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Characterization of Enterocins Produced by *Enterococcus mundtii* Isolated from Humans Feces

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ABSTRACT

The aim of this study was to characterize bacteriocins produced by 70 strains of Enterococcus mundtii.Four strains exhibited antibiotic activity towards Listeria innocua, L. monocytogenes, Lactobacillus plantarum, and Salmonella Enteritidis. They remained active under temperatures of up to 121°C for 20 min, and under pH treatments that varied from 2.0 to 10.0. Antimicrobial activity was maintained during the storage test for 60 days under freezing. The kinetics of production revealed the peak activity of 1600 AU /mL during the logarithmic growth phase and the molecular weight found was approximately 3.0 kDa. The characterization of the products with antimicrobial activity indicated their proteic nature, presenting a typical kinetics of primary metabolite and a molecular weight similar to many purified enterocins.

Key words: *Enterococcus mundtii*, enterocins, antimicrobial activity, biopreservative

INTRODUCTION

Bacteriocins are ribosomally synthesized peptides and proteins which may inhibit the growth or eliminate certain bacterial species, affecting the permeability of the membrane or even interfering with essential roles played by the cell, such as DNA replication and translation (Bennick et al., 1998). The study of bacteriocins produced by lactic acid bacteria (LAB) has gained much attention recently. The potential use of LAB as non-toxic biopreservation agents in the industrial processing of human food and animal feeds is chiefly due to the fact that they inhibit the growth of pathogenic and degradative bacteria (Cintas et al., 2000). Bacteriocins are subdivided into four classes, in terms of their biochemical and genetic characteristics. Class I bacteriocins comprises the lantibiotics, small molecules characterized by the presence of residues of two modified amino acids, lanthionine, and methyl-lanthionine, both added post-translationally. They have drawn much attention due to nisine, the most widely studied lantibiotic produced by *Lactococcus lactis,* which is used as food preservative in several countries (Kawamotto et al., 2002). Class II bacteriocins are heat-stable non-lantibiotic bacteriocins, whose molecular size is under 5 kDa there are three subdivisions (Cintas et al., 1997). Class IIa is defined in terms of similarity of amino acid sequences present in the N-terminus and by the strong antilisterial activity. Due to the antimicrobial activity towards *Listeria monocytogenes*, this subgroup holds noteworthy potential as food biopreservatives (Ennahar et al., 2000). This class assembles the majority of the

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enterocins classified to date, including mundticin, the only enterocin produced by *E. mundtii* so far characterized. Class IIb comprises the bacteriocins whose activity is dependent on the complementary activity of two different peptides, and class IIc is formed by non-lantibiotic bacteriocins that failed to be ranked under classes IIa and IIb (Casaus et al., 1997; Franz et al., 1999). Class III bacteriocins are large size (above 30 kDa), heat-labile proteins. Class IV is formed by complex proteins that require association with lipids and carbohydrates in order to exhibit antimicrobial activity (Lauková et al., 1998).

Since *E. mundtii* enterocins have been scarcely studied, this study was designed to characterize the bacteriocins produced by *E. mundtii* for their antimicrobial activity spectrum, evaluating their antimicrobial activity measured in Arbitrary Units (AU /mL), their sensitivity to heat, pH, storage conditions and proteolytic enzymes. Molecular size and the kinetics production curve were also determined.

MATERIALS AND METHODS

Bacterial strains

This study used 70 *E. mundtii* strains, from the Department of Microbiology Collection (Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Brazil). Strains were from animal and clinical and nonclinical human isolates. In order to establish the antimicrobial activity spectrum of enterocins, 16 Gram-positive and 12 Gram-negative bacterial strains, related to food poisoning; two yeasts, isolated from clinical samples, and two filamentous fungi, isolated from rice, were used (Table 1).

