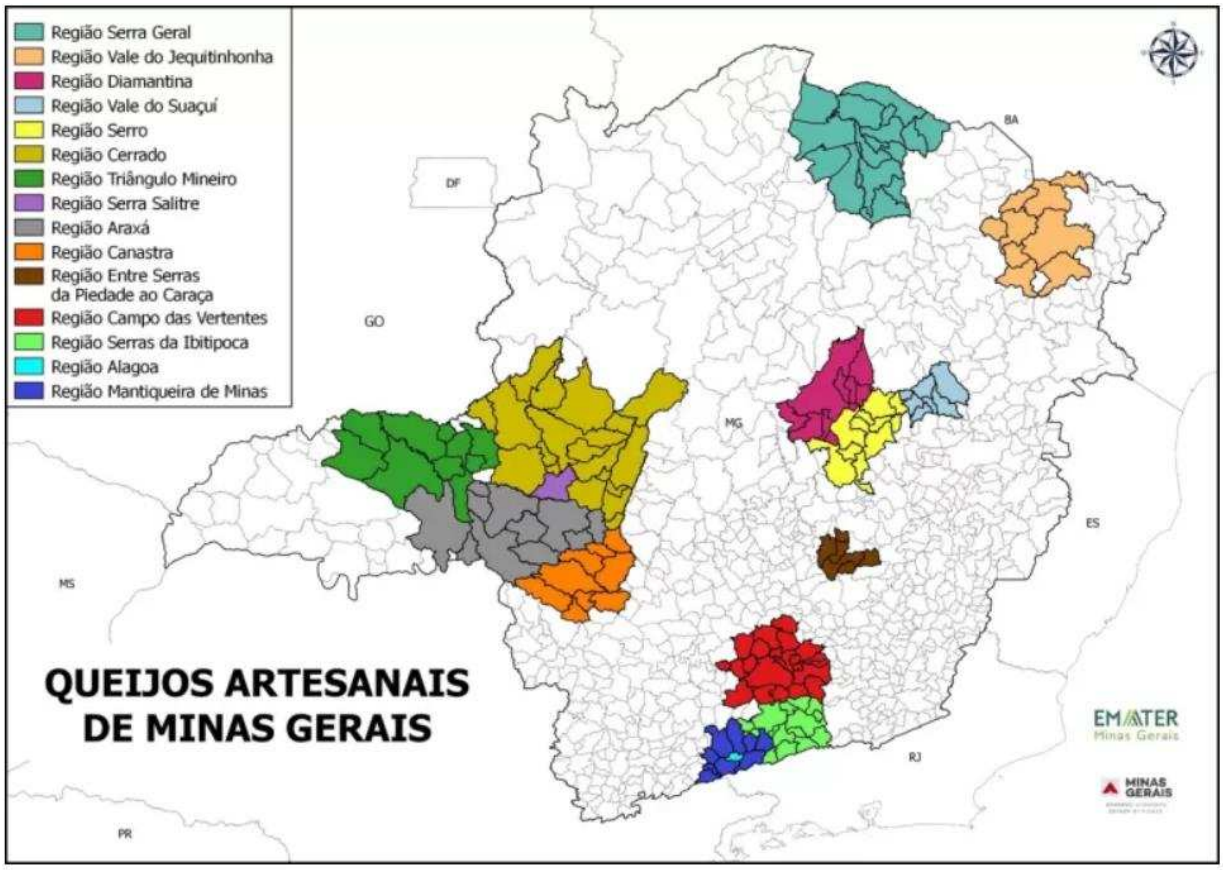
Segundo a Portaria do Instituto Mineiro de Agropecuária (IMA) nº 2303, de 20 de maio de 2024, considera-se o QMA, o queijo elaborado a partir do leite de vaca, integral, fresco, cru, hígido e recém ordenhado, com utilização de cultura láctica natural, que apresente consistência firme, cor e sabor próprios, massa uniforme, isenta de corantes e conservantes, com ou sem olhaduras mecânicas, produzido dentro do estado de Minas Gerais, conforme a tradição histórica e cultural da região de origem (IMA, 2024).

A produção de queijos no Brasil é muito expressiva e neste contexto, o estado de Minas Gerais se destaca. Segundo o Relatório de 2022 do Sistema Safra Agroindústria da Empresa de Assistência Técnica e Extensão Rural do Estado de Minas Gerais (EMATER), o Estado possuía 3.103 agroindústrias produtoras de QMA, com produção estimada de 21,8 mil toneladas por ano, representando 65,2% da produção dos queijos artesanais das agroindústrias familiares (EMATER, 2022).

Atualmente, existem 15 regiões identificadas e reconhecidas como produtoras de queijos artesanais no território do estado. Dez são regiões produtoras do Queijo Minas Artesanal (QMA): Araxá, Campo das Vertentes, Canastra, Cerrado, Diamantina, Entre Serras da Piedade ao Caraça, Serra do Salitre, Serro, Triângulo Mineiro, Serras da Ibitipoca – e cinco produtoras de outros tipos de queijos artesanais mineiros: Alagoa, Mantiqueira de Minas, Serra Geral do Norte de Minas, Vale do Jequitinhonha e Vale do Suaçuí (Figura 1) (IMA, 2024). A região das Entre Serras da Piedade ao Caraça foi reconhecida como produtora de QMA em 2022 e é composta pelos seguintes municípios: Santa Bárbara, Caeté, Catas Altas, Rio Piracicaba, Bom Jesus do Amparo e Barão de Cocais. E o queijo Entre Serras tem esse nome porque é produzido entre as Serras da Piedade ao Caraça (IMA, 2022).

Segundo informações do IMA (2024), o queijo da região Entre Serras possui as seguintes características: forma redonda, borda reta ou arredondada, com variações de altura entre 4 e 5 centímetros, com diâmetro entre 13 e 15 centímetros e peso entre 600 a 900 gramas e altura de 5 centímetros ou com 10 e 12 centímetros de diâmetro e peso entre 200 a 400 gramas.

Figura 1 – Mapa das regiões produtoras de QMA em Minas Gerais.



Fonte: (“Queijos Artesanais | MG.GOV.BR - Agricultura”, [s.d.])

As etapas de fabricação do QMA são, em sua maioria, comuns nas regiões onde é produzido. O processo de fabricação deve começar logo após a ordenha, com o leite à temperatura ambiente, sem passar por resfriamento ou tratamento térmico. Ele deve ser oriundo da fazenda produtora do queijo, obtido por ordenha manual ou mecânica, sendo necessária à sua filtração. Para a fermentação, utiliza-se o “pingo” (fermento natural endógeno) e o coalho para coagulação. Na prensagem, utiliza-se apenas o processo manual, e a salga é realizada a seco. O tempo de maturação segue o período estipulado para cada microrregião que possua pesquisas científicas ou, na ausência desses estudos, pelo maior período determinado geral, para a região por exemplo, o tempo de maturação é de 22 dias (Costa *et al*., 2022).

Para Kamimura *et al.* (2019), os queijos artesanais brasileiros têm grande importância histórica e socioeconômica, especialmente porque sua comercialização em todo o país permite avivar a conexão entre o produto e a cultura popular, essencial

para a preservação desse patrimônio. Isso vai de encontro quando vemos a crescente valorização dos QMA nas regiões em que são produzidas e no quanto isso contribui para o reconhecimento e valorização territorial.

4.2 Diversidade de BAL em QMA

As BAL participam de um grupo de microrganismos que apresentam diversas características morfológicas, metabólicas e fisiológicas semelhantes. Esse grupo de bactérias são responsáveis por uma série de processos fermentativos essenciais na produção de QMA e, embora possuam como principal função a produção de ácido lático no decorrer do processo fermentativo, elas têm uma atuação muito importante na maturação do queijo, visto que suas enzimas estão envolvidas de modo direto nos processos de proteólise, lipólise e conversão de aminoácidos em compostos aromáticos (Slattery *et al.*, 2010; Tamang *et al*., 2016, Hosken *et al*., 2023).

As BAL podem ser classificadas de acordo com sua temperatura de crescimento ideal (mesófilas ou termofílicas) e com o tipo de fermentação láctica (homofermentativas e heterofermentativas). BAL homofermentativas (*Pediococcus, Streptococcus, Lactococcus, Vagococcus* e alguns Lactobacilos) são consideradas culturas iniciadoras, sendo essenciais, pois acidificam o leite, iniciam o processo de coagulação, originando o queijo e liberando compostos enzimáticos responsáveis pela lipólise, proteólise e conversão de aminoácidos, que contribuem para a formação de sabores característicos. Por outro lado, BAL heterofermentativas (*Leuconostoc, Oenococcus, Weissella, Carnobacterium* e alguns Lactobacilos) que produzem, além do ácido láctico, substâncias como os ácidos acético, butírico e propiônico, bem como álcoois. Este grupo microbiano também metaboliza compostos como dióxido de carbono, peróxido de hidrogênio, acetaldeído e diacetil (Holzapfel e Wood, 2014; Lahtinen *et al.*, 2011). Todo esse processo resulta em características sensoriais típicas de cada produto artesanal, como textura e sabor (Camargo *et al*., 2021a; Hosken *et al*., 2023).

Embora as BAL compreendam uma ampla diversidade de bactérias, *Lactococcus, Streptococcus, Leuconostoc, Enterococcus* e o grupo dos Lactobacilos são os mais comumente identificados em queijos artesanais. Alguns estudos realizados no Brasil ainda demonstram que, além da região de origem, a microbiota

láctica prevalente nos estabelecimentos produtores também pode determinar as características dos queijos artesanais (Camargo *et al.*, 2021b).

Além disso, algumas cepas podem produzir substâncias antimicrobianas, principalmente bacteriocinas, sendo esses importantes na modulação do crescimento de microrganismos patogênicos, podendo inibir ou inativar os mesmos (Pineda *et al*., 2020). Portanto, a diversidade dessas bactérias láticas contribui para a riqueza e complexidade sensorial desses queijos, uma vez que a composição deste grupo de bactérias é diretamente influenciada pelas características geográficas das regiões produtoras e sendo uma das principais responsáveis pelas características dos queijos durante a sua produção e maturação (Nero *et al*., 2021).

