Characterisation of Antilisterial Bacteriocin-Like Substance Produced by *Enterococcus mundtii*

Kateřina SOLICHOVÁ, Ivana SLOŽILOVÁ, Iva JEBAVÁ, Barbora UHROVÁ and Milada PLOCKOVÁ

Department of Dairy and Fat Technology, Faculty of Food and Biochemical Technology, Institute of Chemical Technology Prague, Prague, Czech Republic

Abstract

Solichová K., Složilová I., Jebavá I., Uhrová B., Plocková M. (2012): **Characterisation of antilisterial bacteriocin-like substance produced by *Enterococcus mundtii*.** Czech J. Food Sci., **30**: 89–97.

The antilisterial activity of *E. mundtii* 1282 strain and its cell-free neutralised supernatant was observed against five persistent *L. monocytogenes* strains using the agar spot method. The enterococcal metabolite was consequently characterised as a proteinaceous substance – the bacteriocin-like substance, which was heat-stable at heating to 100°C for 30 min, stable at pH 2–12, and still active after eight-week long storage at 6°C and –20°C. The bacteriocin-like substance reached the highest activity of 6400 AU/ml after ten hours of cultivation of *E. mundtii* 1282 strain, when it was at the stationary phase of its growth. *E. mundtii* 1282 strain produced the bacteriocin-like substance: in BHI broth with pH 4–6, at the cultivation temperature 12–45°C, and in BHI broth with 1–6% (w/w) NaCl.

Keywords: *Enterococcus*; *enterocin*-like substance; *Listeria monocytogenes*; persistent

Listeria monocytogenes is known to contaminate food processing plants for prolonged periods of time, e.g. in a meat processing plant for 4 years (Nesbakken *et al*. 1996), in a slaughtering plant for 16 months (Rørvik *et al*. 2003), in a fresh sauce industry for 17 months (Pourshaban *et al*. 2000). Significantly extended periods of contamination are typical of dairy industry, e.g. in a cheese plant for 7 years (Unnerstad *et al*. 1996), in an ice cream plant for 7 years (Miettinen *et al*. 1999). This prolonged contamination is often caused by a few dominating strains or persistent strains (Unnerstad *et al*. 1996; Pourshaban *et al*. 2000; Hoffman *et al*. 2003). The occurrence of persistent strains coming from dairy and non-dairy materials is common in the dairy plant environment and, on the contrary, persistent strains are usually not found in raw materials (Miettinen *et al*. 1999). Persistent strains also show remarkably higher adherence to stainless steel surfaces in contrast to non-persistent strains (Chae & Schraft 2000; Lattore *et al*. 2011), and there are also differences in the susceptibility to disinfectants between the persistent and non-persistent *L. monocytogenes* strains (Lundén *et al*. 2008; Purkrtová *et al*. 2010). Variants resistant to nisin A, nisin Z, pediocin PA-1, divergicin M35, and to a bacteriocin-like compound produced by *Bifidobacterium thermophilum* subsp. *infantis* RBL67 were developed from *L. monocytogenes* LSD530. Moreover, the acquired bacteriocin resistance generally decreased its antibiotic sensitivity (Naghmouchi *et al.* 2007). The potency to inhibit the biofilm-forming listerias is important

from the manufacturers point of view, because the persistent strains represent a serious hazard. Unpredictable release of part of the biofilm containing *L. monocytogenes* can contaminate the final product and cause listeriosis after the consumption. This serious disease has emerged as an atypical food-borne illness causing major public health concern because of the severity of the disease (meningitis, septicaemia, and abortion), a high fatality rate (20–30% of cases), a long incubation time, and the predilection for the individuals who have an underlying condition, which leads to the impairment of T-cell-mediated immunity (Aller-berger 2003).

Among the bacteriocins produced by lactic acid bacteria (LAB), nisin, a class I bacteriocin, has demonstrated antilisterial activity and has been the first bacteriocin to be characterised and the only one approved for use in food applications. However, the emergence of nisin-resistant *L. monocytogenes* mutants has been reported (Martínez *et al.* 2005; Naghmouchi *et al.* 2007). Thus, novel bacteriocins of LAB are of interest for their potential use.

The class IIa (or pediocin-like) bacteriocins are a large group of heat-stable antibacterial peptides produced by LAB typified by their potent antilisterial activity (Drider *et al.* 2006), thus the current attention is especially focused on their study and application. Enterocins are bacteriocins produced by enterococci and according to the traditional classification usually belong to the class IIa bacteriocins. The ability of enterocins to inhibit *Listeria* spp. is explained by a close relationship between enterococci and *Listeria* (Moreno *et al.* 2006) and is well-known. Most enterocins are produced by *E. faecium* and *E. faecalis* strains isolated from different food sources (Veljovic *et al.* 2009),

but are produced by other species as well, e.g. *E. mundtii* (Campos *et al.* 2006; Ferreira *et al.* 2007; Settani *et al.* 2008), *E. casseliflavus* (Sabia *et al.* 2002). Some enterococcal bacteriocins can be grouped with typical bacteriocins produced by LAB according to the traditional classification, whereas others are atypical and structurally distinct from the general classes of bacteriocins. These atypical enterocins recently played an important role and prompted reclassification of the class II bacteriocins into a new scheme as suggested by Franz *et al.* (2007).