Screening of bacteriocin activity

Initially, a triage procedure was carried out for the 70 strains using five indicator bacteria: *Listeria innocua* ATCC 33090, *L. monocytogenes* ATCC 15313, *L. welshimeri* DMIC/UFRGS, *Corynebacterium fimi* NCTC 7547 and *Micrococcus luteus* ATCC 4698. The double layer test (Tagg et al., 1971) was used. The *E. mundtii* isolates were spotted onto a Petri dish containing Trypticasein soya agar (TSA), and then incubated at 35° C for 24 h. Subsequently, each dish was overlaid with semi-solid TSA agar previously inoculated with a 10^6 cell/mL solution of each indicator bacterium. After another incubation period at 35° C for 24 h, inhibition halos were observed. All strains that inhibited the growth of at least one of the five indicator bacteria, underwent hydrogen peroxide test, using double layer method in anaerobic conditions, and the bacteriophage assay described by Lewus et al. (1991). These procedures were adopted to rule out hydrogen peroxide and bacteriophage action as inhibitors of bacterial growth.

Preparation of the cell-free culture supernatant

The strains that presented antimicrobial activity but with a negative result for hydrogen peroxide and bacteriophage tests were used to prepare the cell-free culture supernatant. The strains were incubated in MRS broth (Accumedia) at 35° C for 18 h under agitation. Subsequently, the pH of the culture was adjusted to 6.2 with NaOH 1M. The culture was then centrifuged at 3000g for 15 min, and sterilized by filtration through a 0.22-µm-pore size membrane (Schleicher and Schuell).

Quantitative analysis of the antimicrobial activity of the cell-free culture supernatant

The quantitative analysis of the antimicrobial activity was measured in Arbitrary Units per milliliter (AU/mL). Antimicrobial activity was evaluated using the agar well diffusion method, with *L. innocua* ATCC 33090 as indicator strain at a concentration of 10^6 cell /mL. Serial 1:1 dilutions in phosphate buffer (PBS) of the supernatant were carried out. Aliquots of 80 µL from these dilutions were inoculated into the wells. Aliquots of the crude supernatant underwent the same procedure. After 10 minutes at 4ºC for diffusion, the agar plates were incubated at 35° C for 18 h, after which inhibition halos were measured. All the experiments were carried out in triplicate, with three different supernatants prepared as described before. An Arbitrary Unit (AU/mL) was defined as the reciprocal of the highest dilution that produced an inhibition halo larger than 2 mm in diameter (Kawamoto et al., 2002).

Determination of the antimicrobial activity spectrum of the cell-free culture supernatant

The indicator bacteria used to establish the antimicrobial activity spectrum of the cell-free supernatant are listed in Table 1. The agar well diffusion method was performed as described,

with 80 µL of the cell-free culture supernatant and 10^6 cells/mL of the indicator bacteria cell culture. After incubation at 35°C for 18 h, inhibition halos were measured. A cross-inhibition test was carried out, in which cell-free supernatants were tested against their own bacteriocin-producing strains.

Sensitivity to heat, pH variation, storage conditions and proteolytic enzymes

Aliquots of the cell-free supernatant were exposed to heat treatments of 40, 50, 60, 70, 80, 90 and 100° C for 30 min, and at 121° C for 10 and 20 min. Aliquots of the supernatants after treatment as well as a control aliquot (supernatant without treatment) were inoculated using the agar well diffusion method. In a separate experiment, cell-free culture supernatants were adjusted to values between pH 2 and pH 10 using buffers. The agar plates were incubated at 35°C for another 24h. Controls were prepared adding PBS instead of the buffer with predefined pH. In order to confirm the proteic nature of the bacteriocin, sensitivity to proteolytic enzymes trypsin, proteinase K and lysozyme at 0.1 and 1 mg /mL were assessed in a concomitant experiment. The agar well diffusion method was used to measure the activity of the supernatants after treatments. The bacteriocin stability under storage was assessed using two sets of aliquots of the cell-free supernatants: one set was kept refrigerated at 4° C, and the other kept frozen at –20° C for 60 days. The antimicrobial action was determined every 15 days, and expressed in AU/mL using the agar well diffusion method. All tests used 10^6 cells/mL preparations of *L. innocua* ATCC 33090 as indicator strain. Plates were incubated at 35° C for 18 h and inhibition halos were measured in mm. All tests were carried out in duplicate using three different supernatants.