4.3 Potencial tecnológico de BAL

Dentre os potenciais tecnológicos de BAL, destaca-se a fermentação, que é um método conveniente para melhorar a qualidade nutritiva dos alimentos e conceder atributos sensoriais importantes ao produto final (Huang, Lai e Chou, 2011). A classificação das culturas láticas é dividida em BAL *starter* (SLAB) ou iniciadoras, por terem a capacidade de metabolizar a lactose e, BAL *non-starter* (NSLAB) ou não iniciadoras/adjuntas, se evidenciando durante o processo de fabricação e período de maturação dos queijos (Hosken *et al*., 2023).

Além disso, a produção de ácido lático pela microbiota *starter* durante a fabricação do queijo, facilita a ação do coalho e auxilia na expulsão do soro, além do desenvolvimento das características sensoriais do queijo e o controle do crescimento de patógenos (Camargo *et al.*, 2021b; Hosken *et al*., 2023). A produção de proteases é outra importante propriedade tecnológica de BAL, pois pode aumentar a capacidade do coágulo em ligar água devido à quebra da rede proteica, melhorando o desenvolvimento da textura dos queijos (Lima *et al.*, 2021; McSweeney, 2004).

As BAL podem produzir também compostos aromáticos, como diacetil ou acetoína, que são gerados como produtos finais do metabolismo do citrato, dando ao queijo sabores amanteigados e odores intensos (Mukisa *et al*., 2017). Além dessas características, elas podem produzir ácido acético, etanol, várias enzimas e compostos antimicrobianos (Hati, Mandal e Prajapati, 2013).

Outra importância fundamental de BAL é sobre seu papel na inocuidade dos alimentos, decorrente da síntese de substâncias antimicrobianas, responsáveis por

produzir um microambiente desfavorável a diversos microrganismos contaminantes, inclusive aqueles com potencial patogênico, sendo este fator a base de inúmeros métodos de conservação de alimentos por fermentação (Priyodip, Prakash e Balaji, 2017).

Atualmente, as indústrias de laticínios buscam cada vez mais produtos novos e aprimorados com base em fermentações de BAL, e estão constantemente em busca de cepas que possam contribuir com características sensoriais nos alimentos, pois elas possuem um potencial enriquecedor para o produto. Dentre isso, podemos citar a elaboração de fermentos a partir desses isolados, onde o estudo das características tecnológicas das BAL são fundamentais para definir qual tipo de fermento melhor se adequa para aplicação industrial.

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CAPÍTULO I

Microbiological characterization of artisanal cheese from Entre Serras da Piedade ao Caraça region, Minas Gerais, Brazil

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Title page

Microbiological characterization of artisanal cheese from Entre Serras da Piedade ao Caraça region, Minas Gerais, Brazil

Caracterização microbiológica de queijos artesanais da região de Entre Serras da Piedade ao Caraça, Minas Gerais, Brasil

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Abstract

Minas Artesanais cheeses (MAC) are traditionally made with raw milk in several regions of Minas Gerais, Brazil, and undergo a ripening process that allows lactic acid bacteria (LAB) to act as bioprotectors, contributing to the microbiological safety of the product. Aiming to evaluate the quality and microbiological safety of MAC from the Entre Serras da Piedade ao Caraça region, this study sought to correlate the presence of LAB during the MAC ripening process with the inhibition or absence of pathogens. For this purpose, cheeses from a single batch were collected on the day of production (time 0) and after 7, 14, 21, 30, 45 and 60 days of ripening, from four MAC producers (A, B, C and D). At the ripening times (0, 30 and 60), analyses were performed for *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp. and *Listeria monocytogenes*, in addition to the LAB count at all ripening times. The cheeses from producers A, C and D were considered microbiologically safe for consumption after 30 days of ripening, while those from producer B reached this safety after 60 days of ripening. However, the presence of pathogens, even within legal limits, highlights the need to review the good manufacturing practices adopted in these cheese production facilities. The LAB population remained above 10^6 CFU/g throughout the ripening period, contributing to pathogen control. It is noteworthy that this is the first study that uses cultivation-dependent techniques to microbiologically characterize MAC from the Entre Serras da Piedade ao Caraça region.

Index terms: cheese terroir, food safety, indicator microorganisms, lactic acid bacteria, raw milk cheese.

Resumo

Os queijos Minas Artesanais (MAC) são feitos tradicionalmente com leite cru em diversas regiões de Minas Gerais, Brasil, e passam por um processo de maturação que permite que as bactérias ácido-láticas (BAL) atuem como bioprotetores, contribuindo para a segurança microbiológica do produto. Com objetivo de avaliar a qualidade e a segurança microbiológica dos MAC da região de Entre Serras da Piedade ao Caraça, este estudo buscou correlacionar a presença de BAL durante o processo de maturação de MAC com a inibição ou ausência de patógenos. Para isso, foram realizadas coletas de queijos de um único lote no dia da produção (tempo 0) e após 7, 14, 21, 30, 45 e 60 dias de maturação, de quatro produtores de MAC (A, B, C e D). Nos tempos de maturação (0, 30 e 60), foram realizadas análises para de *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp. e *Listeria monocytogenes*, além da contagem de BAL em todos tempos de maturação. Os queijos dos produtores A, C e D foram considerados seguros microbiologicamente para consumo após 30 dias de maturação, enquanto os do produtor B atingiram essa segurança após 60 dias de maturação. No entanto, a presença de patógenos, mesmo dentro dos limites legais, ressalta a necessidade de revisar as boas práticas de fabricação adotadas nestas instalações de produção de queijos. A população de BAL manteve-se superior a 10^6 UFC/g, durante todo o período de maturação, contribuindo para o controle de patógenos. Destaca-se que este é o primeiro estudo que que utiliza técnicas dependentes de cultivo para caracterizar microbiologicamente os MAC da região de Entre Serras da Piedade ao Caraça.

Termos de indexação: terroir de queijo, segurança alimentar, microrganismos indicadores, bactérias lácticas, queijo de leite cru.

Introduction

Minas Artesanal Cheese (MAC), produced in the state of Minas Gerais, is one of the most famous artisanal cheeses in Brazil, whose production method was recognized as Intangible Cultural Heritage of Minas Gerais and Brazil by the Instituto do Patrimônio Histórico e Artístico Nacional (IPHAN) in 2008. MAC is defined as matured cheese produced from raw milk, salt and natural yeast, called “pingo”. Pingo is the whey that runs off the cheese made the day before, which contains microorganisms that give MAC unique sensory and microbiological characteristics (Castro et al., 2016; Kamimura et al., 2020). Cheese processing must begin no later than ninety minutes after the start of milking and respect cultural and regional traditions passed down from generation to generation (IMA, 2022). The final product has soft consistency, compact texture, yellow color, moderate odor and flavor intensity related to specific geoclimatic and production characteristics (IMA 2024).

The State of Minas Gerais has several microregions recognized as traditional in the production of MAC. In 2022, Entre Serras da Piedade ao Caraça is the tenth region officially recognized by the Instituto Mineiro de Agropecuária (IMA) as a producer of MAC, which includes the municipalities of Catas Altas, Barão de Cocais, Santa Bárbara, Rio Piracicaba, Bom Jesus do Amparo, and Caeté (IMA, 2022; IMA 2024). Camargo et al. (2021a) used a culture-independent method and provided initial insights into the bacterial diversity of MAC from the Entre Serras da Piedade ao Caraça region. The core bacterial microbiota of cheese samples includes not only lactic acid bacteria (LAB), but also spoilage and potentially pathogenic bacteria.

For microbiological quality and safety of cheeses, several aspects must be considered, such as the quality of raw milk, control of hygienic-sanitary conditions during production, storage, and marketing of the product (Silva et al., 2023). When

these aspects are not observed, cheeses can carry pathogenic microorganisms, including *Salmonella* spp., *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes* (Camargo et al., 2021b; Castro et al., 2016). One way to biocontrol these pathogens is to use the native microbiota of artisanal cheeses to inhibit or eliminate these microorganisms. One example is lactic acid bacteria (LAB), which are the dominant microorganisms in these cheeses and can produce a variety of compounds that have been shown to be effective in inhibiting the growth of pathogens (Campagnollo et al., 2018). Among the antimicrobial compounds, organic acids (mainly lactic acid), hydrogen peroxide and bacteriocins stand out, which can inhibit the deterioration and growth of pathogenic bacteria. They also contribute to the flavor of the cheese and can play a probiotic role (Castro et al., 2016).

Considering the importance of MAC consumption and its potential as a vehicle for foodborne pathogens, this study aimed to characterize the microbiological quality and safety of MAC in the region of Entre Serras da Piedade ao Caraça, in addition to correlating LAB with the biocontrol of these microorganisms throughout the ripening process, since there is little information available on the microbiological qualities of this region.

Material and Methods

Study design and sample collection

Four producers (A, B, C and D) from the Entre Serras da Piedade ao Caraça region (Minas Gerais, Brazil; Figure 1) were included in the study. Their obtained milk from cows by manual milking and herds are fed with pasture, silage and concentrate. A total of 28 MAC samples were obtained, seven from each producer (obtained from a unique lot monitored during ripening), on production day (time 0) and after 7, 14, 21,

30, 45 and 60 days of ripening. MAC were ripened by each producer under environmental conditions, at an average temperature of 20 ºC and a relative humidity of 80%. All the samples (n = 28) were transported under refrigeration for microbiological analysis.

Microbiological analysis procedures

MAC samples were subjected to microbiological analysis over a 60-day ripening period. Pathogen enumeration or detection was conducted on days 0, 30, and 60, while LAB were enumerated on days 0, 7, 15, 21, 30, 45, and 60. Portions of 25 g of each sample were aseptically collected and transferred to sterile bags with 225 mL of citrate solution 2% (w/v). Then, the samples were homogenized in a stomacher (10^{-1} dilution) and 10-fold diluted in saline solution 0.85% (w/v). Selected dilutions of each sample were plated, according to the protocols. Based on the obtained results, counts were expressed as log CFU/g.

Escherichia coli enumeration

Aliquots of 1 mL were plated onto Petrifilm™ EC Cont Colif count plate (EC; 3M Microbiology, St. Paul, MN, USA) and incubated at 35 ± 1 ºC for 48 h (Curiale et al., 2002).

Staphylococcus aureus enumeration

Aliquots of 1 mL were plated onto Petrifilm™ Staph Express count plate (STX; 3M Microbiology) and incubated at 35 ± 1 ºC for 24 h; thermonuclease production was identified by DNAse disk (Viçosa et al., 2010).