The aim of this study was to determine the antilisterial activity of an enterocin-like substance (ELS), produced by *Enterococcus mundtii* 1282 strain, against *Listeria monocytogenes* persistent strains, and to test the effects of extrinsic parameters (cultivation temperature, pH, and NaCl addition) on its production, as well as to characterise its technologically important traits (pH and thermal stability, influence of enzymes, stability during storage).

MATERIAL AND METHODS

Microorganisms and media. All microorganisms used are summarised in Table 1 and were cultivated aerobically in BHI broth (Himedia Pvt Ltd, Mumbai, India) at 37°C for 18 hours.

Preparation of culture supernatant. The strain with antilisterial activity was grown in accordance with the optimal conditions of its cultivation as given above. The culture (5 ml) was centrifuged at 3680× g for 15 min at 4°C, the cell-free supernatant was neutralised to pH 6.0–6.5 using NaOH (100 g/l solution) and heated at 90°C for 10 min to inacti-

Table 1. Origin of selected microorganisms

Strain	Source	Characterisation
E. mundtii 1282	VFU Brno, CR	raw goat milk
L. monocytogenes L-2296	Milcom a.s., Prague, CR	persistent, raw cow milk, serovar 1/2a
L. monocytogenes L-2299	Milcom a.s., Prague, CR	persistent, raw cow milk, serovar 1/2a
L. monocytogenes L-2300	Milcom a.s., Prague, CR	persistent, raw cow milk, serovar 1/2a
L. monocytogenes L-2297	Milcom a.s., Prague, CR	persistent, raw cow milk, serovar 1/2a
L. monocytogenes Lm-31*	Milcom a.s., Prague, CR	persistent, manufacturing dairy plant equipment
L. innocua Ln-03	DBM, ICT Prague, CR	bovine brain, NCTC 11288, serovar 6a
L. innocua Ln-06*	DBM, ICT Prague, CR	salad

*serovar not determined yet

vate the remaining cells and enzymes. The cell-free neutralised supernatant (CFNS) was used in further experiments concerning the bacteriocin-like substance characterisation (Franz *et al.* 1996).

Screening of antilisterial activity was performed according Kučerová *et al.* (2007); all experiments were done in BHI soft (7 g/l) agar.

Growth and ELS production. An overnight culture was inoculated (1% v/v) into BHI broth and incubated at 37°C for 18 hours. Every two hours samples were taken and the changes in pH, optical density (615 nm), and counts (CFU/ml) were recorded. The ELS activity (AU/ml) against *L. innocua* Ln-06 in CFNS was determined, as described by Chumchalová *et al.* (1998).

All experiments dealing with the effects of different extrinsic parameters were done in BHI medium and in triplicate, and *L. innocua* Ln-03 and Ln-06 strains were used as indicator strains. The effects of the cultivation temperature (12, 20, 30, 37, and 45°C) and medium pH (pH 2, 3, 4, 5 and 6) were determined according to Ogunbanwo *et al.* (2003a) and the influence of NaCl addition (1, 2, 3, 4, 5, and 6% w/w) into the cultivation medium was estimeted as published by Chumchalová *et al.* (1998).

ELS was characterised by its sensitivity to different enzymes, pH, heat treatment, and storage stability. All experiments were carried out in BHI medium and in triplicate, and *L. innocua* Ln-03 and Ln-06 strains were used as indicator strains. The influence of enzymes was established according to Kang and Lee (2005), the enzymes tested were: catalase, proteinase K (both Sigma Aldrich, St. Louis, USA), protamex (protease; Novo Nordisk A/S, Bagsvaerd, Denmark), flavourzyme (aminopeptidase; Novozymes A/S,

Bagsvaerd, Denmark), protease, α-chymotrypsi, lysozyme (all Sigma Aldrich, St. Louis, USA), and validase (a mixture of fungal lipase and proteases; Valley Research, South Bend, USA). Their concentrations in CFNS were 0.1 and 1.0 mg/ml. Heat stability (5, 15, 20, 30, 40, 50, 60, 70, 80, 90, and 100°C for 10, 30, 60 min, and 121°C/15 min) was determined according to Jennes *et al.* (2000). The influence of the pH (2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12) treatment was tested according to Schillinger *et al.* (1993). The enterocin stability during its storage (−20°C and 4°C) was estimated according to Ogunbanwo *et al.* (2003b).