Bacteriocin Production kinetics

Bacteriocin production kinetics was determined for *E. mundtii* strains 39 and 115, which were chosen in the light of the results of cross-inhibition analyses. The antimicrobial activity, expressed in AU/mL, and sensitivity to storage conditions were also carried out. Strains were grown in MRS broth (Accumedia) at 35° C for 18 h, under constant agitation. Aliquots of 100 µL were retrieved every 1h. Antimicrobial activity (AU/mL) was evaluated using the agar well diffusion method, and *L. innocua* was the indicator microorganism used. Bacterial growth was followed by 620 nm absorbance analysis, the pH of the culture was also determined.

Molecular weight analysis

The molecular weight of bacteriocin was determined by 16% SDS-PAGE of the cell-free culture supernatant (Ausubel et al., 1987). Polyacrylamide gels were loaded with two aliquots of each cell-free supernatant. After electrophoresis gels were cut in halves, one half was stained with Coomassie blue to observe molecular size, and the other half was thoroughly washed in sterile distilled water and plated onto a thin TSA agar layer and overlaid with a layer of TSA 0.7% agar, previously inoculated with indicator bacterium *L. innocua*. The Petri dishes were incubated at 35° C for 18 h for further inspection of inhibition halos.

Statistical analysis

The results of sensitivity to heat, pH variations and storage conditions were subjected to analysis of variance (Mixed Hierarchical ANOVA) at a probability of $p < 0.05$.

RESULTS

Screening of the bacteriocin-producing strains

After the screening of 70 *E. mundtii* strains against the five indicator bacteria, 15 strains (21.4%) exhibited antimicrobial activity against at least one of the indicator strains. Out of the 15 strains detected, seven had originally been obtained from human feces samples, four from pig feces samples, and four from water samples. The test to detect bacteriophages did not produce any positive result. The hydrogen peroxide test revealed eight strains with negative results, of which six strains were from human feces samples and two from pig feces samples. These eight strains were used to prepare cell-free supernatants, with which the antimicrobial activity was confirmed using *L. innocua* as indicator microorganism. Out of the eight strains tested, four produced active antimicrobial cell-free supernatants (Table 1), all of them obtained from human feces samples.

These four strains were considered bacteriocin producers and underwent characterization assays.

Table 1: Indicators strains used and results of the antimicrobial activity test for the cell free supernatants of *E. mundtii* strains.

Indicator strains and source	**Inhibition halos in mm**			
	Cell-free supernatant of strains			
	5	39	42	115
Bacteria				
Bacillus cereus ATCC 33018 and ATCC 33019	-	-	-	-
B. subtilis ATCC 23856 and ATCC 19659	-	-	-	-
Corynebacterium fimi NCTC 7547	-	-	-	-
Lactobacillus fermentum ATCC 9338	-	-	-	-
L. plantarum ATCC 10012	3	3	3	-
Listeria monocytogenes ATCC15313	7	7	7	8
L. monocytogenes ATCC 19112	7	8	8	8
L. innocua ATCC 33090	8	8	8	8
L. ivanovii DM/UFRGS	6	7	7	6
L. seeligeri DM/UFRGS	5	4	7	7
Micrococcus luteus ATCC 4698	-	-	-	-
Paenibacillus peoriae [a]	-	-	-	-
P. validus [b]	-	-	-	-
Staphylococcus aureus ATCC 12692	-	-	-	-
Enterobacter aerogenes ATCC 13048	-	-	-	-
Escherichia coli ATCC 25922 and ATCC 23229	-	-	-	-
Pseudomonas fluorescens DM/UFRGS	-	-	-	-
P. putida ATCC 15175	-	-	-	-
P. aeruginosa ATCC 15442	-	-	-	-
Salmonella Enteritidis [c]	5	5	5	5
S. Enteritidis DM/UFRGS	7	6	8	6
S. Typhimurium ATCC 14028	-	-	-	-
Xanthomonas anoxopodis ATCC 8718	-	-	-	-
Shigella sonnei DM/UFRGS	-	-	-	-
Burkholderia cepacia ATCC 17759	-	-	-	-
Cross-inhibition				
E. mundtii 5	-	-	-	5
E. mundtii 39	-	-	-	4
E. mundtii 42	-	-	-	-
E. mundtii 115	-	-	-	-
Yeasts and Filamentous Fungi				
Candida tropicalis [d]	-	-	-	-
Candida albicans [d]	-	-	-	-
Aspergillus fumigatus [e]	-	-	-	-
Aspergillus orizae [e]	-	-	-	-

