

CAN ELECTROPHORETIC TYPES OF *LISTERIA MONOCYTOGENES* INDUCE DIFFERENT SENSITIVITY TO LACTIC ACID BACTERIA BACTERIOCINS?

DIMITRIJEVIĆ MIRJANA, TEODOROVIĆ V., KARABASIL N., TEODOROVIĆ RADISLAVA
and BUNČIĆ OLIVERA

Faculty of Veterinary Medicine, Belgrade

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*The aim of this study was to find if electrophoretic types (ETs) of *Listeria monocytogenes*, typed by multilocus enzyme electrophoresis (MEE), can induce different sensitivity to lactic acid bacteria bacteriocins. Bacteriocins are extracellular peptides or protein molecules, produced by lactic acid bacteria, which not only have bactericidal or bacteriostatic effects, on usually closely related bacterial strains, but also they may have destructive effects on some not so closely related Gram positive bacteria, for example *Listeria monocytogenes*.*

*Listeria monocytogenes is commonly found in the intestines of humans and animals, in milk, soil, leafy vegetables and in food processing environments. These bacteria have been isolated in a variety of foods, including raw and cooked poultry, meat, seafood, salads and sandwiches. Many techniques for typing of *Listeria monocytogenes* in foodstuffs, have been developed for the purpose of identification of the origin of infection for epidemiological and epizootological studies.*

*Among the 98 examined isolates of *Listeria monocytogenes* (50 clinical/human and 48 from food of animal origin) 32 electrophoretic types have been detected. Bacteriocins, which we have used in the study, originated from the following lactic acid bacteria: *Lactobacillus sake* 148, *Lactococcus* UW, *Lactobacillus sake* 706, *Pediococcus* 347 and *Lactobacillus sake* 265. In this study, on the basis of a dendrogram, our results indicate that a reliable relationship between genetic distance of *Listeria monocytogenes* electrophoretic types and their sensitivity to lactic acid bacteria bacteriocins cannot be found. MEE may, however, be of future benefit in establishing links between isolates from human disease cases and thus be useful in establishing the epidemiology of not only sporadic cases, but of outbreaks of listeriosis, as well.*

*Key words: *Listeria monocytogenes*, multilocus enzyme electrophoresis, lactic acid bacteria bacteriocins, electrophoretic types.*

INTRODUCTION

Listeria monocytogenes are foodborne bacteria that enter the intestines of warmblooded animals including same food animals and humans following the ingestion of contaminated food.

The disease caused by *Listeria monocytogenes* is called listeriosis, which causes approximately 2500 serious cases and 500 deaths in the United States each year (CDS Morbidity and Mortality Weekly Report, 2000). People that are more likely to be infected are: over 65 years of age, patients suffering from diabetes, AIDS, kidney disease, cancer, or who take immunosuppressive medications. Manifestation of illnesses include meningitis and sepsis. Infected pregnant women may experience only a mild, influenza-like illness; however, infections during pregnancy can lead to premature delivery, miscarriage, stillbirth, or serious infection of the newborn. Symptoms may begin from one day to several weeks following infection.

Listeria monocytogenes is commonly found in the intestines of human and animals, in milk, soil, leafy vegetables and in food processing environments. These bacteria have been isolated in a variety of foods, including raw and cooked poultry, meat, seafood, salads and sandwiches. This microorganism can grow slowly at refrigerator temperatures. Several factors are thought to play a role in the occurrence of foodborne human listeriosis, including host immunocompetence (Rocourt, 1994), as well as the characteristics of the particular *Listeria monocytogenes* strain involved (Ryser, 1999).

