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Enterococcus faecium in artisanal ripening cheese: technological and safety aspects

Enterococcus faecium em queijo maturado artesanal: aspectos tecnológicos e de segurança

Enterococcus faecium en queso de curado artesanal: aspectos tecnológicos y de seguridad

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Abstract

The aims of this work were to identify and characterize for some important technological properties of the *E. faecium* throughout the ripening process of cheese. The study involved evaluating the technological potential of six isolates of *E. faecium* (EFM55, EFM67, EFM9A, EFM16A, EFM19A and EFM44) from artisanal cheeses as their probiotic characteristics and technological perspective. Concerning the antimicrobial sensibility, 71% of the *E. faecium* tested were sensitive to all antimicrobials. No other medicine inhibited the active of *E. faecium*, with the exception of dipyrone for the EFM19A and EFM9A strains. The isolates showed good antagonistic activity against gram-negative, as EFM9A, EFM55 and EFM67 strais with better activity. The highest fermentation was observed in 42 ºC, showed the pH variation of 4.15 to 5.15, after 48 hours of fermentation. All isolates showed pH reduction at 20 ºC. However, all isolates produced higher titratable acidity at a temperature of 42ºC. The isolates EFM55 and EFM9A has had probiotic actived, biochemical and susceptibility parameters desirables, aim it's technological potential in the production of dairy products.

Keyword: Drug sensitivity; Antagonistic activity; Fermentation.

Resumo

Este trabalho teve como objetivo caracterizar propriedades tecnológicas do isolado *Enterococcus faecium* ao longo do processo de maturação de queijos. O estudo envolveu avaliar o potencial tecnológico de seis isolados de *E. faecium* (EFM55, EFM67, EFM9A, EFM16A, EFM19A e EFM44) de queijos artesanais quanto às suas características probióticas e perspectiva tecnológica. Em relação à sensibilidade antimicrobiana, 71% dos *E. faecium* testados foram sensíveis a todos os antimicrobianos. Nenhum outro medicamento inibiu a atividade de *E. faecium*, com exceção da dipirona para as cepas EFM19A e EFM9A. Os isolados apresentaram elevada atividade antagônica contra gram-negativos, com destaque para os isolados EFM9A, EFM55 e EFM67. A maior taxa de fermentação ocorreu a 42 ºC, apresentando variação de pH de 4,15 a 5,15, após 48 horas. Todos os isolados apresentaram redução do pH a 20 ºC. No entanto, à temperatura de 42 °C, todos desenvolveram a maior acidez titulável no tempo de 48 horas. Os isolados EFM55 e EFM9A também apresentaram

características probióticas, bioquímicas e de suscetibilidade desejáveis, visando seu potencial tecnológico na produção de laticínios.

Palavras-chave: Sensibilidade a medicamentos; Atividade antagônica; Fermentação.

Resumen

Este trabajo tuvo como objetivo caracterizar propiedades tecnológicas del aislado *Enterococcus faecium* durante la maduración de quesos. El estudio evaluó el potencial tecnológico de seis aislados de *E. faecium* (EFM55, EFM67, EFM9A, EFM16A, EFM19A y EFM44) de quesos artesanales, según sus características probióticas y perspectiva tecnológica. En relación a la sensibilidad antimicrobiana, 71% de los *E. faecium* analizados fueron sensibles a todos los antimicrobianos. Ningún otro medicamento inhibió la actividad de *E. faecium*, excepto la dipirona para las cepas EFM19A y EFM9A. Los aislados mostraron elevada actividad antagónica contra gram-negativos, con destaque para los aislados EFM9A, EFM55 y EFM67. La mayor tasa de fermentación fue observada a 42 ºC, siendo la variación de pH de 4,15 a 5,15, después de 48 horas. Todos los aislados presentaron reducción del pH a 20 ºC. Sim embrago, a la temperatura de 42 °C, todos desenvolvieron la mayor acidez titulable en el tiempo de 48 horas. Los aislados EFM55 y EFM9A también presentaron características probióticas, bioquímicas y de susceptibilidad satisfactorias, teniendo en cuenta su potencial tecnológico en la producción de derivados lácteos.