RESULTS AND DISCUSSION

The *E. mundtii* 1282 strain isolated from goat raw milk had previously shown antilisterial activity against *L. innocua* (12 strains) and *L. monocytogenes* CCM5576 due to the production of ELS (Kučerová *et al.* 2009). Thus, this strain was selected for further experiments. It was revealed that this ELS has a potential to inhibit also five persistent *L. monocytogenes* strains (Table 2 and Figure 1). Due to this fact, this enterocin-like substance could be a promising natural tool for ensuring the safety of hazardous food with typical incidence of *L. monocytogenes* taking into consideration the demand of consumers for chemically untreated foods. The inhibition zones obtained in the tests against both *L. monocytogenes* and *L. innocua* species were almost the same. Other authors also

Table 2. Inhibition of persistent *L. monocytogenes* strains by *E. mundtii* 1282

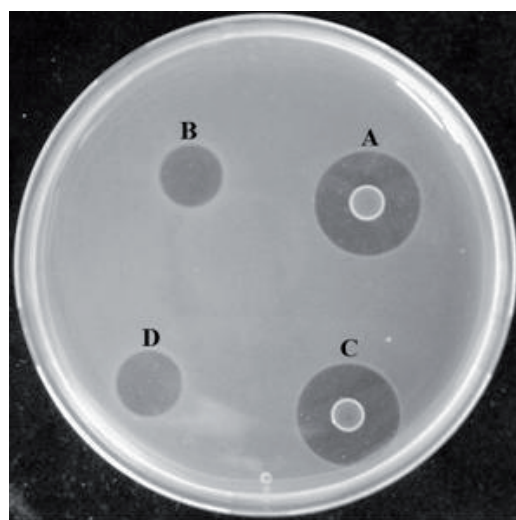| Indicator strain	Diameter of inhibition zone (mm)	
	live cells	CFNS
L-2296	20 ± 1	13 ± 3
L-2299	18 ± 2	13 ± 2
L-2300	21 ± 4	12 ± 1
L-2297	19 ± 2	13 ± 1
Lm-31	20 ± 2	11 ± 1
L. innocua Ln-03	20 ± 1	10 ± 1
L. innocua Ln-06	20 ± 1	14 ± 1

*results published by Kučerová *et al.* (2009)

Figure 1. Antilisterial activity of *E. mundtii* 1282 strain against *L. monocytogenes* L-2292 strain by: A, C – live cells of *E. mundtii* 1282; B, D – CFNS of *E. mundtii* 1282

determined similar sensitivity of different *Listeria* species to a particular enterococcal bacteriocin (Sabia *et al.* 2002; Ferreira *et al.* 2007). Considering these circumstances, *L. innocua* Ln-03 and Ln-06 strains, being less problematic to handle with respect to safety, were used in this study as model strains for specific characterisation of the bacteriocin-like substance instead of pathogenic *L. monocytogenes* strains.

The factors influencing bacteriocin production are most important when using bacteriocinogenic cultures. Bacteriocin production *in situ* may, among others, depend greatly on physicochemical factors (e.g. pH, temperature, a_w, CO_2, O_2, redox potential, time of incubation) as well as on the food-related factors (e.g. the food structure, buffering capacity, composition, additives, antimicrobials, and processing conditions that may indirectly damage bacterial cells, as well as thermal treatments and other treatments intended to reduce the microbial load). Sometimes, at least ten-fold higher bacteriocin concentrations must be added to foods in order to achieve an equivalent inhibitory effect. The efficacy of bacteriocins in foods will greatly depend on the number of food-related factors (e.g. food processing conditions, storage temperature, food pH, and bacteriocin instability at pH changes, inactivation by food enzymes, limited stability of bacteriocin during food shelf life, etc.) that in

Table 3. Effect of selected extrinsic parameters on the production of ELS produced by *E. mundtii* 1282 when tested against *L. innocua* Ln-03 and Ln-06 strains

Growth condition		Inhibition zone (mm)			
		Ln-03		Ln-06	
		live cells	CFNS	live cells	CFNS
Temperature (°C)	12	13 ± 2	6 ± 0	12 ± 1	7 ± 1
	20	19 ± 0	12 ± 1	19 ± 0	12 ± 0
	30	18 ± 1	13 ± 1	20 ± 0	12 ± 1
	37	18 ± 0	12 ± 1	19 ± 0	10 ± 1
	45	12 ± 2	3 ± 2	15 ± 1	7 ± 1
pH	4	0 ± 0	0 ± 0	4 ± 3	3 ± 2
	5	17 ± 0	10 ± 1	18 ± 0	10 ± 2
	6	18 ± 0	12 ± 2	18 ± 1	12 ± 0
NaCl (%, w/w)	1	19 ± 1	9 ± 2	19 ± 0	11 ± 1
	2	16 ± 0	6 ± 0	18 ± 0	7 ± 2
	3	17 ± 0	6 ± 0	18 ± 0	7 ± 0
	4	18 ± 0	7 ± 1	18 ± 0	7 ± 1
	5	18 ± 0	6 ± 0	18 ± 0	8 ± 0
	6	17 ± 0	6 ± 0	18 ± 0	7 ± 0