Bacteriocins are extracellular peptides or protein molecules, produced by lactic acid bacteria, which have, on usually closely related bacterial strains, bactericidal or bacteriostatic effects (Jack *et al.*, 1995). They may have destructive effects on some, not so closely related Gram positive bacterial species, for example *Listeria monocytogenes* (Muriana, 1996; Dimitrijevic, 1998). Some authors found that sensitivity/resistance to the bacteriocins could not be correlated with the strain origin, MEE type (Larsen and Norrung, 1993), or serotypes (Ferreira and Lund, 1996; Rasch and Knochel, 1998; Dimitrijevic, 1999). Different electrophoretic types (ETs) of *Listeria monocytogenes* were found by a number of authors (Norrung and Skovgard, 1993; Boerlin and Piffareti, 1991; Avery *et al.*, 1996). MEE has been used in establishing the relationships between the source of isolates and contamination pathways. Therefore, the aim of this study was to find if electrophoretic types (ETs) of *Listeria monocytogenes*, typed by multilocus enzyme electrophoresis (MEE) can induce different sensitivity to the lactic acid bacteria bacteriocins.

MATERIALS AND METHODS

A total of 98 isolates of *L.monocytogenes* had consisted of 50 clinical/human and 48 from the foodstuff of animal origin. Clinical/human types were from blood, liquor, infected human feces (carriers or patients). Types were isolated and identified from the foodstuff of animal origin by the McLain and Lee method (1989). Method of Multilocus enzyme electrophoresis includes: preparation of

enzyme extracts, enzyme electrophoresis, enzyme staining and data analysis. Statistical analysis of data was performed using a program provided by Dr. B. Norrung. Genetic diversity (h) for an enzyme locus was calculated by the following formula:

$$h = (1 - \sum x_i^2) / n-1$$

where x_i is frequency of the i -th allele and n is the number of ETs. Genetic distance between ETs was expressed as the proportion of loci at which dissimilar alleles occur. Cluster analysis using the average distance method and weighted proportions was used to produce a dendrogram.

Bacteriocins originated from the following lactic acid bacteria were used: *Lactobacillus sake* 148, *Lactococcus UW*, *Lactobacillus sake* 265, *Pediococcus* 347 and *Lactobacillus sake* 706. Assessment of growth inhibition of various types of *L. monocytogenes*, which was influenced by the selected bacteriocins, was performed by the method of diffusion on agar with holes. Each type was assessed three times, so that the statistical analysis was based on three values for every type, and the amortization of agar thickness, on the results. Two incubation temperatures were applied: 37°C, for 24 hours and 4°C for 12 days. For every type, three inhibition values were noted (expressed in millimeters), and they are given as average values in the enclosed tables. Statistical significance of the obtained results between the assessed experimental groups was performed by the random plan method according to the statistical software Statgraphics 5.0 (Statistical Graphic Corporation USA).

RESULTS AND DISCUSSION

Multilocus enzyme electrophoresis (MEE) technique has been used for subspecies typing of *Listeria monocytogenes* isolates and although PFGE proved to be capable of subdividing a number of recurrent and sporadic ETs, the grouping of strains arrived at by PFGE and MEE were in broad agreement. Hence previous conclusions regarding the designation of *L. monocytogenes* strains as recurrent or sporadic remained unaltered. It is considered that PFGE was able to detect minor genetic changes in recurrent ETs which occurred during the time period in which food surveys were carried out (Avery *et al.*, 1996; Harvey J. and Gilmour A., 2001).

The 98 isolates of *Listeria monocytogenes* in this study produced 32 electrophoretic types (ETs), most of which were represented by only one isolate but some of them had as many as 8 isolates (Table 1 and 2).

This is in contrast to previous studies (Bibb *et al.*, 1989), where the electrophoretic types consisted of relatively few isolates. All types of *Listeria monocytogenes* represented in one electrophoretic type were genetically close. Two bacteriocins, originated from *Lactococcus UW* and *Lactobacillus sake* 148 showed no inhibitory activity. There is statistically a significant difference between arithmetical average values of the ETs 18, 29 and 32 according to the ETs 2, 12, 19, 21, 23, 24 and 30 on incubation at 37°C/ 24 hours. For those electrophoretic

types, by analysis of genetic diversity, it was found that they were at level 0.3 and 0.4 (Shema 1) which means a large genetical diversity.