Palabras clave: Sensibilidad a medicamentos; Actividad antagónica; Fermentación.

1. Introduction

Artisanal ripened cheese is the common and traditional cheeses produced in various areas of the Brasil. Several microorganisms can be used in the cheese ripening process, including *Enterococcus* genus. Enterococci are ubiquitous microorganisms present in different environments such plants, soil, water and commensal microbiota of human and animals (Ben Braïek et al., 2017). Enterococci strain occur in different foods, highlighting dairy products (cheeses and raw milk), vegetables, meats and raw fish/sea foods (Elmoslih et al., 2017; Vinderola et al., 2017).

The use of Enterococci in cheeses continuing be controversial, i.e, are considered essential for cheese flavor, but otherwise they can be considered potentially pathogenic microorganisms (Yerlikaya & Akbulut, 2019), that could cause important infections and

diseases such as bacteremia, endocarditis and several infections, besides multiple antibiotic resistances (Franz et al., 2001).

However, because of this anti-inflammatory potential, *Enterococcus* strains is commonly adopted as a probiotic in the treatment of some diseases such as recurrent diarrhea, diarrhea treatment in association with antibiotic medication, viral and chemotherapy, chronic sinusitis and bronchitis, demonstraing highest probiotic activity, when compared with different latic acid bacteria (LAB) (Nueno-Palop & Narbad, 2011). Moreover, some enterococci exhibit antimutagenic, anticarcinogenic, hypocholesterolemic, and immune regulation effects (Foulquié Moreno et al., 2006).

In previous work, Furlaneto Maia et al. (2017) demonstrated that *E. faecium* possessed high ability to survive in the presence of lysozyme and pancreatic enzymes, bile salt, low pH and high auto-aggregation, criteria for potential probiotic strains.

Another important factor of a probiotic is the production of bacteriocins. Enterocins are bacteriocin produzed by *Enterococcus* sp, being characterized as antimicrobial peptides produced *in situ* that exhibit specific molecular properties, including low toxicity and either broad-spectrum or narrow-spectrum activity (Ogaki et al., 2016).

In fermented foods, Enterococci strains play an important role in the ripening of traditional cheeses, probably through proteolysis, lipolysis, and citrate contributing to their typical taste and flavor. A majority of works specify that Enterococcus isolates play a vital role in the development of the sensory properties of this type of food (Nami et al., 2019).

Because of their flavor development and bacteriocin production in ripening cheeses, it has been suggested that Enterococci with desirable technological and metabolic properties could be included as exogenous cultures for various cheeses prodution (Ogier & Serror, 2008; Fernández et al., 2015).

In this research, *Enterococcus* species with probiotic properties were isolated from raw milk and some traditional dairy products by using biochemical, phenotypical and genotypical methods. The aim of this study was to evaluate the technological characteristics and their potential use in ripening cheese production as adjunct culture.

2. Material and Methods

2.1. Biological material

Six *E. faecium* strains (EFM 55, EFM 67, EFM 9A, EFM 16A, EFM 19A E EFM 44) were obtained from soft cheese and raw milk samples over a period of 1 year from 2011 to 2012 as described elsewhere (Furlaneto Maia et al., 2017).

As indicator bacteria, *Salmonella typhimurium* ATCC *14028*, *Salmonella enteritidis* ATCC 13076, *Escherichia coli* enterohemorrhagic (EHEC) 3001, *E. coli* enterotoxigenic (ETEC), *E. coli* enteropathogenic (EPEC), *Enterococcus faecalis* ATCC 29212 and *Listeria monocytogenes* CDC 4555 were used. The strains belong to the Laboratory of Basic and Applied Microbiology of Federal Technological University of Paraná-Londrina, Brazil. Stock cultures were maintained at − 20 °C in brain heart infusion (BHI) (Acumedia-Neogen) broth supplemented with 20% (v/v) glycerol (Gibco).