most cases involve the interaction with the food components, precipitation, inactivation, or uneven distribution of bacteriocin molecules in the food matrix (Gálvez *et al.* 2007).
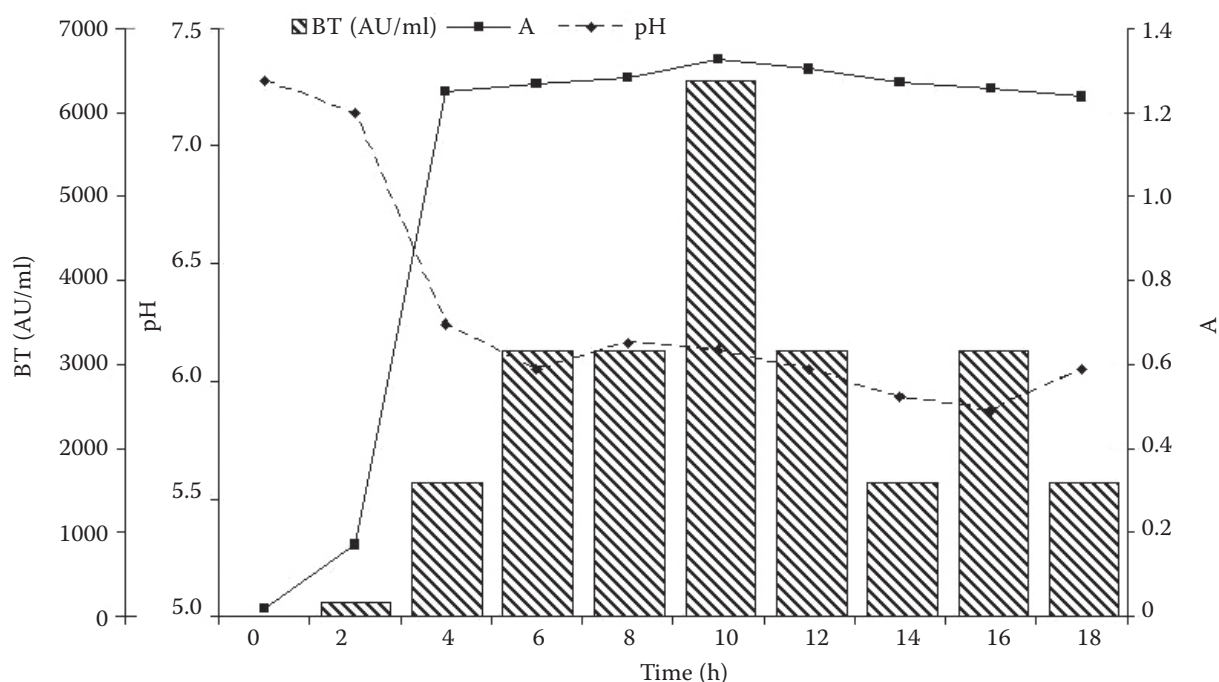

Figure 2. Growth characteristic and bacteriocin titre of *E. mundtii* 1282 strain during its cultivation in BHI broth at 37°C for 18 hours

Table 4. Stability of ELS produced by *E. mundtii* 1282 during its enzyme and pH treatment and storage at temperature −20°C and 6°C when tested against *L. innocua* Ln-03 and Ln-06 strains

Enzyme	Diameter of inhibition zone (mm)			
	Ln-03		Ln-06	
	0.1 mg/ml	1.0 mg/ml	0.1 mg/ml	1.0 mg/ml
Proteinase K	0 ± 0	0 ± 0	0 ± 0	0 ± 0
α-Chymotrypsin	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Protease	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Flavourzyme	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Protamex	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Catalase	7 ± 1	6 ± 1	12 ± 1	11 ± 1
Lysozyme	7 ± 0	6 ± 1	11 ± 1	11 ± 0
Validase	8 ± 1	6 ± 0	11 ± 0	10 ± 0
Days of storage	6°C	−20°C	6°C	−20°C
7	13 ± 0	11 ± 0	11 ± 0	10 ± 0
14	10 ± 0	9 ± 1	10 ± 0	10 ± 0
21	9 ± 1	10 ± 1	11 ± 1	11 ± 0
28	12 ± 0	9 ± 1	11 ± 0	10 ± 0
35	13 ± 1	12 ± 0	11 ± 0	10 ± 1
42	10 ± 0	10 ± 0	14 ± 1	14 ± 0
49	13 ± 0	14 ± 0	13 ± 0	15 ± 1
56	10 ± 1	10 ± 0	14 ± 0	13 ± 0
pH				
2	14 ± 0		17 ± 0	
3	15 ± 1		17 ± 1	
4	17 ± 1		17 ± 1	
5	17 ± 0		17 ± 0	
6	16 ± 1		18 ± 1	
7	17 ± 1		16 ± 0	
8	16 ± 1		17 ± 0	
9	15 ± 1		16 ± 1	
10	15 ± 0		15 ± 0	
11	13 ± 1		14 ± 1	
12	0 ± 0		5 ± 0	
Control*	0 ± 0		0 ± 0	

