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Autolysis of Listeria monocytogenes strains isolated from food and clinical specimens

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Summary

Autolysis of Listeria monocytogenes strains isolated from clinical and food specimens were tested under starvation conditions. Late exponential phase harvested bacterial cells were transferred in potassium phosphate buffer (100 mM, pH 7) and incubated at 37°C to induce lysis. At the end of the 48 h incubation period a heterogeneous autolysis profile was observed among the tested strains. All the clinical strains exhibited high autolysis i.e. above 75%. Meanwhile, the extent of food isolates was not as high as the clinical ones but demonstrated a great variability – ranging between 22 to 88%. An increase in the amount of intracellular markers upon lysis was also measured in the higher autolytic clinical strain – UVF172, compared to low autolytic food strain – UVF114. The results obtained from the study provided evidence that autolysis in L. monocytogenes is strain dependent and revealed that tested clinical isolates had a higher level of autolysis than food isolates.

Keywords: Listeria monocytogenes, food

Listeria monocytogenes is a ubiquitous Gram-positive intracellular bacterial pathogen (7). It is widespread in nature and has been isolated from several ecologic environments including foodstuff, vegetables, soil and water. It is responsible for human and animal listeriosis, an infection that is mainly characterized by meningitis, meningoencephalitis, septicemia, abortion and gastroenteritis (5). Several outbreaks associated with this bacterium have been reported and it is now becoming a major problem mainly for food industry because of frequent contamination of food products (15).

Many previous publications have dealt with the virulence of *L. monocytogenes*. It has been shown that *L. monocytogenes* is able to enter and multiply in phagocytes such as epithelial cells or hepatocytes (8). Even though several virulence determinants including listeriolysin O, phospholipases C, intracellular motility protein ActA, surfaces proteins internalin A and internalin B which are implicated in the pathogenesis of *L. monocytogenes* were described and thoroughly studied, the complete mechanism is not completely elucidated (11). Together with all these factors, it has been shown that autolysins can also contribute to virulence (3). The major extracellular protein p60 of all virulent *L. monocytogenes* strains has been shown to be an autolysin that plays role in cell separation and uptake of the pathogen by mammalian host cells (22). Bacterial autolysins are endogenous enzymes that can hydrolyze covalent bonds in the peptidoglycan of their own cell walls. It has been proposed that these enzymes play roles in a variety of physiological conditions including expansion of the cell wall, cell division and separation, cell wall turnover, flagella formation allowing motility, differentiation and antibiotic induced lysis (4, 18). Cell autolysis is the result of the hydrolysis of sufficient number of bonds in the protective cell wall peptidoglycan.

Transferring bacteria in a starvation environment such as buffer solution is an easy, rapid and widely used technique to induce and study bacterial autolysis (2, 6, 19). The purpose of the present study was to test the autolysis of *L. monocytogenes* strains isolated from clinical and food specimens in optimized buffered system.

Material and methods

Bacterial strains and growth conditions. The *Listeria* strains used in this study and their characteristics are given in tab. 1. They were isolated from foodstuffs and patients and stored in Brain-Heart Infusion Broth (BHI; Difco Laboratories) containing 20% glycerol at –80°C. Growth of the bacteria was performed in BHI broth at 37°C.

Serotyping of the strains was determined by the slide agglutination method as described by the manufacturer (Denka Seiken, Tokyo, Japan).

Determination of autolysis. Late exponential phase cells (Optical Density at 650 nm is about 1,5-2) (Shimadzu UV--Visible Spectrophotometer, UV-1601, Japan) were harvested by centrifugation at 4000 g for 10 min., washed twice with sterile distilled water and resuspended in potassium phosphate buffer (100 mM, pH 7) to have an initial OD_{650} 0.6-0.8. Thereafter the cell suspension was incubated at 37°C and autolysis was measured as the turbidimetric decrease. The extent of autolysis was expressed as the percentage decrease of the OD_{650} after 48 h of incubation and calculated as follows:

$$\% \text{ autolysis} = 100 - (A1/A2 \times 100)$$

where A1 is the lowest OD_{650} and A2 is the maximum OD_{650} measured.