Before use, frozen stock was inoculated into 10 mL in Brain Heart Infusion (BHI) broth (Neogen Culture Media, USA) and incubated at 37 °C for 24 h.

2.2. Antagonist activity

To evaluation of the antagonistic activity, each *E. faecium* isolate was inoculated BHI medium separately and incubated at 37 ° C for 24 hours (Ogaki et al., 2016). The plates were inverted to receive 1 mL of chloroform in the plate covers and remained closed for 20 min. The residual chloroform was evaporated by opening the plates. Through the pour plate method, each indicator strain (1.0 x 10^6 cells/ml) was inoculated into soft BHI agar (0.8%) and was poured into the *Enterococcus* plates forming an overlay. The plates were then incubated at 37°C for 24 h. Inhibition was considered positive when the inhibition halo was greater than 0.5 mm. The assays were performed in duplicate for each type of pathogen studied.

2.3. Purified Enterocin preparation and antagonic test

Bacteriocin (cell-free supernatant – CFS) obtaining and precipitation was performed as described by Rocha et al. (2019). The pH of CFS was adjusted to 6.5 and treated with catalase, to avoid the presence of H2O2 (Ogaki et al., 2016). The CFS was sterilized by

membrane filtration, using 0.22 μm pores with low protein binding capacity (Millipore®). The inhibitory spectrum of activity was obtained using the agar-well assay (De Vuyst & Leroy, 2007) against indicator bacteria. Brain Hearth Infusion (BHI) agar plates were overlaid with BHI soft agar (0.75%) seeded with actively growing cell of the test organism (1.0 x 10^6 cels/mL). The wells were done with tips. Forty microliters of the CFS was added to wells, and then incubated at 37 °C for 24 hours. The sensitivity of the strain in question was evaluated by checking for clear zones around wells and then inhibition zones were scaled in millimeters (mm).

2.4. Antimicrobial susceptibility

The strains were tested against 11 antibiotics using the Kirby-Bauer disc diffusion method. This assay was performed on Mueller Hinton agar (HiMedia Laboratories, Mumbai, India) using gentamicin (10 μg), vancomycin (30 μg), tetracycline (30 μg), erythromycin (15 μg), ampicillin (10 μg), chloramphenicol (30 μg) and ciprofloxacin (5 μg) (Oxoid, Basingstoke, UK). *Staphylococcus aureus* ATCC 25923 was used as control strain and the zones of inhibition were determined using the Clinical and Laboratory Standards Institute (CLSI, 2017).

2.5. Susceptibility to medicaments

Capability to resist medicaments treatment is the one of the characteristics for a good probiotic. *E. faecium* strains were tested for susceptibility to commercially available drugs (Table 1) following protocol described by (Todorov et al., 2011).

The drugs were acquired in a handling pharmacy, solubilized in sterile water at the concentration indicated for use established by the manufacturer. MRS agar (Difco) plates containing 10^6 cfu/mL of *E. faecium* strains were prepared after strain cultivation in MRS broth at 37 °C for 48 h.

Subsequently, filter paper disks (5 μm diameter) were dispensed onto the surface of the medium, in which 10 μl of the resuspended drug was added. The drugs presenting the inhibition zones larger than 2 mm were considered sensitive.

Table 1. List of continuous medications tested against *E. faecium* isolates.

Active principle	Concentration (mg / mL)	Medication group
Acetylsalicylic acid	100	Anti-inflammatory and analgesic
Losartan Potassium	20	Antihypertensive and ACE inhibitor
Dipyrone	100	Analgesic
Paracetamol	200	Analgesic
Enalapril Maleate	4	Antihypertensive
Ibuprofen	40	Anti-inflammatory
Loratadine	2	Antihistamine
Nimesulide	80	Analgesic, Anti-inflammatory and Antipyretic
Omeprazole	4	Anti-ulcer and proton pump inhibitor
Simvastatin	4	Antilipemic

Source: Authors (2020).