*distill water adjusted to the relevant pH values (2–12)

During the 18 h growth of *E. mundtii* 1282 in BHI broth at 37 °C, the pH decreased from 7.28 to 6.05. The counts increased from 5.0×10^6 CFU/ml to 1.2×10^9 CFU/ml, with maximum count 9.5×10^9 CFU/ml after 10 h of cultivation. Detectable levels of the ELS were observed after 2 h of the growth (approximately 2.0×10^2 AU/ml) against *L. innocua* Ln-06. Maximal production of ELS (6.4×10^3 AU/ml) was reached after 10 h of cultivation in the stationary phase of growth (Figure 2). This finding is in accordance with the fact that bacteriocin production is usually associated with the late exponential/early stationary growth phase (Moreno *et al.* 2002; Van den Berghe *et al.* 2006).

Table 5. Influence of heat treatment on the stability of ELS produced by *E. mundtii* 1282 when tested against *L. innocua* Ln-03 and Ln-06 strains

Temperature (°C)	Time (min)	Diameter of inhibition zone (mm)		Temperature (°C)	Time (min)	Diameter of inhibition zone (mm)	
		Ln-03	Ln-06			Ln-03	Ln-06
5	10	13 ± 0	13 ± 0	60	10	14 ± 1	14 ± 1
	30	12 ± 0	12 ± 0		30	13 ± 0	13 ± 0
	60	12 ± 1	12 ± 1		60	11 ± 1	11 ± 1
15	10	13 ± 1	13 ± 1	70	10	13 ± 0	13 ± 0
	30	13 ± 1	13 ± 1		30	12 ± 0	12 ± 0
	60	12 ± 1	12 ± 1		60	12 ± 0	12 ± 0
20	10	13 ± 0	13 ± 0	80	10	13 ± 1	13 ± 1
	30	13 ± 1	13 ± 1		30	12 ± 0	12 ± 0
	60	14 ± 1	14 ± 1		60	10 ± 1	10 ± 1
30	10	12 ± 0	12 ± 0	90	10	12 ± 0	12 ± 0
	30	16 ± 0	16 ± 0		30	11 ± 1	11 ± 1
	60	13 ± 0	13 ± 0		60	10 ± 1	10 ± 1
40	10	10 ± 0	10 ± 0	100	10	12 ± 0	12 ± 0
	30	11 ± 0	11 ± 0		30	8 ± 0	8 ± 0
	60	10 ± 1	10 ± 1		60	0 ± 0	0 ± 0
50	10	11 ± 0	11 ± 0	121	15	0 ± 0	0 ± 0
	30	11 ± 1	11 ± 1	Control		10 ± 1	14 ± 0
	60	13 ± 0	13 ± 0				

Control – untreated CFNS

The effects of the selected extrinsic parameters on the ELS production were tested (Table 3) using the indicator strains *L. innocua* Ln-03 and Ln-06. ELS was produced in BHI broth at all temperatures tested. During the cultivation at extreme temperatures (12°C and 45°C), the growth rate of *E. mundtii* 1282 strain decreased whereby ELS was produced in lower amounts in comparison to the cultivation at optimal temperature. In spite of this decline in the production of ELS during cultivation at lower temperatures, the *E. mundtii* 1282 strain could be utilised as a protective agent of ripened cheese in using it as a bacteriocinogenic culture. The growth of *E. mundtii* 1282 strain in BHI broth at pH adjusted to 2 and 3 was completely suppressed. The cultivation in BHI broth at pH 4 significantly reduced the production of ELS. In our case, the highest ELS production was observed in BHI broth at pH 5.0 and 6.0. As regards the salt tolerance, the addition of NaCl (1–6% w/w) into the cultivation medium did not affect the ELS production at all; higher concentrations of NaCl in the medium only slowed down the growth rate.