Table 1. Electrophoretic types (Ets) among clinical/human isolates of *Listeria monocytogenes*

Mark of type	ET	Number of types	%
126 64269 67977	1	3	6
64416 63711 66091	2	3	6
62433	3	1	2
166	4	1	2
104 65980	5	2	4
63158	6	1	2
96	7	1	2
63588	8	1	2
67675 67992	9	2	4
62846 65017	11	2	4
94 63869 64716	13	3	6
97 98 103	14	3	6
8	15	1	2
99 102	16	2	4
101	17	1	2
95	18	1	2
67143 68614 69873	21	3	6
61632	22	1	2
61763	23	1	2
62124	24	1	2
62728	25	1	2
64084	26	1	2
100 128 64563 65631 66255	27	5	10
105 132 66339 66575 68602 69300	28	6	12
66824	29	1	2
67324 67479	32	2	4

Table 2. Electrophoretic types (Ets) among isolates of *Listeria monocytogenes* from food of animal origin

Mark of type	ET	Number of type	%
53 59 130 134 169	1	5	10.41
168 170	2	2	4.16
171	3	1	2.08
58 167	4	2	4.16
54 55 74 77 92	5	5	10.41
60 62 67 71 79 83 90	6	7	14.58
48 61	7	2	4.16
57	8	1	2.08
56 70592	9	2	4.16
63 82 84 91	10	4	8.33
69	11	1	2.08
78	12	1	2.08
68 70	14	2	4.16
70708	17	1	2.08
70841	19	1	2.08
70172 70916	20	2	4.16
70620 71062	21	2	4.16
70313	26	1	2.08
72 70550 70722	28	3	6.25
66 125	30	2	4.16
127	31	1	2.08

On incubation at 4°C for 12 days (Table 3), bacteriocins had listericidal effects on a larger number of electrophoretic types than on incubation at 37°C/ 24 hours.

The greatest effects lactic acid bacteria bacteriocins is shown on ETs 15 and 18, in which a statistically significant difference $p < 0.01$ between mean values in almost all electrophoretic types. A very significant statistical difference $p < 0.01$ was found among ETs: 7,8,11,13,14,16,19,22,23,25,27,28 and 32.

The lack of correlation of MEE types and source of isolates (human, animal, food or environment) was previously observed (Boerlin and Piffaretti, 1991).

Table 3. Sensitivity of different Ets of *Listeria monocytogenes* to the lactic acid bacteria bacteriocins