2.6. Lipolytic and proteolytic activity

The enzymatic activities of proteolysis and lipolysis were determined by the method of (El-Ghaish et al., 2010; Hankin & Anagnostakis, 1975). The evaluation to the proteolytic activity consisted of the addition of 10 µl of each isolate (1.5 x 10^8 CFU / mL), in plates containing Plate Count Agar (PCA) supplemented with 1% skim milk (Molico® 10%). For the evaluation of lipolytic activity, the isolates were inoculated in Luria-Bertani medium (LB) supplemented with 0.5% Tween 20. Plates from both tests were incubated at 20° C and 42° C for 120 hours and the IE measurement was performed at times 6, 12, 24 hours for the plates incubated at 42° C and at the times 24, 48 and 120 hours for the plates incubated at 20° C.

To improve visualization of the proteolysis halo an aliquot of 5 ml of 5% acetic acid was added to the surface of the plates for 1 minute and the excess discarded. The proteolytic activity was considered positive when transparent halo occurred around the inoculum. The lipolytic activity plates were subjected to refrigeration (4 °C) for 6 hours, after incubation for the verification of halo formation.

2.7. Acidification capacity

E. faecium acidification capability was determined by inoculating of each isolate (1.5 x 10^8 CFU / mL) in 25 mL of 10% (w / v) reconstituted skim milk (Molico®). These solutions

were prepared proportionally to the incubation times (6, 12, 24 and 48 hours), at two temperatures (20 and 42 ºC) according to Psoni et al. (2006). The pH and titratable acidity were measured at each time (AOAC, 2003). The analyzes were performed in duplicate.

2.8. Beta-galactosidase activity

Beta-galactosidase enzyme activity was determined using paper discs impregnated with o-nitrophenyl-β-D-galactopyranose (ONPG Disks from Sigma-Aldrich). For this, a solution was prepared with sterile distilled water and 100 ug ONPG. The filter paper disks (6 mm) were saturated with 10 µl of the ONPG solution and kept in laminar flow for a few minutes to dry the solution. Subsequently, the disks were added / dipped into tubes containing 1.5 x 10^8 CFU / mL of the isolate in physiological solution (0.85%). Followed by incubation for 4 hours at 37 °C. After the disks, we evaluated to the colorimetric alteration, presence and absence of yellow color. The presence of yellow color in the discs is due to the release of the chromogenic compound, o-nitrophene, which indicates the production of β-galactosidase by the isolate. The assays were performed in the dark in order to preserve the enzymatic activity. All tests were done in duplicate.

2.9. Statistical analysis

All experiments were carried out according to the completely randomized design, with one replicate. The data were submitted to analysis of the variance, and the means were compared by the Tukey test at the 5% significance level through the Statistica 7 program (Statsoft®, USA).

3. Results e Discussion

One traditional criterion of a probiotic microorganism is, to be autochthonous of the ecosystem where they will be ingested (Dunne et al., 2001). In this study we evaluated six *E. faecium* strains isolated from soft cheese and raw milk. None of them were no vancomycin-resistant and no pathogenic genes (cylA, asa1, gelE, ace and cpd) were found. The isolates showed good viability at 120 and 240 min of incubation with pH 3.0, and were able to resist 0.3% and 0.1 g/ml of bile salts and pancreatic enzyme (Furlaneto Maia et al., 2017).

The antagonist activity of *E. faecium* isolates were confirmed against foodborne pathogens pathogens (Table 2; Figure 1 A to E).

Table 2. Antagonist activity of *Enterococcus faecium* isolates against foodborne pathogens.