The highest production of ELS in broth at pH 5.0 and 6.0 and minimal salt influence on its production make *E. mundtii* 1282 strain eligible for the protection of both hard and mould cheeses. ELS produced by *E. mundtii* 1282 was completely inactivated by all proteolytic enzymes tested (proteinase K, α-chymotrypsin, protease, flavourzyme, and protamex) at both enzyme concentrations followed. The antagonistic activity of its CFNS was not inhibited by catalase, which indicated that the inhibition observed was not due to hydrogen peroxide. CFNS was also minimally inactivated by lysozyme and validase, which suggests that the peptide did not contain or require a lipid or carbohydrate moieties for the activity, however, more lipolytic and saccharolytic enzymes have to be tested to confirm this finding (Table 4). The complete loss of this enterocin inhibitory potential after the proteolytic enzymes treatment can complicate its utilisation as a biopreservative in food in view of its protein/proteolytic enzymes content. The bacteriocins sensitivity to proteolytic enzymes is common (Kang & Lee 2005; Ghrairi

et al. 2008; Pinto *et al.* 2009) and is related with bacteriocins definition. On the other hand, our ELS could be probably inactivated in human alimentary tract after the consumption of biopreserved foods with no impact on natural enteric microflora. The ELS activity produced by *E. mundtii* 1282 was not affected by the pH treatment (Table 4) at the values of 2–11. Only the extreme pH 12 caused a decrease of its activity against *L. innocua* Ln-06 and complete loss of its activity against *L. innocua* Ln-03. This significant pH-stability allows the application of our ELS as biopreservative in the wide range of foods, and especially the fermented food. As far as the storage-stability is concerned, the stability of *E. mundtii* 1282 ELS was not affected by eight-week long storage at –20°C and 4°C (Table 4). The ability of our ELS to retain its activity during storage suggests it as a suitable biopreservative. The ELS of *E. mundtii* 1282 was not inhibited by any of the applied heat treatments except for 100°C/60 min and 121°C/min (Table 5). After the heat treatment at 100°C/30 min, the ELS activity only decreased. This finding enables e.g. the addition of our ELS directly into the milk for cheese manufacture before pasteurisation without any effect on its activity.

The antilisterial ELS produced by *E. mundtii* 1282 showed typical properties of enterocins, e.g. maximal production during the cultivation of producing strains at their optimal temperature (Aymerich *et al.* 2000; Annamalai *et al.* 2009) and neutral pH (Du Toit *et al.* 2000). Also the noticeable pH stability (Ghrairi *et al.* 2008; Pinto *et al.* 2009), heat-stability (Kawamoto *et al.* 2002; Campos *et al.* 2006), and storage-stability (Sabia *et al.* 2002; Ferreira *et al.* 2007) are typical traits of enterocins. Taking into consideration the attributes of this new ELS produced by *E. mundtii* 1282 strain, the crucial benefit resides in its activity against the persistent *L. monocytogenes* strains and its suitability for the protection of smear-cheese that are often contaminated by this pathogen.

CONCLUSIONS

E. mundtii 1282 strain with previously described antilisterial activity inhibited five persistent *L. monocytogenes* strains. This strain produced a long storage-heat- and pH-stable ELS, which was deactivated by all proteolytic enzymes tested. The tested extrinsic parameters, that allowed simultaneous growth of the strain, had minimal influence on its production. Based on the results obtained, this compound will be purified in the future and used in laboratory conditions for studying its protective effect in smear-cheese model system with persistent *L. monocytogenes* strains as indicators.

References

Allerberger F. (2003): *Listeria*: growth, phenotypic differentiation and molecular microbiology. FEMS Immunology and Medical Microbiology, **35**: 183–189.

Annamalai N., Manivasagan P., Balasubramanian T., Vijayalakshmi S. (2009): Enterocin from *Enterococcus faecium* isolated from mangrove environment. African Journal of Biotechnology, **8**: 6311–6316.

Aymerich T., Artigas M.G., Garriga M., Monfort J.M., Hugas M. (2000): Effect of sausage ingredients and additives on the production of enterocins A and B by *Enterococcus faecium* CTC492. Optimization of *in vitro* production and anti-listerial effect in dry fermented sausages. Journal of Applied Microbiology, **88**: 686–694.

Campos C.A., Rodríquez Ó., Calo-Mata P., Prado M., Barros-Velázquez J. (2006): Preliminary characterization of bacteriocins from *Lactococcus lactis*, *Enterococcus faecium* and *Enterococcus mundtii* strains isolated from turbot (*Psetta maxima*). Food Research International, **39**: 365–364.

Chae M.S., Schraft H. (2000): Comparative evaluation of adhesion and biofilm formation of different *Listeria monocytogenes* strains. International Journal of Food Microbiology, **62**: 103–111.

Chumchalová M., Josephsen J., Plocková M. (1998): The antimicrobial activity of acidocin CH5 in MRS broth and milk with added NaCl, $NaNO_3$ and lysozyme. International Journal of Food Microbiology, **43**: 33–38.

Drider D., Fimland G., Hechard Y., McMullen L.M., Prevost H. (2006): The continuing story of class IIa bacteriocins. Microbiology and Molecular Biology Reviews, **70**: 564–582.