ET	Lactobacillus sake 265		Pediococcus 347		Lactobacillus sake 706	
	37°C/24h*	4°C/12d*	37°C/24h*	4°C/12d*	37°C/24h*	4°C/12d*
1	1.65 ± 1.02	2.27 ± 1.16	1.66 ± 0.77	1.52 ± 0.64	1.05 ± 0.16	1.07 ± 0.23
2	1.88 ± 0.78	5.88 ± 2.84	2.27 ± 0.97	3.25 ± 0.27	0.88 ± 0.22	1.94 ± 0.52
3	1.33 ± 0.46	5.16 ± 0.70	3.33 ± 0.46	1.66 ± 0.94	0.50 ± 0.70	0.50 ± 0.70
4	3.11 ± 4.54	2.66 ± 2.51	1.49 ± 2.04	0.55 ± 0.95	0.61 ± 1.05	0.55 ± 0.95
5	1.57 ± 0.39	3.33 ± 1.41	2.25 ± 0.86	2.42 ± 0.69	1.05 ± 0.16	1.85 ± 0.61
6	1.83 ± 0.45	4.07 ± 2.20	2.83 ± 0.93	3.00 ± 1.02	1.69 ± 1.12	1.68 ± 0.50
7	1.94 ± 0.85	2.77 ± 3.23	2.44 ± 1.22	1.33 ± 1.33	0.83 ± 0.28	1.11 ± 0.84
8	1.00 ± 1.41	5.00 ± 4.24	1.83 ± 1.65	3.08 ± 2.23	1.00 ± 0.00	2.99 ± 1.88
9	1.12 ± 1.03	3.99 ± 2.22	1.37 ± 1.88	1.66 ± 0.23	0.75 ± 0.50	1.16 ± 0.20
10	1.61 ± 1.45	4.55 ± 2.87	2.22 ± 2.03	2.83 ± 1.36	1.00 ± 1.00	2.10 ± 0.42
11	0.33 ± 0.57	9.99 ± 0.57	3.00 ± 2.64	4.44 ± 0.76	1.44 ± 1.70	2.77 ± 1.17
12	0	0	0	0	0	0
13	1.55 ± 1.38	7.21 ± 2.36	3.16 ± 0.60	2.72 ± 0.85	0.83 ± 0.28	1.88 ± 1.01
14	2.75 ± 1.19	5.53 ± 2.17	2.50 ± 0.52	2.26 ± 0.67	0.95 ± 0.58	1.66 ± 0.72
15	2.00 ± 0.00	10.33 ± 0.57	5.00 ± 0.00	4.00 ± 0.00	1.00 ± 0.00	3.00 ± 0.00
16	2.33 ± 0.46	10.66 ± 2.65	4.66 ± 0.28	2.00 ± 2.82	1.25 ± 0.35	2.99 ± 0.47
17	1.50 ± 0.70	2.49 ± 1.64	2.16 ± 1.64	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
18	4.33 ± 0.28	10.66 ± 0.57	3.66 ± 0.28	4.83 ± 0.76	3.50 ± 0.00	3.83 ± 0.28
19	0	7.66 ± 0.57	0	2.33 ± 0.57	0	2.00 ± 0.00
20	0.75 ± 1.06	4.83 ± 0.24	1.50 ± 2.12	2.16 ± 0.47	0.41 ± 0.58	1.83 ± 0.94
21	2.50 ± 0.54	3.04 ± 0.96	1.41 ± 0.49	1.66 ± 0.43	1.00 ± 0.66	0.90 ± 0.20
22	3.00 ± 0.00	9.00 ± 0.00	3.66 ± 0.28	3.00 ± 1.00	1.00 ± 0.00	1.33 ± 0.57
23	0	0	0	0	0	0
24	0	6.00 ± 0.00	0	2.83 ± 0.00	0	1.16 ± 0.28
25	2.00 ± 0.00	9.33 ± 0.00	4.00 ± 0.00	3.83 ± 0.57	1.00 ± 0.00	3.66 ± 0.28
26	3.00 ± 0.00	3.83 ± 5.41	2.16 ± 1.64	1.08 ± 1.52	1.08 ± 0.11	1.00 ± 1.41
27	1.90 ± 0.92	6.80 ± 2.47	3.66 ± 0.64	3.13 ± 0.74	1.37 ± 0.67	1.93 ± 1.06
28	1.80 ± 0.76	7.38 ± 2.87	2.94 ± 0.92	3.05 ± 1.06	0.93 ± 0.45	2.40 ± 0.83
29	3.00 ± 0.00	6.00 ± 0.00	3.83 ± 0.28	3.83 ± 0.28	1.83 ± 0.28	2.83 ± 0.28
30	0.75 ± 1.06	3.66 ± 0.94	1.16 ± 1.64	2.08 ± 0.11	0.50 ± 0.70	1.91 ± 0.35
31	2.66 ± 0.57	8.66 ± 0.57	4.83 ± 0.28	1.00 ± 0.00	1.33 ± 0.28	2.00 ± 0.00
32	2.66 ± 0.28	9.83 ± 1.17	4.16 ± 0.23	3.33 ± 0.46	1.33 ± 0.46	3.24 ± 0.82

