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DIFFERENT SENSITIVITY OF VARIOUS SEROTYPES OF *LISTERIA MONOCYTOGENES* TO LACTIC ACID BACTERIA BACTERIOCINS

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In this study the sensitivity of various serotypes of Listeria monocytogenes towards five lactic bacteria bacteriocins was investigated, at two incubation temperatures (37°C for 24 h and 4°C for 12 days); six serotypes were indentified in 50 clinical/human isolates of L. monocytogenes and eight among 48 isolates from foodstuffs. This microorganism was found in many foodstuffs in numerous studies during the last decade. Among the methods for typing Listeria, the greatest attention has been dedicated to serological typing, which defines the basic characteristics of Listeria antigens. Namely, it is well known that bactericideal or bacteriostatic effects of bacteriocins are not only expressed towardse closely related bacterial species, but also towards less closely related Gram positive bacteria, such as L. monocytogenes. Bacteriocins can be eventually added to food, with the aim of decreasing the risk of listeriosis to the minimum. It was discovered that the bacteriocins, originating from Lactococcus UW and Lactobacillus sake 148 did not express inhibitory effects on any Listeria serotypes, while those bacteriocins originating from Lactobacillus sake 265, Pediococcus 347 and Lactobacillus sake 706 had a listericidal effect towards almost every assessed serotype. The highest bactericid effect was expressed by bacteriocin towards serotypes 4c and 4, at 37°C after 24h incubation and towards serotypes 1/2b and 4b after 12 days incubation at 4°C. Thus, the incubation temperature and time influenced the inhibitory effects of bacteriocins.

Key words: inhibition zone, lactic acid bacteria bacteriocins, Listeria monocytogenes, serotyping

INTRODUCTION

Lactic acid bacteria (LAB), generally considered as “food-grade” organisms, show special promise for selection and implementation as protective cultures. Practical applications of protective cultures concern particular food commodities that either constitute novel systems with respect to packing and/or composition, or represent special hygienic risks. It was concluded that these bacteria offer an additional (and acceptable) processing parameter for improving the safety and assuring the quality of a given food. They achieve their protective

role by competition for food and/or by producing bacteriocins and other antimicrobial substances (Klaenhammer, 1993), which have bactericidal and bacteriostatic effects, usually towards closely related bacterial species (Jack *et al*., 1995). However, it was found that they may also have a destructive effect on some, not so closely related, Gram positive bacterial species, including *L. monocytogenes* (Muriana, 1996; Dimitrijević, 1998), the cause of listeriosis. Food of animal origin is often a carrier of the infection (Farber,1991; Farber and Peterkin, 1991; Dimitrijević and Teodorović, 1998).

Great epidemics of listeriosis in humans have happened after consumption of food of plant origin, milk, dairy products, and meat and meat products (Bader, 1993). In France, after consumption of pork tongue in gelatin, an epidemic of listeriosis, encompassed 279 cases; 63 of which were lethal and 22 with miscarriage (WHO, 1993). The ubiquitous nature of *Listeria monocytogene*s makes total exclusion of the initial contamination in the foodstuff very unreal (Gahan, 1991). This emphasises the extraordinary importance of understanding factors which may reducte its numbers in foodstuffs (Crawford, 1989). Taking into account that *L. monocytogenes* is a cause of serious human disease, for which the minimal infective dose is not yet established, and its ubiquitous nature and wide spread distribution in foodstuffs, it would be very interesting to assess its sensitivity to lactic acid bacteria bacteriocins, which can eventually be added to food (Eckner, 1992;). When bacteriocin combinations were tested in a meat system, the results indicated that more than one LAB bacteriocin in combination may be effective in preventing the spontaneous emergence of a bacteriocin-resistant *Listeria* population (Vignolo, 2000).

For the purpose of identification of infection for epidemiological-epizootological studies, among all the methods for typing of *L. monocytogenes*, greatest attention was dedicated to serological typing, which defines the basic characteristics of Listeria antigens. Many authors have developed reference methods for serotyping listeria, and have presented the basic characteristics of antigens that are present in *Listeria spp.* (Ralovich, 1984; Seeliger and Jones, 1986; Seeliger, 1987). There are 16 serotypes of *L. monocytogenes* and related species, defined by various combinations of somatic and flagellar antigens.

Much research has focused on the problems of why most food – borne listeriosis outbreaks have been caused by *L. monocytogenes* serovar 4b, rather than serovars ½ a and ½ b (McLauchlin, 1987; Rocourt, 1994), although the majority of *L. monocytogenes* isolates from foods belong to serogroup 1 (Autio *et al*., 1990; Ojeniyi *et al*., 1999.). The influece of lactic acid bacteria bacteriocins on growth of nine serotypes of *L. monocytogenes* was investigated in this work.