Mesophilic LAB enumeration

Aliquots of 1 mL were plate-poured onto de Man, Rogosa and Sharpe (MRS; Kasvi, São José dos Pinhais, PR, Brazil) agar and incubated at 30 ± 1 ºC for 48 h, under anaerobic conditions (ISO 15214, 1998).

Listeria monocytogenes detection

Furthermore, for *L. monocytogenes* detection, 25 g of each MAC sample were added to Half Fraser broth (BD-Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and incubated at 30 ± 1 ºC for 24 h. Then 0.1 mL aliquot of the culture was transferred to Fraser broth (BD) and incubated at 35 ± 1 ºC for 48 h. Aliquots of the cultures were streaked onto Palcam and Oxford agar plates (both from Oxoid Ltd., Basingstoke, England) and incubated at 35 ± 1 ºC for 24 and 48 h (ISO 11290-1, 2017).

Salmonella spp. detection

For *Salmonella* spp. detection, 25 g of each sample were added to 225 mL of lactose broth (Oxoid) and incubated at 35 ± 1 ºC for 24 h. Then, 0.1 mL and 1 mL aliquots of the culture were respectively transferred to Rappaport-Vassiliadis broth (incubated at 42 ± 1 ºC for 24 h) and Tetrathionate broth (incubated at 35 ± 1 ºC for 24 h) (both from Oxoid). The obtained cultures were streaked onto Xylose Lysine Desoxycholate agar, Bismuth Sulfite agar and Hektoen Enteric agar plates (all from Oxoid), being incubated at 35 ± 1 ºC for 24 h (Wehr & Frank, 2004). The obtained results were expressed as presence or absence in 25 g.

Statistical analyses

Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's test, with a significance level of $p < 0.05$. Statistical analyses were conducted using GraphPad Prism software (version 8.4.3; GraphPad Software, USA).

Results and Discussion

Pathogen analysis in MAC

In 2022, the Instituto Mineiro de Agropecuária (IMA) recognized the Entre Serras da Piedade ao Caraça region as a MAC producer, establishing a minimum ripening period of 22 days (IMA, 2022). According to IMA Ordinance No. 2303 (IMA, 2024), of May 20, 2024, microbiological standards were set for coliforms at 35°C, coliforms at 45°C, coagulase positive staphylococci, *Salmonella* spp., and *L. monocytogenes*. Most MAC production occurs in facilities with limited infrastructure and ripening process is employed to mitigate risks. This step is part of the identity and contributes to cheese safety, particularly for those made with raw milk (Camargo et al., 2021b). For this, four MAC producers from the Entre Serras da Piedade ao Caraça region were chosen for microbiological analyses of the cheeses produced.

IMA Ordinance No. 2303 establishes acceptable limits in MAC for coliforms at 35 ºC (≤ 5000 CFU/g or ≤ 3.7 log CFU/g) and for coliforms at 45 ºC (≤ 500 CFU/g or ≤ 2.7 log CFU/g). *Escherichia coli* was analyzed in the MAC samples, as it is an important indicator of recent fecal contamination and belongs to the coliform group. The cheeses from all producers analyzed at time 0 showed *E. coli* counts around 6 log CFU/g (Figure 1A). However, after 30 days of ripening, *E. coli* was not detected in the samples, except

in the cheese from producer C. After 60 days of ripening, the cheese from producer C also showed no presence of *E. coli* (Figure 1A).

Considering the microbiological standards established by Brazilian legislation (IMA, 2024), which has an acceptable value (≤ 1000 CFU/g or ≤ 3 log CFU/g) for *S. aureus*, the cheeses from producer D did not present *S. aureus* at any time analyzed (Figure 1B). For producers A, B and C, the count for *S. aureus* in MAC at time 0 was around 6 log CFU/g. With the ripening process, there was inhibition or reduction in the counts, and the cheeses from producers A and C were within the acceptable limits at 30 days of ripening, and the cheeses from producer B after 60 days of ripening (Figure 1B).

Due to the high risk to public health, IMA Ordinance No. 2303 establishes the total absence of pathogens in 25 g cheese samples for the analysis of *Salmonella* spp. and *L. monocytogenes* in MAC. All MAC samples showed the absence of *Salmonella* spp. and *L. monocytogenes* (Table 1).

According to the microbiological criteria established by IMA Ordinance No. 2303, it is concluded:

- Producers A, C and D: The cheeses comply with the legal standards in force after 30 days of maturation.
- Producer B: After 30 days of maturation, the cheeses are unfit for consumption, as they do not meet the standard established for *S. aureus*. However, after 60 days of maturation, the cheeses comply with the legal standards in force.

Dores, Nobrega & Ferreira (2013) conducted a random sampling of MAC produced on eight farms in Serra da Canastra, collecting cheeses two days after manufacturing. They found that *E. coli* and *S. aureus* counts decreased to levels

compliant with regulations when the cheeses were ripened at room temperature (25 ºC) for a minimum of 22 days (in dry or rainy seasons). On the other hand, Campos et al. (2021) evaluated the microbiological characteristics of Canastra MAC samples (n = 78) after 22 days of ripening. A high number of non-compliances to the Brazilian regulation was observed, 42% of the samples exceeded the limit for coagulase positive staphylococci and 18% for *E. coli*. Firmo et al. (2023) identified that 24.2% of cheeses produced in different regions analyzed were unfit for consumption, as they presented high counts of spoilage microorganisms and the presence of pathogens, even after ripening.

LAB analysis in MAC

LAB counts ranged from 6.47 to 8.05 log CFU/g for cheeses from producer A (Figure 3A), 6.68 to 8.35 log CFU/g for cheeses from producer B (Figure 3B), 6.81 to 8.27 log CFU/g for cheeses from producer C (Figure 3C), and 6.12 to 8.42 log CFU/g for cheeses from producer D (Figure 3D). These counts were obtained throughout the ripening period (0 - 60 days) and evidenced the stability of the LAB population (Figure 3). High LAB population (> 6.0 log CFU/g) was also reported by Almeida et al. (2020) and Nero et al. (2021) in MAC samples from the Serro region, Minas Gerais.

Ripening is a very important stage in cheese production, as it is a complex phenomenon that involves physical, biochemical and microbiological changes that contribute to ensuring the quality, identity and safety of the product (Camargo et al., 2021a; 2021b). The safety obtained during ripening is achieved mainly by the action of microorganisms present in raw milk and in natural starter culture (“pingo”). Some microorganisms present, especially LAB, have the ability to produce antimicrobial compounds, such as bacteriocins with a wide range of effectiveness, particularly

against pathogenic and spoilage microorganisms (Perez, Zendo & Sonomoto et al., 2022). In addition, LAB have a function in the fermentation process, converting lactose into lactic acid. This acidification contributes to cheese preservation by establishing an environment that can inhibit the growth of undesirable microorganisms (Coelho, Malcata & Silva, 2022).

Previous studies have revealed nonconformities in MAC samples from other regions of Minas Gerais, and our results corroborate their findings. Ultimately, this demonstrates the need for adequate hygiene practices, good manufacturing practices, and rigorous control in the production of artisanal cheeses, in addition to reinforcing the importance of ripening as a critical process to ensure the quality and microbiological safety of products.

Conclusions

This study is the first to use the culture-dependent method for the microbiological characterization of MAC from the Entre Serras region, Minas Gerais. Highlighting the presence of pathogens at the beginning of the ripening process and the subsequent inhibition or absence of these pathogens at the end of the ripening period, which is attributed to the sustained presence of LAB, which contribute to the control of these pathogens, making MAC safe for consumption, in addition to contributing to the unique sensory characteristics.

Author Contribution

Conceptual idea: Costa, E. A.; Fusieger, A.; Carvalho, A. F. C.; Methodology design: Fusieger, A.; Freitas, R.; Rodrigues, R. S.; Data collection: Rodrigues, F. F.C.; Costa,

E. A.; Data analysis and interpretation: Rodrigues, F. F.C.; Salgado, C. A.; and Writing and editing: Rodrigues, F. F.C.; Fusieger, A. Salgado, C. A.; Carvalho, A. F. C.

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Table 1. Pathogen analysis in MAC of the four producers from the Entre Serras da Piedade ao Caraça region.

Producer	Day	*Salmonella* (in 25 g)	*L. monocytogenes* (in 25 g)
A	0	Absence	Absence
	30	Absence	Absence
	60	Absence	Absence
B	0	Absence	Absence
	30	Absence	Absence
	60	Absence	Absence
C	0	Absence	Absence
	30	Absence	Absence
	60	Absence	Absence
D	0	Absence	Absence
	30	Absence	Absence
	60	Absence	Absence