*Mean value of inhibition zone ± standard deviation

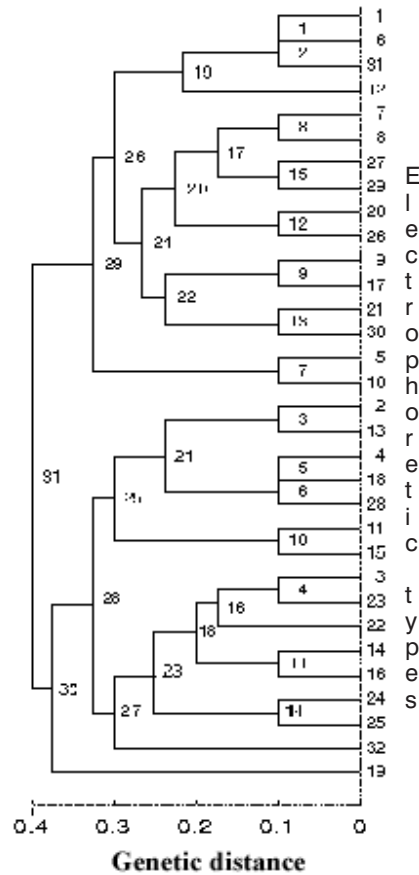


Figure 1.

However, in this study, on the basis of a dendrogram, our results indicate that a reliable relationship between genetic distance of *Listeria monocytogenes* electrophoretic types and their sensitivity to lactic acid bacteria bacteriocins cannot be found. Others authors state that sensitivity/resistance to bacteriocins could not be correlated to strain origin, or MEE type (Larsen and Norrung, 1993). Studies targeting specifically serotype 4b and 1/2 are lacking. MEE may be of future benefit in establishing links between isolates from human disease cases and thus be useful in establishing the epidemiology of not only sporadic cases, but of outbreaks of listeriosis, as well.

Address for correspondence:
 Mirjana Dimitrijević
 Faculty of Veterinary medicine
 Bul oslobođenja 18, 11000 Beograd
 Serbia&Montenegro

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MOGU LI ELEKTROFORETSKI TIPOVI *LISTERIA MONOCYTOGENES* USLOVITI RAZLIČITU OSETLJIVOST PREMA BAKTERIOCINIMA MLEČNO-KISELINSKIH BAKTERIJA?

DIMITRIJEVIĆ MIRJANA, TEODOROVIĆ V, KARABASIL N, TEODOROVIĆ RADISLAVA i BUNČIĆ OLIVERA

SADRŽAJ

Cilj ovog ispitivanja je bio da se ustanovi da li elektroforetski tipovi (ET) *Listeria monocytogenes*, tipizirani metodom multilokus enzimske elektroforeze, mogu usloviti različitu osetljivost prema bakteriocinima mlečno-kiselinskih bakterija. Bakteriocini su ekstracelularno oslobođeni peptidi, ili proteinski molekuli, stvoreni od strane mlečno-kiselinskih bakterija, koji imaju baktericidne ili bakterioostatske efekte, obično prema srodnim bakterijskim vrstama, ali takođe mogu imati i destruktivne efekte prema nekim manje srodnim Gram pozitivnim bakterijama, kao što je *Listeria monocytogenes*. *Listeria monocytogenes* se uglavnom nalazi u crevima ljudi, u mleku, zemljištu, listastom povrću i proizvodnoj okolini. Ova bakterija je takođe izolovana iz razne hrane, uključujući sirovu i kuvanu živinu, meso, morske plodove, salate i sendviče. U svrhu identifikacije izvora infekcije za epidemiološke i epizotološke studije, razvijeno je mnogo tehnika za tipizaciju *Listeria monocytogenes* iz hrane.

Među 98 izolata *L. monocytogenes* (50 kliničkih/humanih i 48 iz namirnica animalnog porekla) ustanovljeno je 32 elektroforetska tipa. Bakteriocini koji su korišćeni u studiji, poreklom su od sledećih mlečno-kiselinskih bakterija: *Lactobacillus sake* 148, *Lactococcus UW*, *Lactobacillus sake* 706, *Pediococcus* 347 and *Lactobacillus sake* 265. U ovom radu, na osnovu dendograma, naši rezultati ukazuju da se ne može naći odgovarajuća povezanost između genetske distance elektroforetskih tipova *L. monocytogenes* i njihove osetljivosti prema bakteriocinima mlečno-kiselinskih bakterija. Multilokus enzimska elektroforeza (MEE) se može ubuduće koristiti za uspostavljanje veza među izolatima od obolelih ljudi i biti korisna za ustanovljavanje epidemiologije kako kod sporadičnih slučajeva, tako i kod epidemija listerioze.