MATERIALS AND METHODS

A collection of 98 isolates of *L. monocytogenes* consisted of 50 clinical/human and 48 isolates from foodstuffs of animal origin. Clinical/human serotypes were isolated from blood, liquor, infected human feces (carriers or ill persons). Serotypes were isolated and identified from foodstuffs of animal origin by the McLain and Lee method (1989). *L. monocytogenes* was typed by slide

agglutination with antiserum to serogroup 1 and 2 (Difco, MI, USA), and further sub-serotyping was performed in the Pasteur Institute in Paris (Institute Pasteur, Centre National de Reference des Listeria, Centre Collaborateur de 1 OMS pour la listeriose d origine alimentarie, Paris, France) by the methods of Jocelyne Rocourt. Bacteriocins originated from the following lactic acid bacteria: *Lactobacillus sake 148*, *Lactococcus UW, Lactobacillus sake 265, Pediococcus 347* and *Lactobacillus sake 706*. Growth inhibition of the various serotypes of *L.monocytogenes*, under the influence of the selected bacteriocins, was assessed by the method of radial diffusion in agar. Each serotype was examined three times, so the statistical analysis was based on three values for every serotype, which reduces the effect of differences in agar thickness. Two incubation temperatures were used: 37°C, for 24 hours and 4°C for 12 days. For every serotype, three inhibition values were noted (expressed in millimeters), and they are presented as mean values in the tables. The statistical significance of differences in the obtained results between the experimental groups was determined by the random plan method using the statistical software pack, Statgraphics 5.0 (Statistical Graphic Corporation USA).

RESULTS AND DISCUSSION

All isolates of clinical/human origin were sorted in to six serotype groups, while the isolates from the foodstuffs belonged to eight serotype groups. The highest number of isolates, regardless of the origin, contained serotype *1/2a*, while the other serotypes differed depending whether they were found in clinical/human or foodstuff isolates (Figure 1). It was documented that the bacteriocins, originating from Lactococcus UW and Lactobacillus sake 148 did

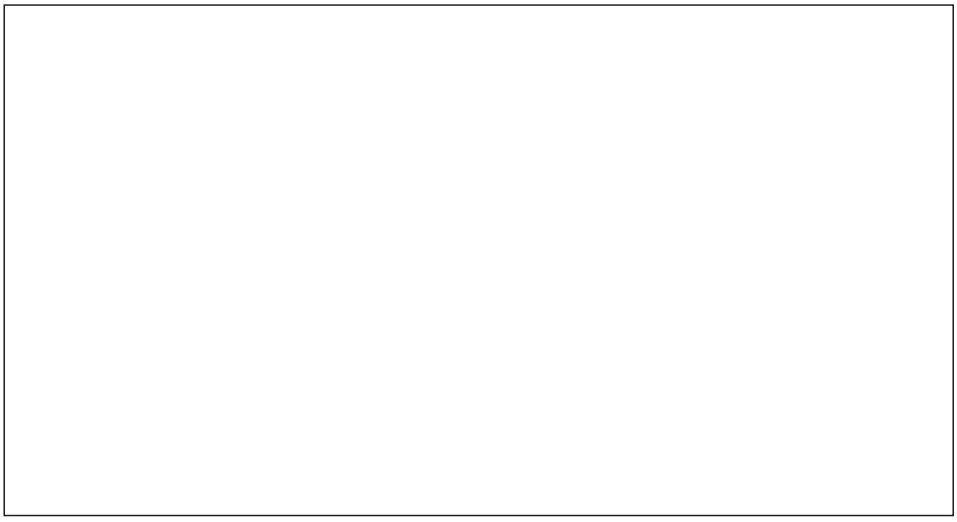

Figure 1. Proportion of different serotypes of L. monocatogenes found in isolates from two sources.

not express inhibitory effects on any *Listeria* serotypes, while those bacteriocins originating from *Lactobacillus sake* 265, *Pediococcus* 347 and *Lactobacillus sake* 706 had a listericidal effect toward almost every assessed serotype.

There have been many examinations of the sensitivity of *L. monocytogenes* serotypes to bacteriocins (Rasch and Knochel, 1998; Dimitrijević, 1999), but up to now sensitivity to bacteriocins could not be correlated with the MEE type (Larsen and Norrung, 1993), or serovar (Fereira and Lund, 1996; Rasch and Knochel, 1998; Dimitrijevic, 1999; Buncic *et al*., 2001) The sensitivity of our serotypes of *L. monocytogenes* towards some bacteriocins was assessed at 37°C for 24h and during cooling at 4°C for 12 days. It was found that the serotypes of *L. monocytogenes* were more or less sensitive to the examined bacteriocins regardless of their origin. Thus, during incubation at 37°C for 24h, bacteriocins expressed the highest inhibitory effect towards serotypes 4c and 4 (Table 1).