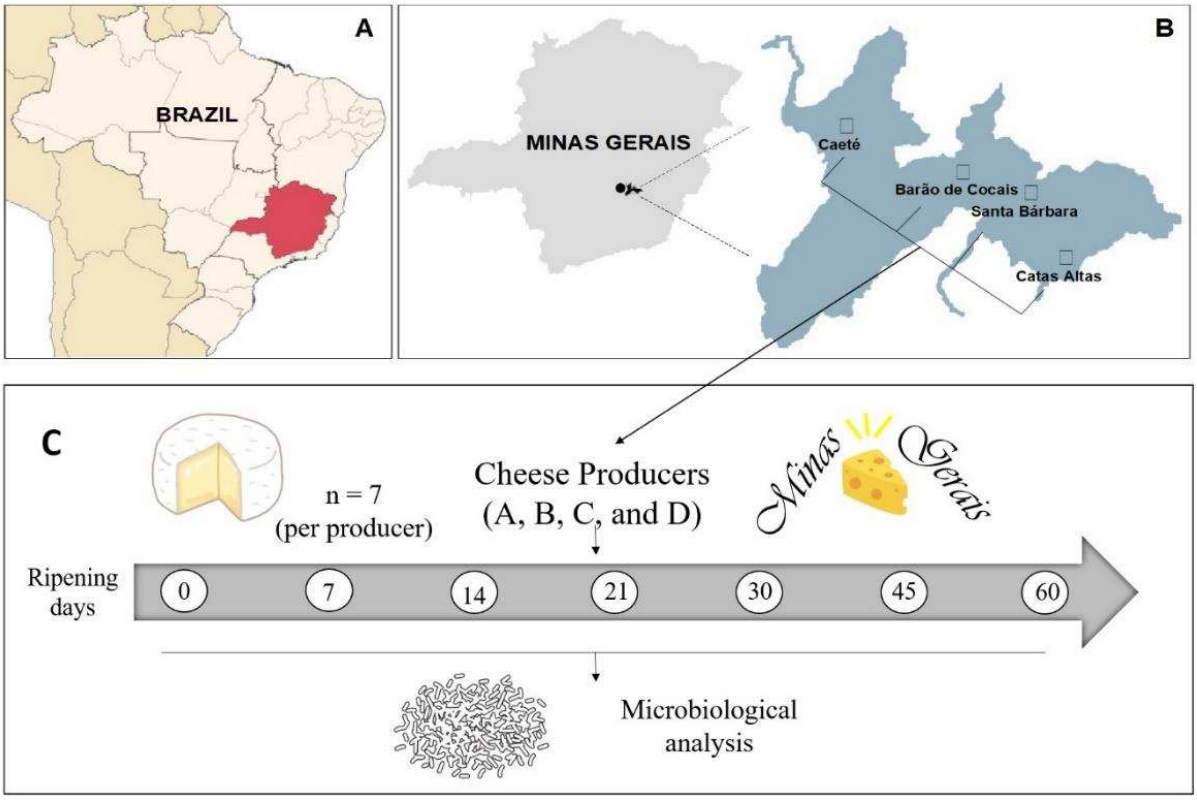

Figure 1. Geographical location of artisanal cheese from Entre Serras da Piedade ao Caraça region, Minas Gerais, Brazil. Localization of Minas Gerais (MG) state in Brazil (red area) **(A)**. Entre Serras da Piedade ao Caraça region and the municipalities where the cheese production was analyzed **(B)**. Study Design **(C)**.

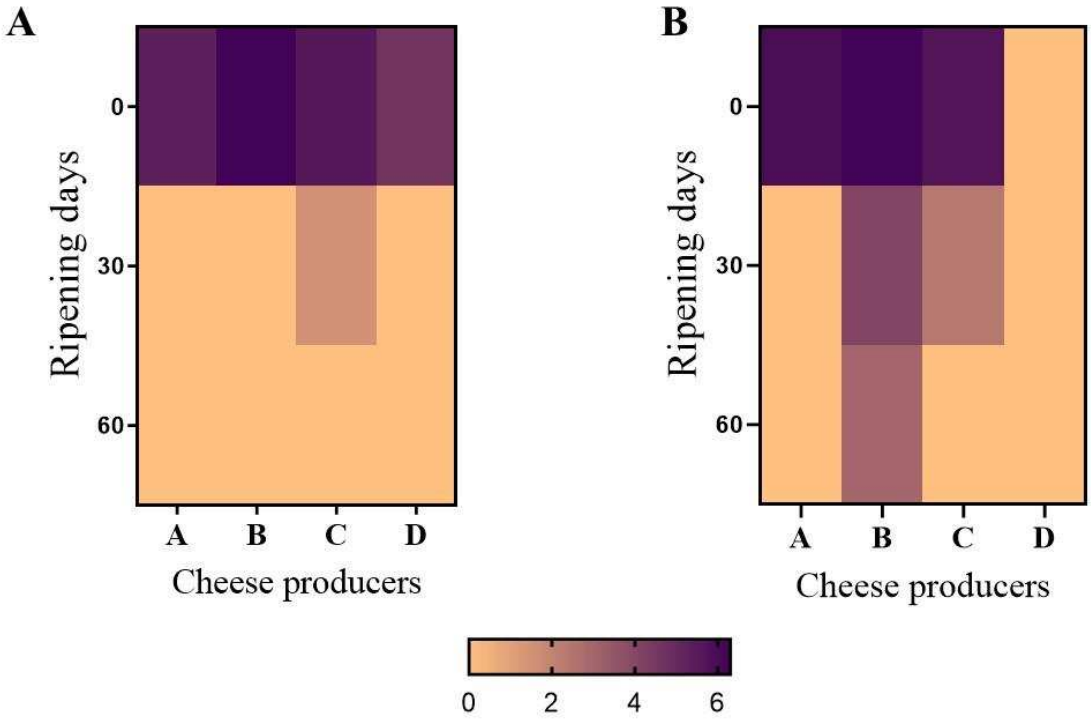

Figure 2. Logarithmic counts (log CFU/g) of *Escherichia coli* **(A)** and *Staphylococcus aureus* **(B)** throughout the ripening period (0, 30 and 60 days) for four different cheese producers (A, B, C and D). The color intensity indicates the variation in bacterial count in each sample; Purple: Highest count, Beige: No growth.

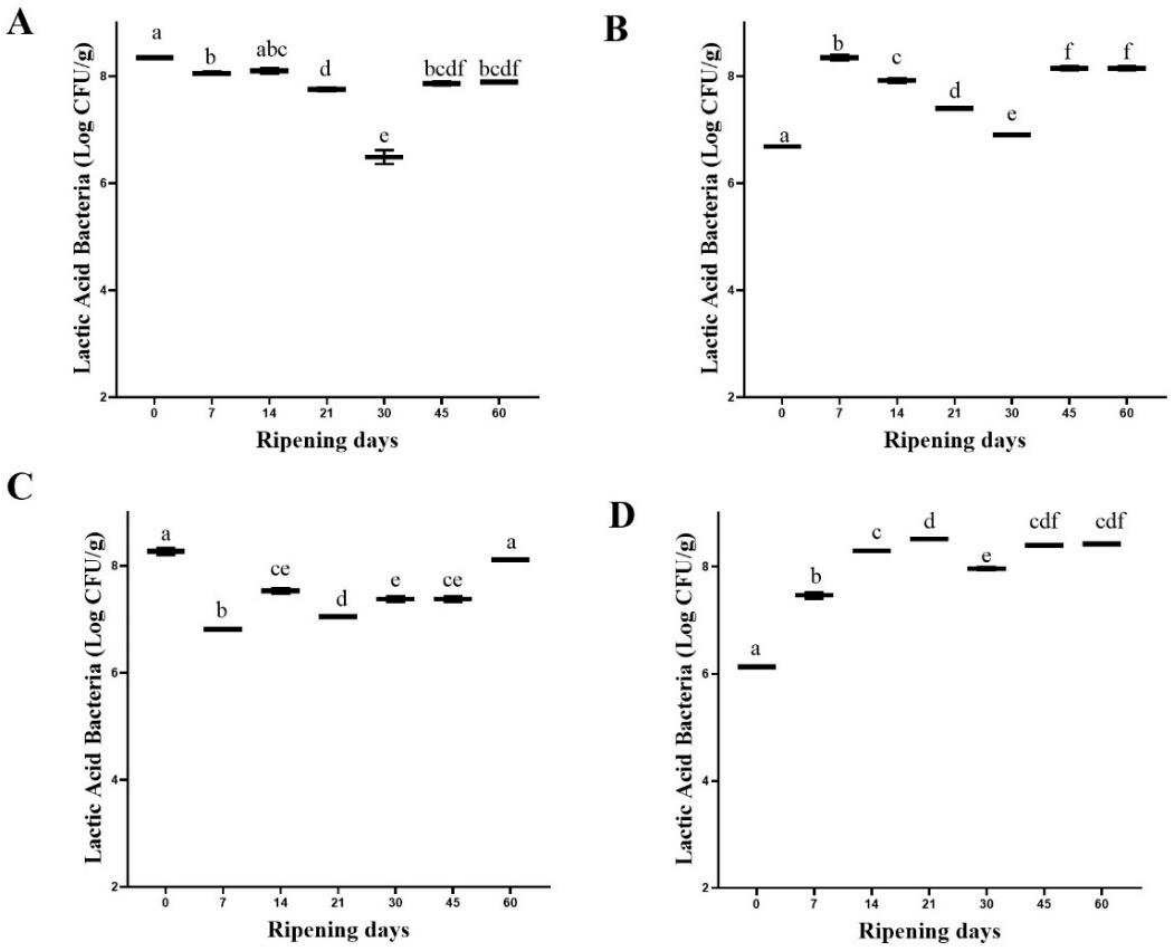

Figure 3. Counts expressed in Log CFU/g of Lactic Acid Bacteria of MAC samples from producer A **(A)**, producer B **(B)**, producer C **(C)**, and producer D **(D)** over 60 days of ripening. Different letters above the error bars indicate a statistically significant difference by Tukey's test ($p < 0.05$), and equal letters indicate no statistically significant difference

CAPÍTULO II

Molecular identification and technological potential of *Lacticaseibacillus paracasei* isolated from Minas artisanal cheese from the Entre Serras da Piedade to Caraça Region

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Title page

Molecular identification and technological potential of *Lacticaseibacillus paracasei* isolated from Minas artisanal cheese from the Entre Serras da Piedade to Caraça Region

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Abstract

Minas artisanal cheese (MAC), a culturally significant product from Minas Gerais, Brazil, has been produced for over two centuries. The Entre Serras da Piedade ao Caraça region, recently recognized for its MAC production, presents unique terroirs contributing to the cheese's distinctive characteristics. This study aimed to identify and evaluate the technological potential of lactic acid bacteria (LAB) isolated from MAC produced in this region, focusing on the species *Lacticaseibacillus paracasei*. Out of 956 LAB isolates, 739 were confirmed as LAB through 16S rRNA partial gene sequencing, with *Lactiplantibacillus* and *Lacticaseibacillus* being the most prevalent genera. *L. plantarum* and *L. paracasei* were the dominant species, aligning with previous studies from other MAC producers regions of Minas Gerais. Genetic profiling using rep-PCR of 197 *L. paracasei* strains revealed intra-species diversity that may influence cheese ripening. Furthermore, 55 *L. paracasei* strains were selected for the technological evaluation. Most strains exhibited strong acidification capacity, diacetyl production, and proteolytic activity, essential for flavor and texture development in cheese. The variability in these characteristics suggests that different strains could contribute uniquely to the sensory properties of MAC. This study contributes to understanding of the microbial diversity of MAC from the Entre Serras da Piedade ao Caraça region and, to the best of our knowledge, is the first to evaluate the technological potential of promising *L. paracasei* strains from this traditional cheese.

Keywords: Lactic acid bacteria. Microbiota. Starter culture. Technological potential.