Strains a to d are from Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul a - strains isolated from soil; b - Strains isolated from water; c - Strains isolated from cheese; d - Strains isolated from clinical samples provided by HIV+ patients; e - Strains isolated from rice, Instituto de Ciência e Tecnologia dos Alimentos, Universidade Federal do Rio Grande do Sul.

Antimicrobial activity of the cell-free supernatant

All cell-free culture supernatants obtained exhibited the same antimicrobial activity spectrum, inhibiting *L. innocua* ATCC33090*, L. monocytogenes* ATCC 15313, *L. ivanovii, L. seeligeri, Lactobacillus plantarum* ATCC 10012 and 2 *Salmonella* Enteritidis strains, isolated from cheese (Table 1). The cross-inhibition test carried out against the antimicrobial strains themselves, revealed supernatant 115 to inhibit *E. mundtii* strains 5 and 39, yet without inhibiting its own strain or strain 42 (Table 1).

Quantitative analysis of antimicrobial activity of cell-free supernatants expressed in AU /mL

The antimicrobial activity of the four strains was determined for three supernatants obtained on different dates, under the same growth conditions. A considerable variation in AU results was

observed for the three supernatants obtained for the same strain. This was observed especially for *E. mundtii* 115, which produced 100 AU/mL for the first supernatant, and remarkably, an antimicrobial activity that was 4 times higher for the other 2 supernatants. *E. mundtii* 5 presents the lowest AU results and *E. mundtii* 115 presented the highest ones (Table 2).

Table 2 - Antimicrobial activity results (AU/mL) for the three supernatants prepared with the four *E. mundtii* strains, using *L. innocua* ATCC 33090 as indicator strain

Antimicrobial activity (AU/mL)			
Isolates	**Supernatant**		
	1	**2**	**3**
E. mundtii 5	100	100	200
E. mundtii 39	200	200	400
E. mundtii 42	200	200	400
E. mundtii 115	100	400	400

Sensitivity to heat, pH, storage conditions and proteolytic enzymes

The test carried out to establish the sensitivity of bacteriocins to heat revealed that supernatants maintained antimicrobial activity at all temperatures adopted. The supernatant produced by *E. mundtii* strain 39 was an exception since it lost all antimicrobial activity when kept at 121° C for 20 min (Fig. 1A). Antimicrobial activity was more affected at 121° C for all supernatants.

The pH sensitivity test showed that antimicrobial activity was not entirely lost along the pH range adopted (Fig. 1B). Yet, the greatest decrement in activity was observed at pH 2 for the four supernatants tested. Supernatants 5 and 42 suffered some degree of antimicrobial activity loss at alkaline pHs, whereas supernatants 39 and 115 produced but a narrow increase in inhibition halos. Antimicrobial activity was the same or even higher at neutral pH when compared to the control experiment. The increased inhibition halos under alkaline pH conditions could be due to alterations in peptide diffusion in such conditions.

As regards the storage test, no supernatant lost completely the antimicrobial activity initially exerted during the 60 day refrigeration and freezing experiments. All supernatants were proved to be more stable when frozen (-20° C), exhibiting little antimicrobial activity loss during the 60 day period adopted (Fig. 1D). The same supernatants kept under refrigeration (4° C) for the same period exhibited a more considerable antimicrobial activity loss (Fig. 1C). The greatest detriment was observed for supernatant 39, either under refrigeration or freezing. Supernatant 115 was considered the most stable among the four tested under both storage conditions. The test to measure sensitivity to proteolytic enzymes showed that all supernatants were rendered inactive by trypsin and proteinase K at the two concentrations tested. Lysozyme did not completely inactivate any culture supernatant.