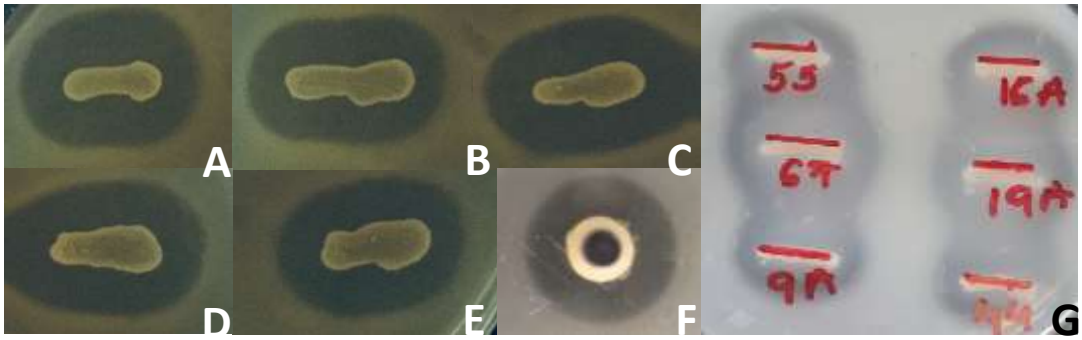
Isolates	Pathogens						
	S.typhimurium	*S. enteritidis*	EHEC	ETEC	EPEC	*E.faecalis*	*L. monocytogenes.*
EFM 19 A	-	+	+	-	+	-	-
EFM 44	-	+	+	+	+	-	+
EFM 55	+	+	+	+	+	-	+
EFM 9 A	+	+	+	+	+	+	+
EFM 16 A	-	-	+	-	+	-	-
EFM 67	+	+	+	-	+	+	+

* + positive result, - negative result. *Salmonella typhimurium* ATCC *14028*, *Salmonella enteritidis* ATCC 13076, enterohemorrhagic *Escherichia coli* (EHEC) 3001, enterotoxigenic *Escherichia coli* (ETEC), enteropathogenic *Escherichia coli* (EPEC), *Enterococcus faecalis* ATCC 29212 and *Listeria monocytogenes* CDC 4555. Source: Authors (2020).

Of all, EFM 9A presented antagonistic activity against all, and EFM55 and EFM67 strains had good antagonistic activity against six pathogens to the seven studied.

The results with enterocin corroborated with the results obtained in antagonistic activity, confirming that the bacterial inhibition was due to the action of the peptide (Figure 1 F). Furthermore, expressive inhibition was found in front of *S. enteretidis* (83.33%) and *S. typhimuiruim* (50%), and *L. monocytogenes* (66.66%). Underline that *Enterococcus* sp. have a close phylogenetic relationship between them, contribute to the control of this pathogens in the cheeses production (García et al., 2004). Whereas, only 33.33% of the *E. faecium* isolates were effective against *E. faecalis*, corroborating with previous studies that showed low efficiency in front of these specie (Furlaneto Maia et al., 2017).

Figure 1. Spot-on-lawn methodology showing the translucid halo around the colony of EFM 9 A strain against *Salmonella typhimurium* ATCC *14028* (A); *Salmonella enteritidis* ATCC 13076 (B); enterohemorrhagic *Escherichia coli* (EHEC) (C); *Enterococcus faecalis* ATCC 29212 (D); *Listeria monocytogenes* CDC 4555 (E), F: Agar diffusion bioassay of bacteriocin from EFM 9 A strain against *L. monocytogenes*; G: Skim milk agar showing expression of proteolytic activity by six *E. faecium* strains, at 20 °C.



Source: Authors (2020).

Among the pathogenic bacteria tested, it was observed the greater antagonistic activity of the isolates tested from Gram-negative bacteria group than Gram-positive group. Since, the EHEC and EPEC *E. coli* serotypes were sensitive to all isolates tested, and the ETEC serotype showed sensitivity to 50% of them. These results are promising, as food EHEC control is laborious, due to its particular characteristics, such as the high tolerance to acid environments, unlike that observed in other enterobacteria (Öncül & Yıldırım, 2019).

Moreover, *Salmonella* and *E. coli* serotypes it was recurrently referred to be pathogens with high clinical relevance for human and animal health, besides promoting a lot of damage in the food industry (Nguyen & Sperandio, 2012). In this way, the *E. faecium* antagonistic action described in our study, must be useful for technological applications in the food industry as a putative biopreservative in adjunct culture of cheeses.