Introduction

Minas artisanal cheese (MAC) is produced by small farmers of different regions situated in Minas Gerais state, Brazil, for more than two centuries. Currently, MAC is well-known and highly consumed among Brazilians, being considered a “cultural patrimony” in the country [1, 2]. The Entre Serras da Piedade ao Caraça region, Minas Gerais, was recognized as a producer of MAC in 2022 and includes the municipalities of Catas Altas, Barão de Cocais, Santa Bárbara, Rio Piracicaba, Bom Jesus do Amparo, and Caeté [3].

The production of this cheese dates back to the 18th century, coinciding with the onset of mining excavations in Minas Gerais during the “Gold Period”. By the 1950s, the production of Entre Serras cheese had nearly disappeared. However, with the growing interest in cultural heritage and traditional food cultures, Minas Gerais has revived its production as a viable alternative for dairy farmers [4]. The Entre Serras cheese has a soft consistency, compact texture with the occurrence of eyes, yellow color, moderate flavor intensity, and moderate odor. It is round in shape, with a straight or rounded edge, varying in height between 4 and 5 cm, with a diameter between 13 and 15 cm and a weight between 600 and 900 g, or with a height of 5 cm and a diameter between 10 and 12 cm and a weight between 200 and 400 g [5].

The geographical and edaphoclimatic conditions of Minas Gerais state provide different *terroirs* for cheese making [2]. Several studies have characterized the diversity of lactic acid bacteria (LAB) in MAC from different producing regions of Minas Gerais, such as Serro, Serra Geral and Serra da Canastra [2, 6, 7]. To date, the Entre Serras da Piedade ao Caraça region has been the focus of few studies [4]. The LAB microbiota present in MAC plays a crucial role in the sensory characteristics, ripening process and safety [8, 9].

Among the microorganisms found in artisanal cheeses, the genus *Lacticaseibacillus* has been reported in several studies [10–13]. Strains of *Lacticaseibacillus* were previously referred to as *Lactobacillus casei* group and, currently, *Lacticaseibacillus* genus consists of 26 species and among them, *L. casei*, *L. paracasei*, *L. rhamnosus*, *L. chiayiensis*, and *L. zeae* are phenotypically and phylogenetically closely related species [14]. These species hold significant economic importance due to their potential use in fermented dairy products, where they enhance texture and flavor. Additionally, their bioactive metabolism offers health benefits, making them valuable as probiotics and starter cultures [15, 16].

In MAC, the discovery and isolation of LAB, such as *Lacticaseibacillus* spp., are significant as they may uncover new strains with unique and beneficial properties for dairy industry applications, or broaden their use in the development of new functional foods with enhanced sensory and rheological characteristics, as well as improved microbiological safety [8]. Thus, the present study aimed to identify presumptive LAB isolates obtained from MAC in the Entre Serras da Piedade ao Caraça region and to evaluate the technological potential of isolates identified as *Lacticaseibacillus paracasei*.

Materials and Methods

Isolates

The isolates were previously obtained from a study that isolated presumptive LAB from four different MAC producers from the Entre Serras da Piedade ao Caraça region (Minas Gerais, Brazil) [17]. Briefly, a total of 28 MAC samples were obtained, seven from each producer (obtained from a unique lot monitored during ripening), on production day (time 0) and after 7, 14, 21, 30, 45 and 60 days of ripening. Presumptive

LAB were isolated using different protocols, being in de Man, Rogosa, and Sharpe agar (MRS; Kasvi, São José dos Pinhais, Paraná, Brazil) at 30 and 37 ºC for 48 h in aerobiosis and anaerobiosis; in M17 agar (Himedia, Mumbai, India) at 35 ºC for 48 h in aerobiosis; and in Mayeux, Sandine and Elliker agar (MSE; Biokar Diagnostic, Beavais, France) at 22 ºC for 96 h in aerobiosis and anaerobiosis [17].

For the present study, we selected presumptive LAB isolates obtained from a single MAC producer, corresponding to a total of nine hundred and fifty-six (n=956). The isolates were stored at -80 ºC in MRS broth (Kasvi) supplemented with glycerol at 20% (v/v; Exôdo Cientifica, Sumaré, SP, Brazil) in the bacterial culture collection at InovaLeite (Laboratório de Leite e Derivados, Universidade Federal de Viçosa). Before use, isolates were transferred to MRS broth, incubated overnight under the same isolation conditions, and checked for purity in MRS agar after incubation. Plates with pure colonies were subjected to catalase and Gram staining test. Isolates with bacilli and cocci morphology, Gram-positive staining, and catalase-negative reaction were selected for further analysis.

Identification of LAB by 16S rRNA gene sequencing

The selected isolates were subjected to DNA extraction using Wizard Genomic DNA Purification Kit (Promega Corp., Madison, WI, USA), following the manufacturer's recommendations. The molecular identification was done by amplification of 16S rRNA gene through PCR using the primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACGGCTACCTTGTTACGACTT-3′). Reactions were composed of 12.5 µL of Go Taq Green Master Mix 2x (Promega), 0.25 µL of each primer (10 µmol/L), 2 µL of DNA (50 ng/µL), and ultra-pure water (Promega) to complete the final volume of 25 µL. The PCR amplification was performed using the following program: 95 ºC for 5 min,

followed by 30 cycles of denaturation at 95 ºC for 30 s, annealing at 55 ºC for 30 s, extension at 72 ºC for 1 min and 40 s, and a final extension cycle was performed at 72 ºC for 5 min [18].

The obtained PCR products were analyzed on 1% (w/v) agarose gels in 0.5x TBE buffer and stained with UniSafe Dye according to manufacturer recommendations (Uniscience Corp., Miami Lakes, FL, USA); 1 kb DNA ladder (Promega) was used as a molecular size marker. Electrophoresis was conducted at 75V for 40 min, and DNA fragments were visualized using an LPIX UV transilluminator (Loccus Biotechnology, São Paulo, SP, Brazil) to detect a PCR product of approximately 1466 bp. *Staphylococcus aureus* ATCC 6538 was used as positive control. PCR products were sequenced by Macrogen Inc. (Seoul, South Korea) and the obtained nucleotide sequences were edited using Molecular Evolutionary Genetics Analysis (MEGA) software version 11.0.11 (Pennsylvania State University, State College, PA, USA). Isolates identification was performed using the Basic Local Alignment Search Tool for Nucleotides (BLASTn) against the nucleotide BLAST, with search limited to sequences from type material, available on the National Center for Biotechnology Information (NCBI) website (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Rep-PCR fingerprinting

Isolates identified as *Lacticaseibacillus paracasei* by 16S rRNA gene sequencing (showing >99% similarity with at least 90% coverage) were subjected to repetitive extragenic palindromic PCR (rep-PCR) analysis using the single primer GTG_5 (5′-GTGGTGGTGGTGGTG-3′), following the protocol described by Versalovic et al. [19] with adaptations. Briefly, PCR amplification was carried out in a thermal cycler, with the following conditions: 94 ºC for 5 min as an initial step, 94 ºC for 1 min, annealing

at 38 ºC for 1 min and 15 s, then 65 ºC for 8 min for the next 30 cycles, and 65 ºC for 16 min to conclude the amplification.

The PCR products were electrophoresed on 1.2% (w/v) agarose gel for 2 h and 30 min at a constant voltage of 60 V in 0.5x TBE buffer. The gels were stained using Diamond™ Nucleic Acid Dye (Promega) and 1 kb DNA ladder (Promega) was used as a molecular size marker. The rep-PCR profiles were visualized under ultraviolet light using an LPIX UV transilluminator (Loccus Biotechnology), followed by digital image capturing. The resulting fingerprints were analyzed by the BioNumerics software version 6.6.11 (Applied Maths, Sint-Martens-Latem, Belgium). The similarities among profiles were calculated using the Pearson correlation, and a dendrogram was constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) at 5% optimization and 1.5% tolerance. A cut-off value of 90% similarity was applied to compare the DNA fingerprint patterns.

Technological tests

Based on the rep-PCR dendrogram, a representative *L. paracasei* strain from each cluster was selected and subjected to technological assays, including proteolytic activity, lactofermentation profile, diacetyl production, and acidifying ability. Before each assay, the strains were cultured in MRS at 37 ºC overnight. All analyses were conducted in triplicate, with two independent repetitions.

For proteolytic activity, aliquots of 10 µL from previously cultured strains were pipetted onto the surface of Plate Count Agar (PCA; Kasvi, São José dos Pinhais, SP, Brazil) enriched with reconstituted skim milk (10% w/v; Nestlé, São Paulo, SP, Brazil) and incubated at 37 ºC for 4 days. Proteolytic activity was indicated by a clear zone

around the colonies and *Pseudomonas fluorescens* 07A was used as a positive control [20, 21].

For lactofermentation patterns and diacetyl production, aliquots (1%, v/v) were inoculated into skimmed UHT milk (Nestlé, São Paulo, SP, Brazil), followed by incubation at 37 ºC for 24 h. Based on the characteristics of the curd formed, the lactofermentation patterns were defined through empirical analysis and classified as: uniform, uniform with presence of whey, uniform and fragile (appearance), broken with presence of whey, and absence of curd [21]. Subsequently, 1 mL of the obtained cultures in milk were transferred to tubes containing 0.5 mL of α-naphthol (1% w/v) and 0.5 mL of KOH (16% w/v) and incubated at 37 ºC for 10 min. Diacetyl production was determined by the formation of a red ring at the top of the tubes [20]. *Lactococcus lactis* subsp. *lactis* bv. diacetylactis ATCC 13675 was used as positive control.

Finally, acidifying activity was assessed by the inoculation of 100 µL into 10 mL of skimmed UHT milk (Nestlé). The pH values and titratable acidity were measured at the time of inoculation (T0), then after 6 (T6), 12 (T12), and 24 h (T24) of incubation at 37 ºC. The pH was obtained by measuring in digital pHmeter (Hanna Instruments, São Paulo, SP, Brazil), while titratable acidity was determined through titration with 0.1N NaOH and expressed as percentage of lactic acid [22, 23]. The acidification rates were calculated based on the variations in lactic acid production at the specified times. Based on the *L. paracasei* strains ability to acidify the milk pH after 6, 12, and 24 h of incubation, they were categorized into 3 groups: (I) high acidification capacity - reduction of more than 2 pH units; (II) moderate acidification capacity - pH reduction from 1.5 to 2.0 pH units; and (III) low acidification capacity - reduction of less than 1.5 pH units [21].