Du Toit M., Franz C.M.A.P., Dicks L.M.T., Holzapfel W.H. (2000): Preliminary characterization of bacteriocins produced by *Enterococcus faecium* and *Enterococcus faecalis* isolated from pig faeces. Journal of Applied Microbiology, **88**: 482–494.

Ferreira A.E., Canal N., Morales A., Bopp Fuentefria D., Corção G. (2007): Characterization of enterocins produced by *Enterococcus mundtii* isolated from humans feces. Brazilian Archives of Biology and Technology, **50**: 249–258.

Franz C.M.P.A., Schillinger U., Holzapfel W.H. (1996): Production and characterization of enterocin 900, a bacteriocin produced by *Enterococcus faecium* BFE 900 from black olives. International Journal of Food Microbiology, **29**: 255–270.

Franz C.M.A.P., van Belkum M.J., Holzapfel W.H., Abriouel H. (2007): Diversity of enterococcal bacteriocins and their grouping in a new classification scheme. FEMS Microbiological Reviews, **31**: 293–310.

Gálvez A., Abriouel H., López R.L., Ben Omar N. (2007): Bacteriocin-based strategies for food biopreservation. International Journal of Food Microbiology, **120**: 51–70.

Ghrairi T., Frere J., Berjeaud J.M., Manai M. (2008): Purification and characterisation of bacteriocins produced by *Enterococcus faecium* from Tunisian rigouta cheese. Food Control, **19**: 162–169.

Hoffman A.D., Gall K.L., Norton D.M., Wiedmann M. (2003): *Listeria monocytogenes* contamination patters for the smoked fish processing environment and for raw fish. Applied and Environmental Microbiology, **66**: 52–60.

Jennes W.; Dicks L.M.T.; Verwoerd D.J. (2000): Enterocin 012, a bacteriocin produced by *Enterococcus gallinarum* isolated from the intestinal tract of ostrich. Journal of Applied Microbiology, **88**: 349–357.

Kang J.H., Lee M.S. (2005): Characterization of a bacteriocin produced by *Enterococcus faecium* GM-1 isolated from an infant. Journal of Applied Microbiology, **98**: 1169–1176.

Kawamoto S., Shina J., Sato R., Eguchi T., Ohmomo S., Shibato J., Horikoshi N., Takeshita K., Sameshima T. (2002): Biochemical and genetic characterization of mundticin KS, an antilisterial peptide produced by *Enterococcus mundtii* NFRI 7393. Applied and Environmental Microbiology, **68**: 3830–3840.

Kučerová K., Chumchalová J., Míková K., Cupáková Š., Karpíšková R., Ho L. (2007): Screening of lactic acid bacteria for antimicrobial properties from mayonnaise-based products and raw materials. European Food Research and Technology, **226**: 265–272.

Kučerová K., Korbová I., Horáčková Š., Šviráková E., Plocková M. (2009): Influence of enterocci and lactobacilli on *Listeria*. Czech Journal of Food Sciences, **27** (Special Issue 2): S2-12–S2-17.

Latorre A.A., Van Kessel J.A.S., Karns J.S., Zurakowski M.J., Pradhan A.K., Boor K.J., Adolph E., Sukhnanand S., Schukken Y.H. (2011): Molecular ecology of *Listeria monocytogenes*: Predominant and persistent strains showed increased *in-vitro* adherence and on-farm persistence in the milking system. Applied and Environmental Microbiology, **77**: 3676–3684.

Lundén J., Tolvanen R., Korkeala H. (2008): Acid and heat tolerance of persistent and nonpersistent *Listeria*

monocytogenes food plant strains. Letters in Applied Microbiology, **46**: 276–280.

Martínez B., Bravo D., Rodriguez A. (2005): Consequences of the development of nisin-resistant *Listeria monocytogenes* in fermented dairy products. Journal of Food Protection, **68**: 2383–2388.

Miettinen M.K., Björkroth K.J., Korkeala H.J. (1999): Characterization of *Listeria monocytogenes* from an ice-cream plant by serotyping and pulsed-field gel electrophoresis. International Journal of Food Microbiology, **46**: 187–192.

Moreno M.R.F., Leisner J.J., Tee L.K., Ley C., Radu S., Rusul G., Vancanneyt M., De Vuyst L. (2002): Microbial analysis of Malaysian tempeh, and characterization of two bacteriocins produced by isolates of *Enterococcus faecium*. Journal of Applied Microbiology, **92**: 147–157.

Moreno M.R.F., Sarantinopoulos P., Tsakalidou E., Vuyst L.D. (2006): The role and application of enterococci in food and health. International Journal of Food Microbiology, **106**: 1–24.

Nagmouchi K., Kheadr E., Lacroix C., Fliss I. (2007): Class I/Class IIa bacteriocin cross-resistence phenomenom in *Listeria monocytogenes*. Food Microbiology, **24**: 718–727.