Determination of released nucleic acids and proteins. In lysing suspension, non-lysed bacteria was eliminated by centrifugation (4000 g for 10 min.), then the supernatant was filtered through 0.22 µm membranes (Millipore S.A., Bedford, MA, USA). The OD of the clarified fluid was measured at 260 nm and 280 nm for nucleic acid and proteins, respectively.

Statistical analysis. Results of triple independent replications are represented. Statistical calculations were processed by students-t test using the MINITAB software statistical program (1).

Tab. 1. Strains of *Listeria monocytogenes* and their autolysis in 100 mM potassium phosphate buffer (pH 7 at 37°C)

Strains	Serotype	Origine	Autolysis (%)		
			2 h	5 h	48 h
UVF114	1/2a	Chicken**	11 ± 1.90	19 ± 1.00	22 ± 1.30
UVF115	4d	Chicken*	8 ± 0.60	17 ± 0.30	27 ± 0.80
UVF136	ND	Chicken*	11 ± 0.10	15 ± 1.50	45 ± 2.35
UVF124	ND	Chicken**	7 ± 1.34	12 ± 2.05	25 ± 0.85
UVF111	ND	Chicken*	9 ± 0.20	23 ± 0.10	53 ± 0.35
UVF109	4a	Chicken**	8 ± 0.25	13 ± 0.20	54 ± 0.20
UVF116	4a	Chicken*	7 ± 0.60	13 ± 1.60	62 ± 0.70
UVF118	4d	Chicken**	8 ± 0.35	17 ± 2.80	58 ± 0.35
UVF119	4	Chicken**	10 ± 0.50	18 ± 1.10	61 ± 4.90
UVF122	4	Chicken**	10 ± 0.85	19 ± 0.25	67 ± 0.45
UVF106	ND	Chicken*	12 ± 0.40	17 ± 0.25	51 ± 0.55
UVF125	ND	Chicken*	10 ± 0.60	16 ± 0.35	50 ± 1.20
UVF107	1/2a	Chicken*	15 ± 0.10	19 ± 0.25	59 ± 0.15
UVF128	4b	Chicken*	10 ± 0.65	16 ± 0.00	62 ± 0.70
UVF129	1/2a	Chicken*	8 ± 0.50	11 ± 0.30	53 ± 0.70
UVF142	1/2a	Chicken**	11 ± 0.45	26 ± 5.45	68 ± 1.00
UVF165	4	Ground meat	6 ± 0.40	9 ± 1.30	63 ± 0.70
UVF164	4	Raw milk	27 ± 1.50	38 ± 0.85	65 ± 2.35
UVF110	ND	Chicken**	5 ± 0.05	13 ± 0.35	77 ± 0.25
UVF131	4	Chicken**	12 ± 0.25	22 ± 1.50	79 ± 4.55
UVF132	1/2a	Chicken*	10 ± 1.35	19 ± 0.80	82 ± 0.85
UVF135	1/2a	Chicken**	14 ± 0.15	29 ± 0.20	76 ± 1.85
UVF166	4	Ground meat	11 ± 0.15	17 ± 0.25	86 ± 2.85
UVF174	4	Ground meat	10 ± 0.20	15 ± 1.75	85 ± 0.40
UVF168	4	Meat	8 ± 0.45	14 ± 0.50	88 ± 0.25
UVF148	4	Meat	6 ± 1.40	15 ± 0.80	80 ± 3.35
UVF126	ND	Chicken*	16 ± 0.75	26 ± 0.20	72 ± 1.85
UVF167	ND	Mussel	10 ± 1.70	16 ± 0.25	72 ± 4.20
UVF147	4	Patient***	5 ± 0.20	17 ± 0.55	75 ± 1.05
UVF151	4	Patient	10 ± 0.35	19 ± 1.20	82 ± 0.15
UVF156	4	Patient	6 ± 0.30	15 ± 2.10	78 ± 0.55
UVF162	4	Patient	7 ± 0.95	14 ± 0.50	81 ± 0.80
UVF170	4	Patient	7 ± 0.55	9 ± 0.50	78 ± 4.30
UVF172	4	Patient	11 ± 0.85	18 ± 0.30	91 ± 1.15
UVF173	4	Patient	10 ± 0.90	14 ± 0.80	75 ± 0.90