Results and Discussion

Out of the 956 presumptive LAB isolates from MAC samples from the Entre Serras da Piedade ao Caraça region, 196 were identified as either Gram negative or catalase positive and were subsequently excluded from the study, leaving 760 Gram positive and catalase negative isolates. These remaining isolates were subjected to 16S rRNA gene sequencing. A total of 21 isolates exhibited poor amplification and were subsequently removed from the study. Therefore, 739 yielding results consistent with LAB, as detailed in **Table 1**. Overall, six genera were identified, being *Lactiplantibacillus, Lacticaseibacillus, Enterococcus, Pediococcus, Weissella*, and *Lactococcus*. The genus *Lactiplantibacillus* was the most frequent within this group (**Table 1**). Lactobacilli are able to grow under the highly selective conditions that exist in cheese, such as low pH and high salt concentrations [8]. Furthermore, *L. plantarum* and *L. paracasei* were the main species found in the MAC samples (**Table 1**). These species are examples of the beneficial microorganisms in cheese production due to their technological and probiotic potential [15, 16].

Nero et al. [6] characterized the LAB microbiota of MAC produced in the Serro region of Minas Gerais using both culture-dependent and -independent methods. Among the 176 isolates, nine genera and eleven species were identified, with Lactobacilli being the dominant genus, and *L. plantarum*, *E. faecalis*, and *L. lactis* were the most predominant species. On the other hand, the culture-independent method showed higher relative frequencies of *L. lactis*. Luiz et al. [24] identified the LAB microbiota in MAC from the Araxá region of Minas Gerais and Lactobacilli represented 72% of the isolates. *L. plantarum* and *L. rhamnosus* were the predominant species; these findings align with the results of our study (**Table 1**).

As described in the materials and methods section, the presumptive LAB isolates from MAC samples from the Entre Serras da Piedade ao Caraça region were sourced from Costa [17]. These samples were also used by Camargo et al. [4] to study bacterial diversity using a culture-independent method. While those studies focused on characterizing MAC samples from four different producers, we specifically selected the presumptive LAB isolates obtained from a single MAC producer (Farm D, as reported by Camargo et al. [4]). Based on the culture-independent method, the MAC samples from Farm D exhibited a relative abundance of *Lactococcus* (≈75%), *Streptococcus* (≈15%) and Lactobacilli (≈5%) [4]. At the species level, *L. lactis* (≈75%), *S. thermophilus* (≈15%) were the most frequent, while *L. casei* accounted for less than 1% [4]. Here, the culture-dependent approach showed *L. plantarum* and *L. paracasei* as the dominant species (**Table 1**). Perin et al. [25] assessed biological ecology of MAC by culture-dependent and -independent methods and some LAB species identified by the selective culture media method were also not identified by culture-independent.

Given the limitations of culture-dependent method, not all LAB can be effectively recovered, and 16S rRNA-based sequencing may detect even non-viable species [6]. Culture-dependent techniques often capture only a small fraction of the microbial community, as certain strains may become stressed and enter a viable but non-culturable state due to environmental factors [26]. The dynamics of microbial communities results from the microbial interactions and various technological and environmental factors, such as pH, temperature, and salinity, which influence and are influenced by microbial activities [27]. Therefore, such analyses may not fully capture the entire microbiota or the symbiotic relationships within complex natural environments, like those found in artisanal cheeses [26, 27]. Even based on different

methodologies, both culture-dependent and -independent approaches introduce biases, yet they offer complementary insights into the microbial ecology of MAC.

As 16S rRNA partial gene sequencing alone does not identify the genetic variability among the strains, isolates identified as *L. paracasei* (showing >99% similarity with at least 90% coverage) were subjected to rep-PCR technique. This method is based on the amplification of specific regions of DNA and has been used to successfully identify the genetic profile of a wide range of LAB isolated from cheeses [23, 28–30]. The dendrogram obtained by rep-PCR clustering of the *L. paracasei* strains isolated from MAC is presented in **Supplementary Fig. 1**. This analysis allowed the identification of intra-species differences and their potential relationship with the ripening time of the cheeses. The 197 strains were grouped into 31 clusters and 31 solitary fingerprint (**Supplementary Fig. 1**). Distinct genetic profiles among the strains were observed, as well as the presence of closely related isolates. There were profiles with more than two similar strains, with strains 591 and 597 showing the highest level of similarity at 99.4%. These isolates were obtained at different ripening times of MAC, 21 and 60 days, respectively. The genotypes of intra-species strains vary according to the ripening time. Consequently, distinct strains observed throughout ripening may impact the characteristics of cheeses.

Furthermore, 55 *L. paracasei* strains were randomly selected, based on rep-PCR fingerprint analysis, for the evaluation of the technological potential, as show in **Table 2**. An important characteristic of LAB is their ability to synthesize diacetyl (2,3-butanedione), a key compound for aroma development in fermented foods, along with other molecules such as CO_2, aldehydes, and acetic acid, produced from citrate via the monophosphate pentose pathway in heterofermentative bacteria [31, 32]. In this study, 43 *L. paracasei* strains (78.19%) demonstrated the ability to produce diacetyl

from milk lactose (**Table 2)**, underscoring their potential as adjunct cultures in cheese production. Margalho et al. [31] also observed *L. paracasei* strains isolated from Brazilian artisanal cheeses showing positive diacetyl production.

All strains exhibit proteolytic activity (ability to degrade milk casein), indicating that *L. paracasei* may positively influence cheese ripening (**Table 2)**. However, it is important to ensure a balanced breakdown of caseins by strains considered as starter cultures in cheese, as excessive proteolysis can lead to undesirable characteristics such as low viscosity and high bitterness [32, 33]. In general, proteolysis is a crucial biochemical process in cheese production, as it generates compounds essential for the development of texture, aroma, and flavor during fermentation and ripening [32, 34]. *L. paracasei* strains isolated from artisanal Pico cheese were associated with proteolytic activity [35], as were Lactobacilli isolates from MAC in the Araxá, Campo das Vertentes, and Cerrado regions [36].

The lactofermentation patterns among the strains vary: 42 strains (76.37%) exhibited a uniform pattern, 10 strains (18.18%) showed uniform patterns with whey, 2 strains (3.64%) had a broken pattern with whey, and 1 strain (1.82%) demonstrated a uniform yet fragile pattern (**Table 2**). A uniform lactofermentation pattern signifies consistent acid production and curd formation, which is desirable in cheese-making. Variations in these patterns may influence the final cheese texture, with strains displaying a broken pattern with whey potentially resulting in a less consistent curd structure. Milk coagulation, the initial stage of cheese production, involves the destabilization of casein micelles, leading to curd formation. This process can be facilitated by coagulant enzymes or bacterial strains that produce lactic acid [35].

All strains of *L. paracasei* were found to be capable of acidifying skim milk, exhibiting different levels of acidification over 24 h (**Table 2**). The pH decrease in

cheese production contributes to ensure proper cheese texture and in the inhibition of undesirable microorganisms [21]. The strains were grouped based on their acidifying potential; Group I displayed the highest acidification ability, where 53 strains (96.36%) achieved a pH drop of more than 2 units after 24 h. Overall, it was observed pH values of 3.78 ± 0.17 to 4.75 ± 0.20 after 24 h (**Table 2**). Titratable acidity values provide further insight into the acid production of the strains, such as strain 500 showing the highest acid production (**Table 2**). The consistency in titratable acidity values across most strains suggests a reliable acidification process, essential for maintaining cheese quality and characteristics. Margalho et al. [36] observed higher acidification rates of *L. paracasei* and *Levilactobacillus brevis* compared to other Lactobacilli isolated from Minas artisanal cheeses. Furthermore, according to Beresford et al. [37] for a LAB to qualify as a starter culture, the LAB must reduce the pH of the milk from ≈6.6 to 5.3 within 6 h. This criterion was met by 23 strains (41.82%), where the pH values ranged from 4.97 ± 0.32 to 5.30 ± 0.10 after 6 h (**Table 2**). In this scenario, the acidification rates of the remaining 32 strains (58.18%) suggest they function as Non-starter LAB (NSLAB).

Finally, most *L. paracasei* strains possess desirable technological characteristics required for use as starter culture, including diacetyl production, proteolytic activity, and adequate acidification. Despite belonging to the same species, these strains demonstrate varying capabilities, highlighting the fact that genetically similar isolates can possess distinct technological properties. Strains with uniform lactofermentation patterns and high acidifying potential are particularly promising for cheese-making applications, as they can contribute to texture and flavor. However, the observed variability in these characteristics emphasizes the necessity of selecting strains based

on the specific requirements of the cheese being produced, where these results provide a basis for the strategic selection of the most suitable *L. paracasei* strains.

Conclusion

Based on the obtained data, we were able to identify and to characterize some technological features of *L. paracasei* strains isolated from MAC from the Entre Serras da Piedade ao Caraça region, Minas Gerais. These results can significantly contribute to understand the microbial diversity of MAC from this region, as well as propose the use of the strains as starter cultures. Additional research is necessary to ensure the safety of the strains from this traditional cheese.

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Statements and Declarations

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Conflict of Interest

The authors have no competing interests to declare that are relevant to the content of this article.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Table 1. Identification by 16S rRNA gene sequencing of the selected LAB isolates (n=739) from MAC samples from the Entre Serras da Piedade ao Caraça region.

Genus	Total	Species	Total
Lactiplantibacillus	513 (69.42%)	*L. plantarum*	440 (59.54%)
		L. pentosus	72 (9.74%)
		L. paraplantarum	1 (0.13%)
Lacticaseibacillus	205 (27.74%)	*L. paracasei*	197 (26.66%)
		L. casei	4 (0.54%)
		L. rhamnosus	4 (0.54%)
Enterococcus	12 (1.62%)	*E. faecalis*	11 (1.49%)
		E. durans	1 (0.13%)
Pediococcus	7 (0.95%)	*P. pentosaceus*	7 (0.95%)
Weissella	1 (0.13%)	*W. viridescens*	1 (0.13%)
Lactococcus	1 (0.13%)	*L. lactis*	1 (0.13%)

Table 2. Technological potential of *L. paracasei* isolated from QMA from the Entre Serras da Piedade ao Caraça region, Minas Gerais.