Table 1. Sensitivity of various serotypes of *L.monocytogenes* at 37°C/24h to all bacteriocins of the examined lactic acid bacteria

Serotype	n	$\bar{X}$	SD	SY	CV	IV
1	180	2.00	1.69	0.13	84.50	0-6.5
1/2a	555	1.87	1.37	0.06	73.26	0-6.5
1/2b	150	2.28	1.47	0.12	64.47	0-5.5
1/2c	45	1.64	0.93	0.14	56.36	0-3.5
3a	15	2.10	0.93	0.24	44.28	1-4
3b	15	2.00	1.41	0.36	70.50	0-4
4	90	2.89	1.99	0.21	68.86	0-9
4b	405	2.09	1.36	0.07	65.07	0-6.5
4c	15	2.97	1.11	0.29	37.37	1.5-5

n ... number of data
$\bar{X}$ ... average value (mm)
SD ... standard deviation
SY ... standard error
CV ... ceficient of variation
IV ... interval of variation

It was found, using the *Lsd test* that there was a statistically very significant difference between the arithmetical mean values for serotypes *4c* and *1/2c* ($p<0.01$), regarding their sensitivity towards the assessed bacteriocins (Table 2).

Statistically significant differences were also found at the same temperature ($p<0.05$) between mean of inhibition zones of serotypes *4c* and *1/2a*, as well as *4* and *1/2c and 4* and *1/2a*. It is obvious that antigenic structure plays an important role in relation to the sensitivity of *L. monocytogenes* toward the bacteriocins of the lactic acid bacteria, during incubation at 37°C for 24h.

When the same serotypes were assessed in an identical manner during cooling at 4°C for 12 days, the obtained values were almost double (Figure 2).

Table 2. Statistical significance of differences in sensitivity to bacteriocins between various serotypes of *L.monocytogenes* grown at 37°C for 24h

Sero-type	$\bar{X}$ (mm)	1/2c	1/2a	3b	1	4b	3a	1/2b	4	4c	4
4c	2.97	1.33**	1.10*	0.97	0.97	0.88	0.87	0.69	0.08	-	-
4	2.89	1.25*	1.02*	0.89	0.89	0.80	0.79	0.61	-	-	-
1/2b	2.28	0.64	0.41	0.28	0.28	0.19	0.18	-	-	-	-
3a	2.10	0.46	0.23	0.10	0.10	0.01	-	-	-	-	-
4b	2.09	0.45	0.22	0.09	0.09	-	-	-	-	-	-
1	2.00	0.36	0.13	0.00	-	-	-	-	-	-	-
3b	2.00	0.36	0.13	-	-	-	-	-	-	-	-
1/2a	1.87	0.23	-	-	-	-	-	-	-	-	-
1/2c	1.64	-	-	-	-	-	-	-	-	-	-

** $p<0.01$ (significance at 99%) * $p<0.05$ (significance at 95%)

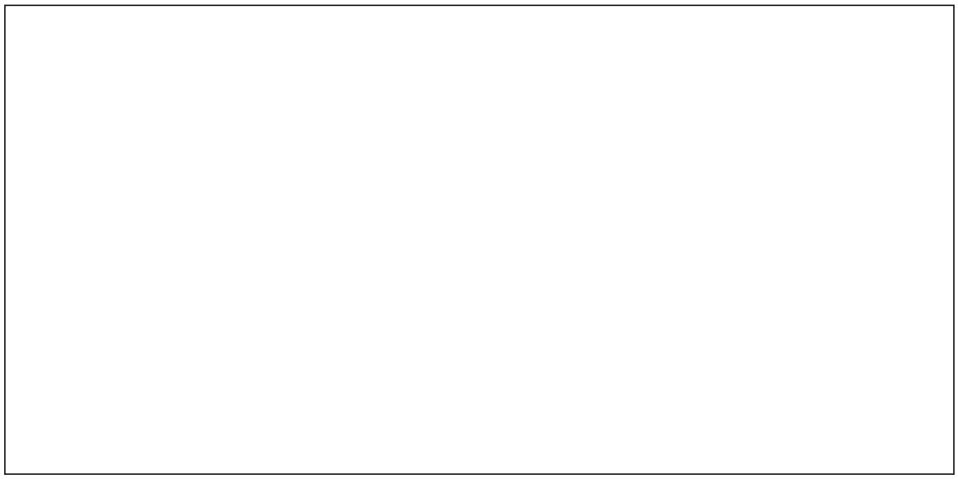

Figure 2. Sensitivity of *L. monocytogenes* serotypes to lactic acid bacteria bacteriocins at two incubation temperatures

All the serotypes were more or less sensitive towards the tested bactericins, as in the previous case. The reactions of the *L. monocytogenes* isolates to the bacteriocins were variable within serovars, which corresponds to previous reports that different *L. monocytogenes* isolates do not behave uniformly when exposed to bacteriocins and significant strain diversity with respect to bacteriocin sensitivity exists (Ferreira and Lund, 1996; Rasch and Knochel,1998). Serotypes

1/2b and 4b expressed the highest sensitivity, while the least sensitivity was shown by serotypes 1/2a, e.g. 1/2c. The Lsd test indicated statistically very significant differences ($p<0.01$) between all the serotypes in relation to serotype 1/2c, except for ½ a where the probability was $p<0.05$ (Table 3).