The development of biopreservation technologies with LAB and/or their metabolites represents protection of food against microbial contamination through the bacterial production of several anti-microbial substances including organic acids, hydrogen peroxide, and bacteriocins (Perin et al., 2013). Food industries has interested to *Enterococcus* spp. whereas that several species are capable of producing antagonistic substances, as enterocins, that controlling or inhibiting the development of pathogenic microorganisms in food (Acuña et al., 2012).

Table 3 presents the data referring to the antibiogram of all *E. faecium* isolates, tested in this study.

Table 3. Resistance and sensitivity profile of *E. faecium* strains to antimicrobials and medicines.

		EFM 55	EFM 67	EFM 9 A	EFM 16 A	EFM 19 A	EFM 44
ANTIMICROBIALS	Gentamycin	S	S	S	S	S	S
	Vancomycin	S	S	S	S	S	S
	Tetracycline	S	S	S	S	S	S
	Erythromycin	R	S	S	S	S	I
	Ampicillin	S	S	S	S	S	S
	Chloramphenicol	S	S	S	S	S	S
	Ciprofloxacin	S	S	S	S	S	I
MEDICAMENTS	Acetylsalicylic acid	-	-	-	-	-	-
	Losartan Potassium	-	-	-	-	-	-
	Dipyrone	-	-	+	-	+	-
	Paracetamol	-	-	-	-	-	-
	Enalapril Maleate	-	-	-	-	-	-
	Ibuprofen	-	-	-	-	-	-
	Loratadine	-	-	-	-	-	-
	Nimesulide	-	-	-	-	-	-
	Omeprazole	-	-	-	-	-	-
	Simvastatin	-	-	-	-	-	-

*(S) sensitivity, (R) resistance; + growth, - cell death. Chloramphenicol (CLO); Ciprofloxacin (CIP); Ampicillin (AMP); Tetracycline (TET); Vancomycin (VAN); Erythromycin (ERI) and Gentamycin (GEN). Source: Authors (2020).

According to the results of the disc diffusion method, all the *E. faecium* isolates demonstrated susceptibility to the majority of the tested antibiotics: gentamicin, vancomycin, tetracycline, erythromycin, ampicillin, chloramphenicol and ciprofloxacin.

On the other hand, the *E. faecium* EFM 55 exhibited resistance to erythromycin and EFM 44 showed intermediate resistance to ciprofloxacin and erythromycin.

Although the acknowledged role of enteroccci as cause of infections in humans is due to the increase of resistance to multiple antibiotics (Giraffa, 2002), recent studies also highlight the predominance of a sensitive phenotype in milk isolates and milk derivatives (Anderson et al., 2018; İspirli et al., 2017).

Moreover, with the exception of dipyrone for the EFM9A and EFM19A *E. faecium* (Table 3), no other drug inhibited the probiotic active of *E. faecium*, suggesting that patients using these probiotics would have their therapeutic activity preserved even with concomitant use of medicaments. However, the probiotic bacteria selection must be submitted to food safety evaluation, it is essential that they showed susceptibility to antimicrobials and drug resistance.