Production kinetics and molecular size of *E. mundtii* 39 and *E. mundtii* 115 bacteriocin

After a 1 h incubation period, it was possible to observe an increase in absorbance for both *E. mundtii* strains 39 and 115. The production of bacteriocins was observed only after a 2 h incubation period. Stationary phase began at approximately 11 h after incubation for strain 39, and roughly 14 h after incubation for strain 115 (Fig. 2). Both strains began to produce bacteriocins during the logarithmic growth phase. Strain 115 presented a peak during the production with 1.600 AU /mL at the end of the logarithmic phase, and partly lost antimicrobial activity during the stationary phase. Strain 39 showed maximum antimicrobial activity of 1.600 AU /mL for up to 18h incubation. Initial pH of cultures was 6.1, and after 18h incubation, it decreased to 4.4.

The molecular weight estimated for bacteriocins produced by *E. mundtii* 39 and 115 was between 2.3 and 3.4 kDa (Fig. 3).

No protein band was observed in the Coomassie blue-stained gel, possibly due to the fact that samples were not concentrated.

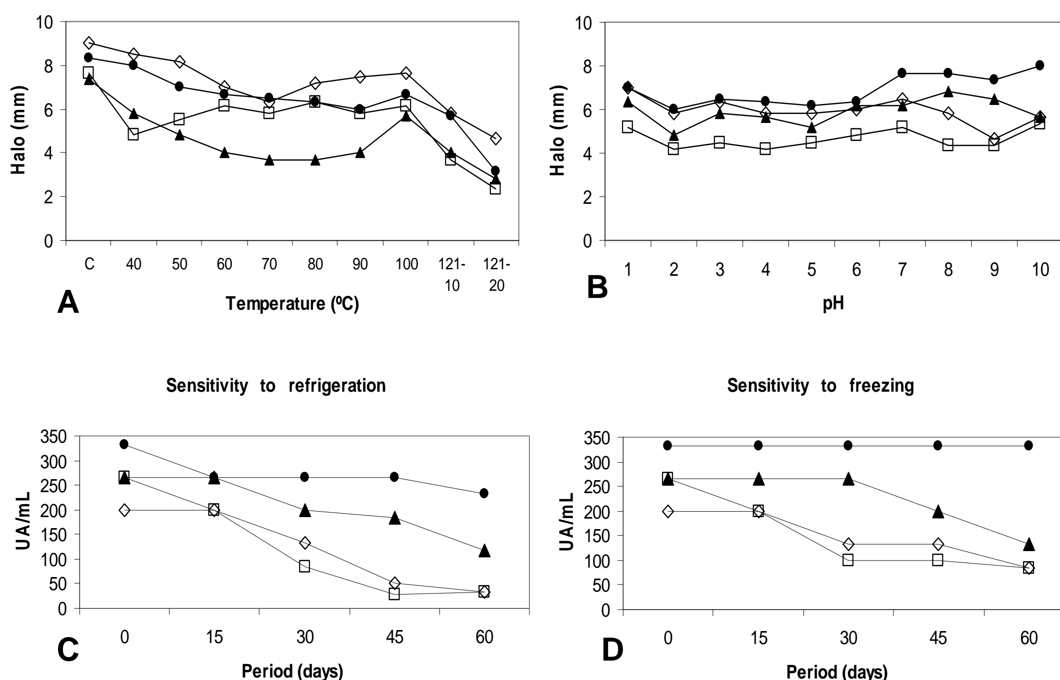

Figure 1 - Results of enterocin sensitivity test of cell-free supernatants produced by *E. mundtii* strains under (A) heat, (B) pH. Results are presented as halo sizes (mm). Temperatures between 40° C and 100° C were applied for 30 min. The temperature of 121° C was applied for 10 and 20 min. Results of enterocin sensitivity test of cell-free supernatants produced by *E. mundtii* strains for (C) refrigeration at 4° C and (D) freezing at –20° C. Results are presented as Arbitrary Units (AU mL^{-1}). The data presented in theses graphics are the means of three supernatants for which diferences were not significant ($p<0.05$). (C=untreated control, ▲= *E. mundtii* 42, = *E. mundtii* 39, ◇= *E. mundtii* 5, ●= *E. mundtii* 115).