Table 3. Statistical significance of differences in sensitivity to bacteriocins between various serotypes of *L.monocytogenes* grown at 4°C for 12 days

Sero-type	$\bar{X}$	1/2c	1/2a	3a	3b	4	1	4c	4b	1/2b	4
1/2b	6.99	2.91**	1.86*	1.56**	1.52**	1.20*	1.01*	0.66	0.52	-	-
4b	6.47	2.39**	1.34**	1.07*	1.00*	0.68	0.49	0.14	-	-	-
4c	6.33	2.25**	1.20*	0.90	0.86	0.54	0.35	-	-	-	-
1	5.98	1.90**	0.85	0.55	0.51	0.19	-	-	-	-	-
4	5.79	1.71**	0.66	0.36	0.32	-	-	-	-	-	-
3b	5.47	1.39**	0.34	0.04	-	-	-	-	-	-	-
3a	5.43	1.35**	0.30	-	-	-	-	-	-	-	-
1/2a	5.13	1.05*	-	-	-	-	-	-	-	-	-
1/2c	4.08	-	-	-	-	-	-	-	-	-	-

** $p<0.01$ (significance at 99%) * $p<0.05$ (significance at 95%)

Serotype 1/2b expressed statistically very significant differences in sensitivity from all serotypes except 4c and 4b. A statistically very significant difference in sensitivity was found for the serotype *4b* ($p<0.01$) in relation to serotype 1/2a, as well as statistically significant differences ($p<0.05$) from serotypes 3a and 3b. Serotype 4c, beside the statistically very significant difference ($p<0.01$) in relation to serotype 1/2c, exhibited significant difference ($p<0.05$) from serotype 1/2a.

According to these results, it can be concluded that the sensitivity of various isolates of *L.monocytogenes* is determined by their antigenic structure and they are generally more sensitive during cooling at 4°C for 12 days, than at 37 °C. A possibility of application of suitable antilisteria factors (bactericins, temperature, and others) with the aim of preventing possible infections, could be predicted. Use of starter cultures with lactic acid bacteria (producing bacteriocins) in meat products, may benefit public health and decrease the risk of Listeria food poisoning (Campanini, 1993).

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ZNAČAJ RAZLIČITIH SEROTIPOVA *LISTERIA MONOCYTOGENES* U POGLEDU OSETLJIVOSTI PREMA BAKTERIOCINIMA MLEČNOKISELINSKIH BAKTERIJA

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SADRŽAJ

U cilju identifikacije izvora infekcije u epidemiološko-epizotooloških istraživanjima, razvijene su brojne tehnike tipizacije Listerija u hrani. Od svih metoda tipizacije, najveća pažnja se posvećuje seološkoj tipizaciji, kojom se definišu osnovne karakteristike antigena Listerija. Poznato je baktericidno ili bakteriostatsko dejstvo bakteriocina ne samo prema srodnim bakterijskim vrstama, već i prema manje srodnim Gram pozitivnim bakterijama, kao što je *L. monocytogenes,* te bi se oni eventualno mogli dodavati u hranu, u cilju smanjenja opasnosti od pojave listerioze na minimum. U ovom radu su prikazani rezultati ispitivanja osetljivosti različitih serotipova *Listeria monocytogenes* prema pet bakteriocina mlečnokiselinskih bakterija na dve temperature inkubacije (37 ^{o}C/24 h i 4 ^{o}C/12 dana). Od 50 kliničkih humanih izolata izdvojeno je 6 serotipova, dok je od 48 izolata poreklom iz namirnica animalnog porekla ustanovljeno 8 serotipova. Ustanovljeno je da bakeriocini poreklom od *Lactobacillus sake 148* nisu ispoljili inhibitorni efekat ni prema jednom serotipu listerija, dok su bakteriocini poreklom od *Lactobacillus sake 265, Pediococcus 347* i *Lactobacillus sake 706* imali listericidni efekat skoro prema svim ispitivanim serotipovima. Bakteriocini su ispoljili najveći inhibitorni efekat prema serotipovima 4c i 4, pri inkubaciji od 37 ^{o}C/24h i prema serotipovima 1/2b i 4b, pri inkubaciji na 4 ^{o}C/12 dana. Takođe je ustanovljeno da je temperatura inkubacije uticala na inhibitorne efekte bakteriocina.