Nesbakken,T., Kapperud G., Caugant D.A. (1996): Pathways of *Listeria monocytogenes* contamination in the meat processing industry. International Journal of Food Microbiology, **31**: 161–171.

Ogunbanwo S.T., Sanni A.I., Onilude A.A. (2003a): Influence of cultural conditions on the production of bacteriocin by *Lactobacillus brevis* OG1. African Journal of Biotechnology, **2**: 179–184.

Ogunbanwo S.T., Sanni A.I., Onilude A.A. (2003b): Characterization of bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OG1. African Journal of Biotechnology, **2**: 219–227.

Pinto A.L., Fernandes M., Pinto C., Albano H., Castilho F., Teixeira P., Gibbs P.A. (2009): Characterization of anti-*Listeria* bacteriocins isolated from shellfish: Potential antimicrobials to control non-fermented seafood. International Journal of Food Microbiology, **129**: 50–58.

Pourshaban M., Gianfranceschi M., Gattuso A., Menconi F., Aureli P. (2000): Identification of *Listeria monocytogenes* contamination sources in two fresh sauce production plants by pulsed-field gel electrophoresis. Food Microbiology, **17**: 393–400.

Purkrtová S., Turoňová H., Pilchová T., Demnerová K., Pazlarová J. (2010): Resistence of *Listeria monocytogenes* biofilms to disinfectant. Czech Journal of Food Sciences, **28**: 326–332.

Rørvik L.M., Aase B., Alvestad T., Caugant D.A. (2003): Molecular epidemiological survey of *Listeria monocy-*

togenes in broilers and poultry products. Journal of Applied Microbiology, **94**: 633–640.

Sabia C., Manicardi G., Messi P., de Niederhausern S., Bondi M. (2002): Enterocin 416K1, an antilisterial bacteriocin produced by *Enterococcus casseliflavus* IM 416K1 isolated from Italian sausages. International Journal of Food Microbiology, **75**: 163–170.

Schillinger U., Stiles M.E., Holzapfel W.H. (1993): Bacteriocin production by *Carnobacterium pisciola* LV 61. International Journal of Food Microbiology, **20**: 131–147.

Settanni L., Valmorri S., Suzzi G., Corsetti A. (2008): The role of environmental factors and medium composition on bacteriocin-like inhibitory substances (BLIS) production by *Enterococcus mundtii* strains. Food Microbiology, **25**: 722–728.

Unnerstad H., Bannerman E., Bille J., Danielsson-Tham M-L., Waak E., Tham W. (1996): Prolonged contamination of a dairy with *Listeria monocytogenes*. Netherlands Milk and Dairy Journal, **50**: 493–499.

Van den Berghe E., De Winter T., De Vuyst L. (2006): Enterocin A production by *Enterococcus faecium* FAIR-E 406 is characterised by a temperature- and pH-dependent switch-off mechanism when growth is limited due to nutrient depletion. International Journal of Food Microbiology, **107**: 159–170.

Veljovic K., Fira D., Terzic-Vidojevic A., Abriouel H., Galvez A., Topisirovic L. (2009): Evaluation of antimicrobial and proteolytic activity of enterococci isolated from fermented products. European Food Research and Technology, **230**: 63–70.

Corresponding author:

Ing. Kateřina Solichová, Ph.D., Vysoká škola chemicko-technologická v Praze, Fakulta potravinářské a biochemické technologie, Ústav technologie mléka a tuků, Technická 5, 166 28 Praha 6, Česká republika
tel. + 420 220 443 275, e-mail: katerina.solichova@vscht.cz

CZECH ACADEMY OF AGRICULTURAL SCIENCES

Těšnov 17, 117 05 Prague 1, Czech Republic
workplace: Slezská 7, 120 00 Prague 2, Czech Republic
e-mail: redakce@cazv.cz
Tel.: + 420 227 010 427, Fax: + 420 227 010 116

Account No. 86634011/0100 KB
IBAN CZ41 0100 0000 0000 8663 4011; SWIFT KOMBCZPPXXX

The Czech Academy of Agricultural Sciences publishes scientific journals dealing with the problems of agriculture, forestry and food sciences and technology. The periodicals are published in English.

Journal	Number of issues per year	Yearly subscription in USD
Plant, Soil and Environment	12	540
Czech Journal of Animal Science	12	660
Agricultural Economics (Zemědělská ekonomika)	12	540
Journal of Forest Science	12	480
Veterinární medicína (Veterinary Medicine – Czech)	12	720
Czech Journal of Food Sciences	6	420
Plant Protection Science	4	140
Czech Journal of Genetics and Plant Breeding	4	160
Horticultural Science	4	160
Research in Agricultural Engineering	4	140
Soil and Water Research	4	140

Subscription to these journals be sent to the above-mentioned address.