DISCUSSION

Enterococcus spp. play an important role in the maturation of some kinds of cheese and in the preparation of some probiotic compositions (Franz et al., 1999). Apart from this, they produce bacteriocins, specifically called enterocins, which inhibit the growth of pathogenic and food-deteriorating bacteria, including the genera *Listeria* and *Clostridium* (Giraffa, 1995). Nevertheless, it is necessary that a strain used commercially was its biological safety duly ascertained. The use of *E. faecalis* and *E. faecium* as biopreservers would be questionable, as both species infected human hosts deleteriously (De Vuyst et al., 2002).

For this reason and due to the paucity of published data on other enterococci species, the *E. mundtii* strains were chosen to characterize the bacteriocins the species produces. During the triage experiment carried out in this study, only a low number (four in a total of 70 strains) of bacteriocin-producing strains was found. This was in accordance with the results of other studies. Du Toit et al. (2000) carried out a study with 92 isolates, of which only seven were enterocin producers (7.6%), and Jennes et al. (2000) found only one enterocin-producing strain among 77 LAB tested.

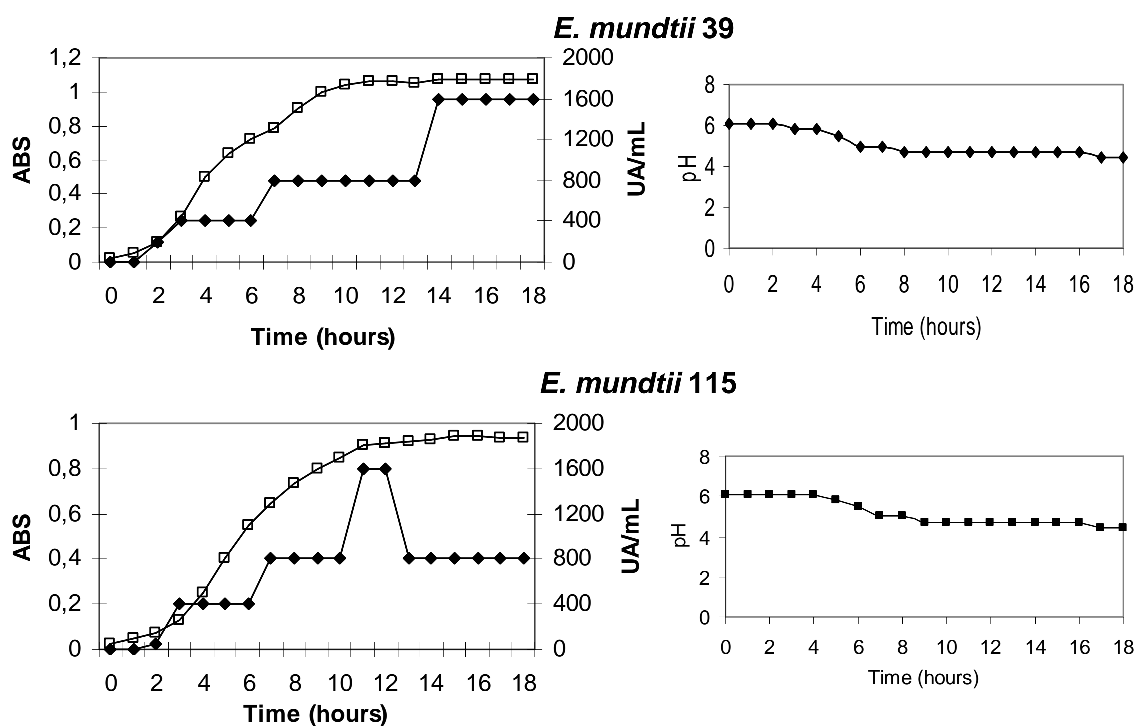

Figure 2 – Growth kinetics of *E. mundtii* 39 and *E. mundtii* 115. Results obtained for antimicrobial activity in AU/mL using the agar well diffusion method and *L. innocua* as the indicator (♦), microbial growth in absorbance (620 nm) ( ) and pH (39 and115) (■), in MRS broth at 35ºC for 18 h.