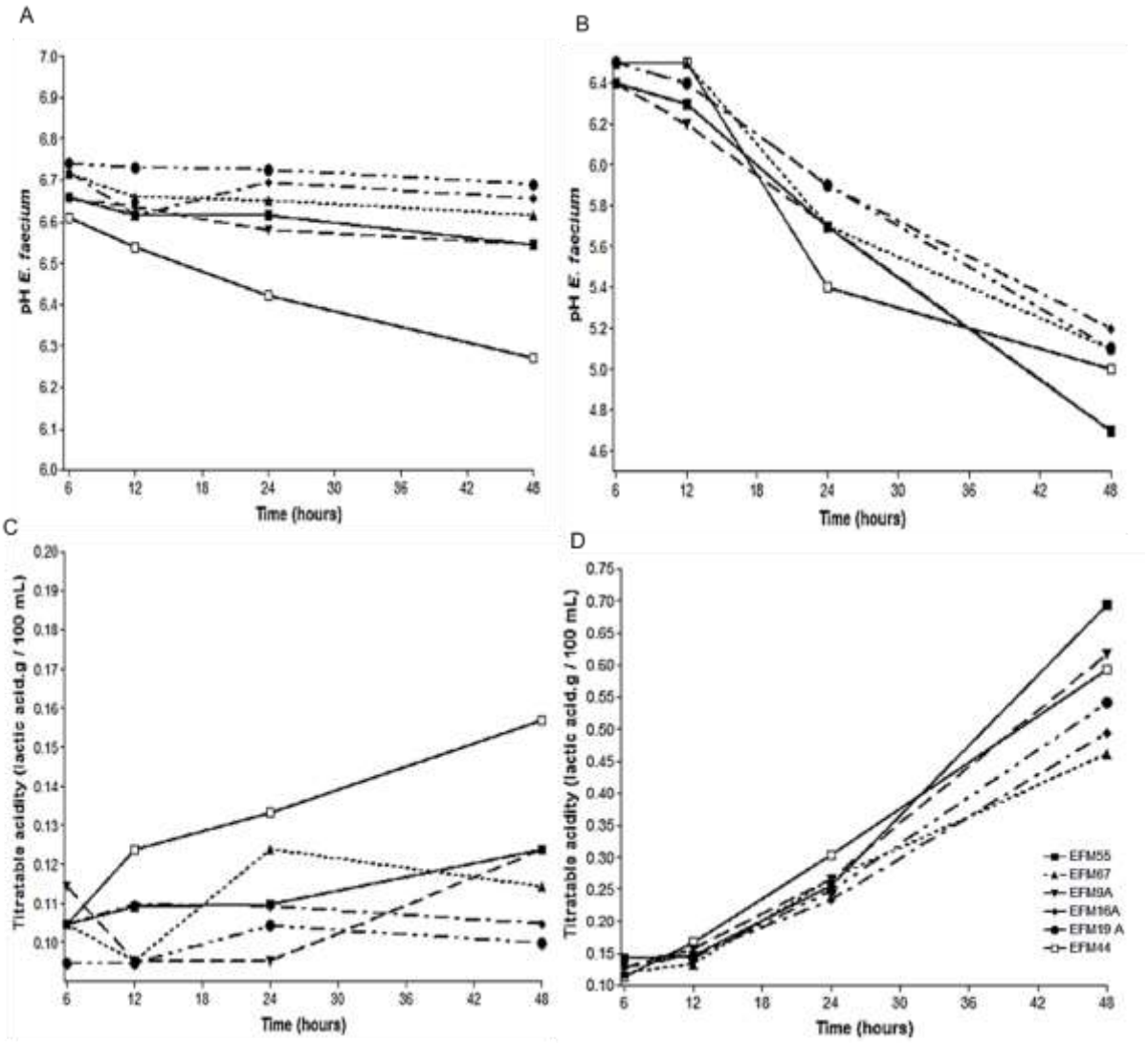
Regarding proteolytic activity, it was observed in all isolates tested after 48 hours of incubation at 20 °C (Figure 1 G), and no proteolytic activity was showed at temperature of 42 °C.

This activity, it is related with cheese quality by improving taste, flavor and functional properties, by promoting hydrolysis of proteins resulting in peptides and aminoacids (Ozturkoglu-Budak et al., 2016). Giraffa (2003), highlight the importance of proteolytic activity of *Enterococcus* sp. isolated in cheese manufacture, contributing with texture and organoleptic properties.

Although our isolates not showed lipolytic activity in LB medium with tween 20, it is not limit the use of these isolates in adjunct culture, since microrganisms with high lipolytic activity such as *Bacillus*, *Arthrobacter* and *Pseudomona*s can be added to catalyze the hydrolysis of fats and oils (Ozturkoglu-Budak et al., 2016).