Strain ID	Diacetyl	Proteolysis	Lactofermentation	pH[1]				Acidifying potential group[2]			Titratable acidity[1]			
				T0	T6	T12	T24	T6-T0	T12-T0	T24-T0	T0	T6	T12	T24
647	+	+	Uniform	6.62 ± 0.03	5.44 ± 0.10	4.60 ± 0.29	3.98 ± 0.08	III	II	I	0.22 ± 0.01	0.38 ± 0.17	0.69 ± 0.07	1.07 ± 0.19
771	+	+	Uniform	6.63 ± 0.02	5.50 ± 0.08	4.44 ± 0.07	4.02 ± 0.13	III	I	I	0.22 ± 0.01	0.41 ± 0.02	0.72 ± 0.18	0.86 ± 0.22
762	+	+	Uniform with whey	6.63 ± 0.02	5.66 ± 0.01	5.25 ± 0.15	4.27 ± 0.03	III	II	I	0.22 ± 0.01	0.37 ± 0.07	0.68 ± 0.06	1.08 ± 0.26
592	+	+	Uniform	6.62 ± 0.03	5.36 ± 0.16	4.45 ± 0.28	4.00 ± 0.06	III	I	I	0.22 ± 0.01	0.40 ± 0.11	0.71 ± 0.06	0.97 ± 0.15
17	+	+	Uniform	6.62 ± 0.03	5.27 ± 0.12	4.41 ± 0.13	3.91 ± 0.05	III	I	I	0.22 ± 0.01	0.39 ± 0.18	0.62 ± 0.21	0.88 ± 0.12
770	+	+	Uniform with whey	6.63 ± 0.02	5.40 ± 0.21	5.13 ± 0.18	4.07 ± 0.02	III	II	I	0.22 ± 0.01	0.41 ± 0.10	0.58 ± 0.18	0.88 ± 0.12
853	-	+	Uniform	6.62 ± 0.03	5.22 ± 0.10	4.39 ± 0.24	3.93 ± 0.02	III	I	I	0.22 ± 0.01	0.42 ± 0.11	0.63 ± 0.19	0.90 ± 0.20
11	+	+	Uniform	6.62 ± 0.03	5.17 ± 0.07	4.35 ± 0.16	3.92 ± 0.02	II	I	I	0.22 ± 0.01	0.50 ± 0.08	0.73 ± 0.17	1.01 ± 0.25
500	+	+	Uniform with whey	6.60 ± 0.01	5.31 ± 0.03	4.49 ± 0.25	3.96 ± 0.03	III	I	I	0.23 ± 0.01	0.49 ± 0.03	0.73 ± 0.03	1.24 ± 0.06
481	+	+	Broken with whey	6.62 ± 0.03	5.34 ± 0.05	4.36 ± 0.20	3.96 ± 0.09	III	I	I	0.22 ± 0.01	0.43 ± 0.12	0.69 ± 0.12	0.86 ± 0.09
568	+	+	Uniform with whey	6.62 ± 0.03	5.40 ± 0.12	4.31 ± 0.20	3.94 ± 0.01	III	I	I	0.22 ± 0.01	0.27 ± 0.05	0.57 ± 0.03	0.87 ± 0.18
80	+	+	Uniform with whey	6.63 ± 0.02	5.67 ± 0.12	5.01 ± 0.12	4.21 ± 0.17	III	II	I	0.23 ± 0.01	0.43 ± 0.04	0.68 ± 0.03	1.16 ± 0.19
300	+	+	Uniform	6.62 ± 0.03	5.30 ± 0.04	4.41 ± 0.18	3.99 ± 0.13	III	I	I	0.22 ± 0.01	0.33 ± 0.04	0.60 ± 0.07	0.86 ± 0.17
368	+	+	Uniform	6.62 ± 0.03	4.97 ± 0.32	4.29 ± 0.23	3.97 ± 0.06	II	I	I	0.22 ± 0.01	0.43 ± 0.15	0.81 ± 0.04	0.92 ± 0.24
668	+	+	Uniform	6.63 ± 0.02	5.59 ± 0.05	4.83 ± 0.02	4.13 ± 0.11	III	II	I	0.23 ± 0.01	0.39 ± 0.05	0.58 ± 0.09	1.20 ± 0.25
58	+	+	Uniform	6.60 ± 0.01	5.20 ± 0.33	4.36 ± 0.09	3.94 ± 0.02	III	I	I	0.22 ± 0.01	0.30 ± 0.03	0.53 ± 0.10	1.04 ± 0.21
38	+	+	Uniform with whey	6.60 ± 0.01	5.35 ± 0.22	4.65 ± 0.27	4.11 ± 0.04	III	II	I	0.23 ± 0.01	0.48 ± 0.05	0.61 ± 0.06	1.16 ± 0.03
74	+	+	Uniform	6.62 ± 0.03	5.13 ± 0.29	4.40 ± 0.21	3.99 ± 0.03	III	I	I	0.22 ± 0.01	0.48 ± 0.07	0.69 ± 0.07	1.03 ± 0.25
15	-	+	Uniform	6.64 ± 0.03	5.91 ± 0.13	5.56 ± 0.08	4.43 ± 0.03	III	III	I	0.26 ± 0.03	0.39 ± 0.07	0.53 ± 0.17	0.84 ± 0.10
630	+	+	Uniform	6.69 ± 0.02	5.46 ± 0.09	4.68 ± 0.42	3.91 ± 0.15	III	II	I	0.26 ± 0.03	0.47 ± 0.03	0.71 ± 0.14	1.07 ± 0.03
666	+	+	Uniform	6.69 ± 0.02	5.42 ± 0.08	4.63 ± 0.31	3.86 ± 0.13	III	II	I	0.26 ± 0.03	0.43 ± 0.03	0.70 ± 0.13	1.10 ± 0.03
81	+	+	Uniform	6.64 ± 0.03	5.76 ± 0.13	5.14 ± 0.20	4.03 ± 0.03	III	II	I	0.26 ± 0.03	0.37 ± 0.02	0.58 ± 0.05	1.05 ± 0.09
547	+	+	Uniform	6.69 ± 0.02	5.32 ± 0.06	4.62 ± 0.38	3.85 ± 0.13	III	II	I	0.26 ± 0.03	0.46 ± 0.01	0.73 ± 0.16	1.14 ± 0.13
480	-	+	Uniform	6.69 ± 0.02	6.19 ± 0.43	5.48 ± 0.13	4.01 ± 0.05	III	III	I	0.26 ± 0.03	0.30 ± 0.06	0.45 ± 0.03	1.02 ± 0.03
234	-	+	Uniform	6.66 ± 0.05	5.02 ± 0.26	4.38 ± 0.02	3.99 ± 0.17	II	I	I	0.23 ± 0.01	0.55 ± 0.05	0.80 ± 0.08	1.16 ± 0.11
147	+	+	Uniform	6.66 ± 0.05	5.02 ± 0.23	4.24 ± 0.04	3.81 ± 0.10	II	I	I	0.26 ± 0.03	0.46 ± 0.07	0.71 ± 0.18	1.05 ± 0.10
417	-	+	Uniform	6.66 ± 0.05	5.14 ± 0.21	4.31 ± 0.12	3.89 ± 0.18	II	I	I	0.26 ± 0.03	0.41 ± 0.08	0.63 ± 0.18	1.10 ± 0.09