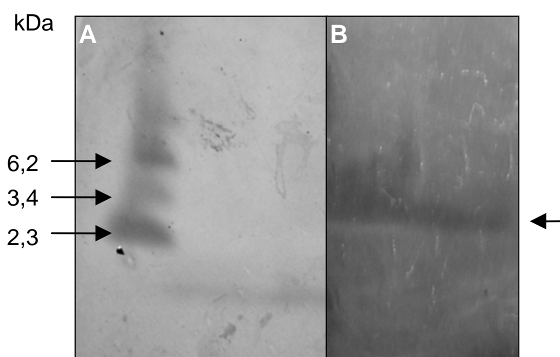

Figure 3 - Characterization of molecular weight of enterocins produced by *E. mundtii* strains 39 and 115. Comassie Blue gel (A) and antimicrobial activity gel (B) using the indicator strain *L. innocua.* The arrows indicate the molecular weight at gel A and the inhibition halo at the gel B.

The enterocins produced by *Enterococcus* sp. typically inhibited Gram-positive bacteria, including the *Listeria* and *Lactobacillus* genera; and this was observed even for the *Enterococcus* genus itself, as reported in the present study. There are only few enterocins that can inhibit Gram-negative bacteria. To our knowledge, the majority of the studies carried out on enterocins have not tested the *Salmonella* genus. The culture supernatants obtained from *E. mundtii* isolates inhibited two *Salmonella* Enteriditis strains. In studies that characterized enterocins as producing antimicrobial activity against *Salmonella* spp., a similar result was found for enterocin 012 produced by *Enterococcus gallinarum*, which inhibited the growth of *Salmonella* Typhimurium

(Jennes et al., 2000; Lauková et al., 1998; 2000; 2004). *Lactobacillus fermentum* was not inhibited by any of the culture supernatants tested, but *L. plantarum* was weakly inhibited. These could be interesting results, as the species mentioned were commonly used in bacteria associations developed as additives to food fermentation processes and probiotic combinations. Todate, only two studies have characterized enterocins produced by *E. mundtii*, and only one of these enterocins was identified and characterized as mundticin (Bennik et al., 1998; Kawamoto et al., 2002). Which inhibited *L. monocytogenes*, *L. plantarum*, and *Enterococcus* strains (Kawamoto et al., 2002). The estimated molecular size of mundticin was 3.4 kDa, which was in accordance with the results found in the present study.

The cross inhibition test revealed that strains 5 and 39 were inhibited by culture supernatant 115, which suggested that this supernatant produced a different enterocin. Yet, in the light of the fact that strain 115 was not inhibited by supernatants 5 and 39, it was possible to conclude that strain 115 was resistant to the supernatants mentioned, and could be producing more than one enterocin.

Enterocins were shown to be consistently stable under high temperatures, different pH ranges, and long storage periods. These characteristics were typical of various enterocins already characterized, such as enterocin A (Aymerich et al., 1996), enterocin B (Casaus et al., 1997), enterocin P (Cintas et al., 1997) produced by *E. faecium*, and enterocin 1071A and 1071B, produced by *E. faecalis* (Balla et al., 2000). The stability exhibited by enterocins under these conditions is a very important feature, since the main use of these peptides lies in biopreservation of fermented foods. The losses in antimicrobial activity after the treatment with proteolytic enzymes reveal the proteic nature of enterocins.

The tests carried out to establish virulence factors and resistance to antimicrobial agents are valuable tools when choosing a safer strain to be used as biopreservative. The four *E. mundtii* strains presented a capsule and produced negative results for hemolysin, gelatinase and adhesin production tests. The resistance profile exhibited by the strains involved between 3 and 5 antibiotics, no strain was resistant to vancomycin. (data not shown).