Explanations: * – thigh samples, ** – skin samples, *** – patient with listeriosis, ND – not detected

Results and discussion

Autolysis of 35 *L. monocytogenes* strains isolated from food and clinical environment was studied under optimized starvation conditions. Strains isolated from food specimens exhibited highly variable autolysis extent ranging from 22% to 88% after 48 h incubation (tab. 1). There was no further autolysis when the incubation time was prolonged. The variability of the autolysis extent of clinical isolates was not as large as the food strains but it was significantly higher ($P < 0.001$). As a function of their autolysis extent three different groups could be categorized: i) lower autolytic strains exhibiting autolysis extent under 50%, ii) moderate autolytic strains exhibiting 50-70% autolysis and iii) higher autolytic group exhibiting higher than 70% autolysis. Whereas the majority of food strains were in moderate autolytic group, all the clinical strains were in higher autolytic group indicating that they are more prone to autolysis. Among the food strains only 10 strains (35%) placed in higher auto-

Tab. 2. Autolysis ($OD_{650\ nm}$) of strains UVF172 and UVF114 in potassium phosphate buffer (100 mM, pH 7 at 37°C) and release of nucleic acids ($OD_{260\ nm}$) and proteins ($OD_{280\ nm}$)

Time (h)	$OD_{650\ nm}$		$OD_{260\ nm}$		$OD_{280\ nm}$	
	UVF114	UVF172	UVF114	UVF172	UVF114	UVF172
0	0.73	0.66	0	0	0	0
2	0.67	0.58	0.06	0.11	0.09	0.07
4	0.61	0.54	0.28	0.19	0.17	0.10
7	0.60	0.42	0.37	0.37	0.20	0.18
19	0.58	0.12	0.47	0.98	0.23	0.62
24	0.58	0.09	0.46	1.06	0.22	0.63
48	0.56	0.07	0.47	1.10	0.17	0.65

lytic group. There was no remarkable correlation between the serotype group and autolysis extent of the strains.

Strains UVF172 and UVF114 representing higher and lower autolytic groups were chosen for studying liberated intracellular components such as liberated nucleic acids and proteins as a consequence of lysis (tab. 2). As expected, higher amounts of intracellular components were released by the strain UVF172 confirming that the OD decrease reflects cellular autolysis leading to release of intracellular content.

In the present study, autolysis of food and clinical strains of *L. monocytogenes* was reported. As previously reported for other pathogenic or nonpathogenic bacteria (6, 9, 10, 12, 21), autolysis of *L. monocytogenes* appears as a strain dependant property. Comparative analysis of food and clinical strains evidenced that clinical strains exhibit relatively higher autolysis extent. Food isolates on the other hand exhibited variable level of autolysis activity. This can be explained by the rich biodiversity of food isolates as reported by genetic finger printing techniques (14). Pathogenicity is also quite heterogeneous among *L. monocytogenes* strains. While some strains are highly virulent some others are hypo- or avirulent (17). This heterogeneity could be linked to the ecological nest of the isolates. Roche et al. (16) reported that hypovirulent or avirulent *Listeria* strains originated mainly from foodstuffs. In the same study, all epidemic and clinical isolates were found virulent. In our study all the clinical strains were isolated from patients having listeriosis.

Implication of autolysins in virulence was described for several Gram-positive bacteria. Mani et al. (13) reported that autolysis-defective mutant of *Staphylococcus aureus* exhibited attenuated virulence in models of endocarditis. It was also reported that glucosaminidase, which is an autolysin of *S. aureus* alters the immune response in mice (20). Similarly, as observed in the present study higher autolysis tendency of clinical strains might be an indication of correlation between pathogenicity and autolysis for *L. monocytogenes* strains.

In the light of the results of the present study, it will be of interest to test the influence of autolysis on virulence of *L. monocytogenes*. To get more insight into this fact, studies aimed at comparing virulence of high and low autolytic strains are currently being performed in our laboratory on immunocompromised mice.

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