The fermentation activity and acidifying capability are presents in figure 2.

Figure 2. Mean values of pH and titratable acidity of *E. faecium* throughout the fermentation time (6, 12, 24 and 48 h). A - pH averages under the temperature of 20 °C. B - pH averages under the temperature of 42 °C. C- Acidity of the strains under the temperature of 20 °C. D - Acidity under the temperature of 42 °C.



Source: Authors (2020).

The fermentation activity of *E. faecium* isolates under different conditions, showed the highest fermentation activity in temperature at the 42 ºC, however it was reduced along the time, resulting in pH variation around 4.15 to 5.15, after 48 hours of fermentation (Figure 2 B).

At temperature of 20 ºC, all isolates had a significant reduction in the pH value during the fermentation time, showed pH 6.3 after 48 hours (Figure 2 A). Both temperatures tested were selected for it is possible application in fermented dairy products (fermented milk and cheese).

The acidifying capability of LAB in fermented dairy products is important to the pH reduction, conservation and safety of dairy products, through the accumulation of lactic acid, being a good attribute for starter cultures. In this perspective the EFM44 isolate was the most efficient in both temperatures, while the EFM55 isolate was effective just in 42 °C; these isolates were able to changed the pH more than 1 unit. However, for the pH noticeable changes were necessary prolonged culture times, since the best acidification values refer to the time of 48 hours.

E. faecium has presented significantly acidification in front of *Enterococcus* species, as *E. faecalis* and *E. durans* (Andrighetto et al., 2001). This limited acidifying ability of *E. faecium* isolates, requiring initiator or homofermentative cultures to act in primary stage to the cheese production. In the initial cheeses stage, it is possible improve the milk pH reduce, which is aid in the coagulation and selection of acid-tolerant cultures (Walstra et al., 2006).

The most efficient temperature regarding the titratable acidity, was 42°C for all isolates, in different times (Figura 1 D). *E. faecium* EFM44 isolate, also stands out regarding its titratable acidity, with a good acidification after 24 hours at 42 °C (0.30%), and after 48 hours at 20 °C (0.15%) (Figure 1 C). However, for the temperature at 42 °C in 48 hours, just the EFM55 and EFM9A *E. faecium* isolates showed acidity higher than 0.60%, suggesting that although all the isolates present acidification capacity, it is necessary use of the starter culture to accelerate the the acidity in fermented milk. This late acidification presented by our *E. faecium* isolates, is advantageous for cheeses with long production and storage time, as Cheddar cheese (Moreno et al., 2003).

Condering that the titratable acidity promoting the inhibition of some pathogens and influence the quality of the cheese produced, and someone starter cultures with acidity levels can incorporated in the probiotics, providing satisfactory acidity levels (El-Garhi et al., 2018). We suggest that, the isolates EFM55 and EFM9A, with probiotic and antagonistic profile described here, have high technological potential in the food industry can be used in the production of rennet cheese, since this model demands to the low titratable acidity.

The presence of the β-galactosidase enzyme was also not detected in all isolates of *E. faecium*. On the other hand, recent studies have already described the occurrence of *E. faecium* strains capable of producing β-galactosidase in dairy products, beneficial feature, especially for the health of lactose intolerant consumers (Badarinath & Halami, 2011; Todorov et al., 2011).

4. Conclusion

This work extends our knowledge of the the of *E. faecium* isolates, with probiotic characteristics, in addition to demonstrating the technological potential of these isolates for producing a cheese with a peculiar flavor. The acidification activity at the 42 ºC temperature was promising, suggesting the possible application of these lines in cheeses with late maturation. In addition, the EFM55 and EFM9A isolates presented acidity above 0.60%, and could be used in cheeses that require low lactic acid concentration. The EFM55 and EFM9A were isolated which showed the greatest potential for use in ripened cheeses. With the results obtained in this paper, we are making cheeses of medium maturity, from a single isolated and a combination of these; and evaluating other technological potentials of these bacteria.

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