The kinetics of production showed primary metabolite kinetics, as the production of enterocins started as of the logarithmic growth phase. Such a finding had previously been reported in studies on enterocins (Torri Tarelli et al., 1994; Franz et al., 1996; Mareková et al., 2003). The decrease in antimicrobial activity observed for strain 115 after a longer incubation time could be due to the degradation of the bacteriocin by proteolytic enzymes present in the medium, or else by the low pH (Torri Tarelli et al., 1994). Another aspect to be considered would be the re-adsorption of enterocin by the cell through its membrane in low pH medium (Franz et al., 1996). Similar results have been found by Herranz et al. (2001) for *E. faecium* P21, in which the activity peak was observed after a 12 h incubation period, as of the early stationary phase and which likewise had a final pH of 4.4. Mareková et al. (2003) analyzed the production of enterocins in *E. faecium* strain EK13 at 37°C without pH adjustments in a fermentation vat. The production started within 1 h incubation period, and the peak activity was at the 7th h of incubation (25,600 AU/mL), with the beginning of the stationary phase at 6 h of incubation. The final pH was 4.4 after a 25 h incubation period. The kinetics of the production of *E. faecium* FAIR – E198 revealed a peak activity of 800 AU/mL after a 5 h fermentation period in MRS broth supplemented with 2% glucose at 37°C and pH kept at 6.5. Under the same conditions, but with pH kept at 5.5, a lower activity was determined (200 AU/mL) after a 10 h incubation period (Sarantinopoulos et al., 2002).

Among the enterocins characterized todate, the majority have been ranked under class IIa, with molecular weight under 10 kDa (Eijsink et al., 2002). Most of the enterocins showed their molecular weight between 2 and 5 kDa. Enterocins A and B are those that have been best characterized and are produced by *E. faecium* strains, with molecular weight of 4.8 and 5.4 kDa, respectively. Other enterocins, such as enterocin 32 (produced by *E. faecium*) has a molecular weight of 5 kDa and enterocin P has 4.5 kDa (Ennahar et al., 2000). All these molecular weight figures are over the molecular weight observed at the present study. However, enterocin O12, produced by *E. galinarum* presented a molecular size of 3.4 kDa (Jennes et al., 2000). Similarly, enterocin ON-157, produced by *E. faecium* strain NIAI 157, showed one of the lowest molecular weight characterized (2.5 kDa, Ohmomo et al., 2000).

The results produced in the present study highlighted the remarkable potential as food

biopreservation agents exhibited by these strains and the enterocins they produced. This was observed in the light of the fact that the enterocins and the strains analyzed showed little sensitivity to high temperatures and pH variations. Also, the capacity to inhibit *Listeria monocytogenes* and *Salmonella* Enteritidis — two very important pathogens associated to food poisoning — reinforced the use of *E. mundtii* in food preservation strategies.

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RESUMO

O objetivo do presente estudo foi caracterizar bacteriocinas produzidas por 70 cepas de *Enterococcus mundtii*. Estas foram caracterizadas quanto a sua atividade antimicrobiana, sensibilidade ao aquecimento, pH, armazenamento e enzimas proteolíticas. Foi também determinada sua cinética de produção e peso molecular. Entre as 70 cepas analisadas, quatro apresentaram atividade antibiótica contra *Listeria innocua, L. monocytogenes, Lactobacillus plantarum,* e *Salmonella* Enteritidis. Esta atividade foi mantida em temperaturas até 121ºC por 20 minutos, e sob condições de pH entre 2,0 e 10,0. A atividade antimicrobiana foi mantida nos testes de armazenamento a -20°C, por 60 dias. A cinética de produção revelou picos de atividade de 1600 AU/mL durante a fase logarítmica de crescimento e o peso molecular foi de aproximadamente 3,0 kDa. A caracterização dos produtos com atividade antimicrobiana revelaram suas naturezas protéicas, cinéticas de metabólito primário e peso molecular semelhante aos das enterocinas já purificadas.

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