408	-	+	Uniform with whey	6.66 ± 0.05	5.18 ± 0.02	4.39 ± 0.16	3.78 ± 0.17	III	I	I	0.23 ± 0.01	0.52 ± 0.05	0.81 ± 0.14	1.15 ± 0.08
28	+	+	Uniform	6.64 ± 0.03	5.29 ± 0.11	4.68 ± 0.25	4.07 ± 0.21	III	II	I	0.23 ± 0.01	0.53 ± 0.01	0.83 ± 0.07	1.08 ± 0.10
65	+	+	Uniform	6.64 ± 0.03	5.65 ± 0.09	5.18 ± 0.09	4.15 ± 0.03	III	III	I	0.23 ± 0.01	0.41 ± 0.03	0.67 ± 0.18	1.38 ± 0.14
562	+	+	Uniform	6.65 ± 0.02	5.27 ± 0.07	4.45 ± 0.07	3.90 ± 0.05	III	I	I	0.23 ± 0.01	0.50 ± 0.01	0.79 ± 0.03	1.05 ± 0.04
726	+	+	Uniform	6.62 ± 0.01	4.97 ± 0.16	4.31 ± 0.10	3.93 ± 0.07	II	I	I	0.23 ± 0.01	0.51 ± 0.05	0.70 ± 0.13	1.00 ± 0.06
14	+	+	Uniform	6.64 ± 0.03	5.27 ± 0.15	4.53 ± 0.18	3.96 ± 0.07	III	I	I	0.22 ± 0.01	0.55 ± 0.03	0.79 ± 0.05	1.05 ± 0.06
154	+	+	Uniform	6.65 ± 0.02	5.16 ± 0.18	4.50 ± 0.11	3.96 ± 0.02	III	I	I	0.23 ± 0.01	0.55 ± 0.04	0.78 ± 0.06	1.05 ± 0.06
603	+	+	Uniform	6.65 ± 0.02	5.21 ± 0.06	4.54 ± 0.10	4.05 ± 0.08	III	I	I	0.23 ± 0.01	0.53 ± 0.06	0.77 ± 0.03	1.01 ± 0.05
743	+	+	Uniform with whey	6.65 ± 0.02	5.41 ± 0.07	4.95 ± 0.12	4.29 ± 0.13	III	II	I	0.23 ± 0.01	0.51 ± 0.02	0.70 ± 0.09	0.94 ± 0.11
153	-	+	Uniform	6.65 ± 0.02	5.14 ± 0.22	4.65 ± 0.24	3.97 ± 0.02	II	II	I	0.23 ± 0.01	0.52 ± 0.01	0.75 ± 0.03	1.02 ± 0.02
264	-	+	Uniform	6.65 ± 0.02	5.21 ± 0.15	4.63 ± 0.16	4.15 ± 0.04	III	II	I	0.23 ± 0.01	0.48 ± 0.03	0.67 ± 0.04	0.92 ± 0.03
585	+	+	Uniform	6.62 ± 0.01	5.26 ± 0.09	4.52 ± 0.03	4.10 ± 0.11	III	I	I	0.23 ± 0.01	0.50 ± 0.03	0.78 ± 0.01	1.11 ± 0.14
749	+	+	Uniform	6.62 ± 0.01	5.20 ± 0.13	4.47 ± 0.08	3.95 ± 0.02	III	I	I	0.22 ± 0.01	0.53 ± 0.04	0.79 ± 0.06	0.96 ± 0.05
622	+	+	Uniform with whey	6.65 ± 0.02	5.71 ± 0.11	5.33 ± 0.36	4.40 ± 0.11	III	III	I	0.23 ± 0.01	0.41 ± 0.03	0.61 ± 0.04	0.94 ± 0.13
759	-	+	Uniform	6.65 ± 0.02	5.35 ± 0.04	4.69 ± 0.26	3.94 ± 0.05	III	II	I	0.23 ± 0.01	0.49 ± 0.07	0.76 ± 0.05	1.05 ± 0.04
891	+	+	Uniform	6.64 ± 0.03	5.23 ± 0.12	4.65 ± 0.25	3.92 ± 0.07	III	II	I	0.23 ± 0.01	0.54 ± 0.27	0.72 ± 0.03	0.93 ± 0.21
185	+	+	Uniform	6.65 ± 0.02	5.30 ± 0.10	4.77 ± 0.22	4.08 ± 0.08	III	II	I	0.23 ± 0.01	0.52 ± 0.09	0.75 ± 0.02	0.99 ± 0.03
703	+	+	Uniform	6.64 ± 0.03	6.50 ± 0.06	6.22 ± 0.05	4.75 ± 0.20	III	III	II	0.22 ± 0.01	0.26 ± 0.02	0.33 ± 0.04	0.66 ± 0.10
257	+	+	Uniform	6.65 ± 0.02	5.33 ± 0.10	4.70 ± 0.28	4.00 ± 0.08	III	II	I	0.23 ± 0.01	0.50 ± 0.03	0.75 ± 0.05	1.00 ± 0.04
686	-	+	Uniform	6.65 ± 0.02	5.32 ± 0.09	4.57 ± 0.16	4.23 ± 0.35	III	II	I	0.23 ± 0.01	0.49 ± 0.02	0.74 ± 0.01	0.94 ± 0.13
690	+	+	Uniform	6.65 ± 0.02	5.35 ± 0.17	4.66 ± 0.31	3.97 ± 0.04	III	II	I	0.23 ± 0.01	0.54 ± 0.03	0.78 ± 0.05	1.04 ± 0.05
757	+	+	Uniform and fragile	6.65 ± 0.02	5.74 ± 0.03	4.97 ± 0.22	4.18 ± 0.10	III	II	I	0.23 ± 0.01	0.47 ± 0.01	0.67 ± 0.04	0.93 ± 0.04
691	+	+	Uniform	6.65 ± 0.02	5.33 ± 0.21	4.55 ± 0.20	3.99 ± 0.07	III	I	I	0.23 ± 0.01	0.49 ± 0.02	0.77 ± 0.02	1.04 ± 0.07
219	+	+	Uniform with whey	6.65 ± 0.02	5.76 ± 0.06	5.41 ± 0.14	4.10 ± 0.09	III	III	I	0.23 ± 0.01	0.42 ± 0.00	0.58 ± 0.03	1.06 ± 0.08
401	+	+	Broken with whey	6.65 ± 0.02	5.87 ± 0.02	5.42 ± 0.13	4.58 ± 0.10	III	III	II	0.23 ± 0.01	0.40 ± 0.03	0.62 ± 0.07	0.84 ± 0.11
242	+	+	Uniform	6.65 ± 0.02	5.32 ± 0.09	4.52 ± 0.07	3.92 ± 0.01	III	I	I	0.23 ± 0.01	0.53 ± 0.02	0.70 ± 0.11	1.03 ± 0.03
722	-	+	Uniform	6.65 ± 0.02	5.32 ± 0.30	4.61 ± 0.32	4.03 ± 0.09	III	II	I	0.23 ± 0.01	0.50 ± 0.05	0.73 ± 0.09	1.04 ± 0.04
730	-	+	Uniform	6.65 ± 0.02	5.24 ± 0.05	4.63 ± 0.24	4.12 ± 0.28	III	II	I	0.23 ± 0.01	0.52 ± 0.05	0.74 ± 0.04	1.01 ± 0.06

[1]Values reported are the means ± standard deviations. [2]Classification of abilities to acidify milk as described by Fusieger et al. [21]. Results: +, positive; −, negative.

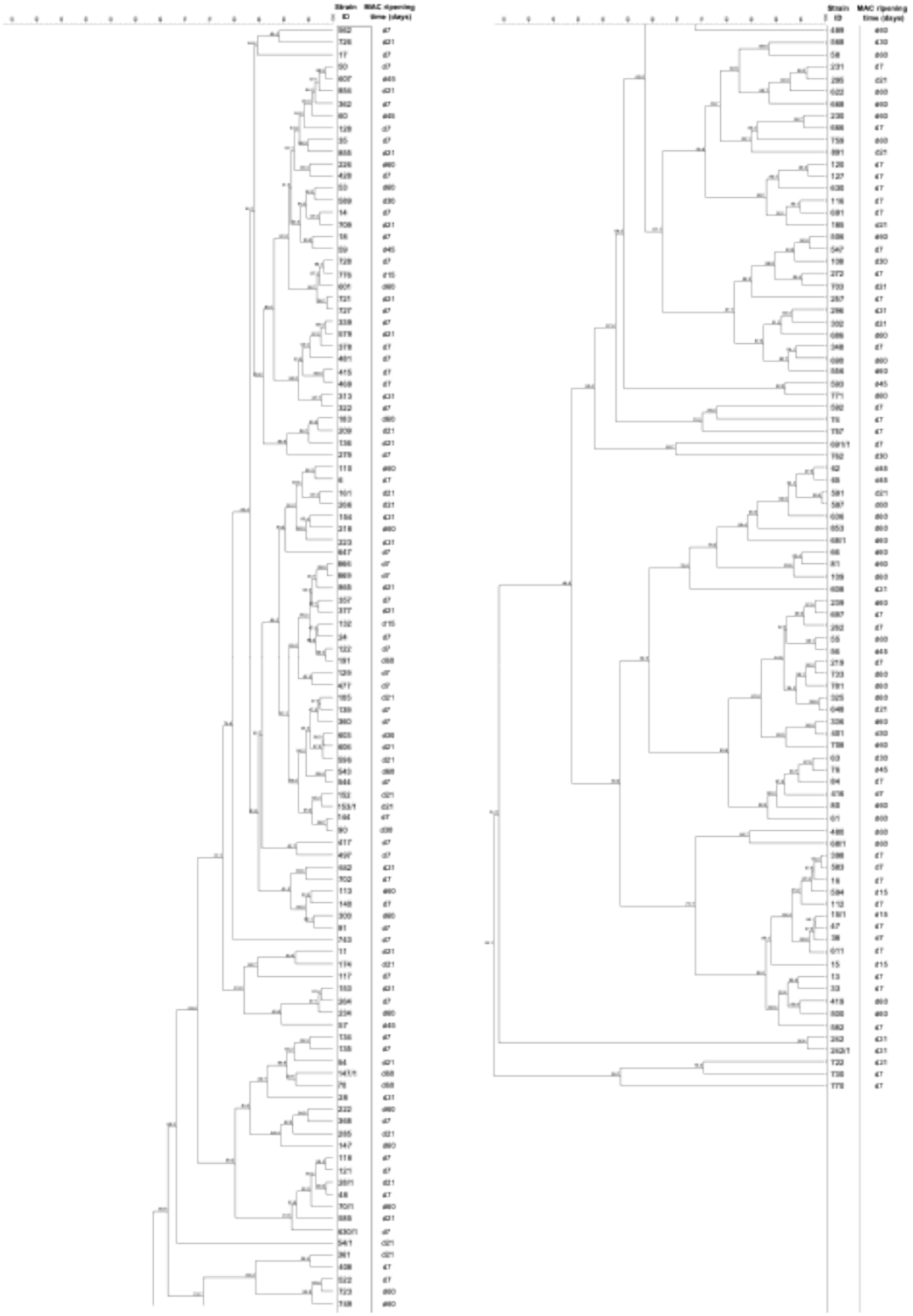

Figure 1. Dendrogram of rep-PCR (GTG)5 fingerprints based on UPGMA clustering among L. paracasei strains (n=197) isolated from Minas artisanal cheese at varying ripening days from the Entre Serras da Piedade to Caraça Region. Node values represent the percentage of similarity.

CONCLUSÕES GERAIS E PERSPECTIVAS

Este estudo realizado com BAL isoladas de QMA da região Entre Serras da Piedade ao Caraça, permite as seguintes conclusões: a presença de BAL ao longo da tapa de maturação dos queijos podem contribuir para a redução de patógenos e contaminantes, além disso, a diversidade de BAL da região é rica e ainda pouco explorada, o que sugere a possibilidade de encontrar outros isolados em queijos com características e tempos de maturação variados, contribuindo assim para o *terrior* da região. A fazenda em estudo, por sua vez, apresentou dominância dos isolados de um gênero específico, mas que podem ser melhores explorados em busca de um diferencial para sua utilização em alimentos, pois essas bactérias podem ter características benéficas ou até mesmo inovadoras para a indústria.

Dos 55 isolados analisados nos testes tecnológicos, foi possível observar que a maioria demonstrou bom potencial tecnológico, sendo possível sua utilização pela indústria de lácteos. Mas, tendo em vista que *L. paracasei* é uma NSLAB, a aplicação tecnológica desta bactéria depende das funções que vão exercer no produto final, ou seja, qual objetivo da implementação deste microrganismo no produto e, em qual produto ele será adicionado. Dessa forma são necessários estudos mais avançados e detalhados sobre a aplicação desses microrganismos nos lácteos.

Portanto, o estudo alcançou os seus objetivos, contribuindo para as pesquisas em uma região de QMA ainda pouco explorada. Estudos mais aprofundados e com a aplicação desses isolados são necessários para melhor caracterizar e definir o potencial tecnológico de BAL dessa região Entre Serras da Piedade ao Caraça – MG.