

Rapid Evaluation of Bacteriolysis of *Listeria innocua* and *L. welshimeri* Strains Isolated from Foods

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Received: 16.06.2004

Abstract: This study was performed to determine the bacteriolytic extent of 10 strains of *Listeria innocua* and 9 strains of *L. welshimeri* isolated from foods. Bacteriolysis was measured at 650 nm by spectrophotometer. For this purpose, late exponential phase cells were transferred into potassium phosphate buffer (100 mM, pH 7.0) and incubated at 37 °C. While the bacteriolysis range of *L. innocua* strains was 48% to 76% after 48 h of incubation, *L. welshimeri* isolates exhibited broader bacteriolytic variabilities, from 10% to 67%. A preliminary analysis of the bacteriolysis of *L. innocua* and *L. welshimeri* may be useful in detailed evaluations of the bacteriolytic system within the genus *Listeria*.

Key Words: *Listeria innocua*, *Listeria welshimeri*, bacteriolysis

Gıdalardan İzole Edilen *Listeria innocua* ve *L. welshimeri* Suşlarının Bakteriyolizinin Kısa Süre İçerisinde Değerlendirilmesi

Özet: Bu çalışma, gıdalardan izole edilen 10 adet *L. innocua* ve 9 adet *L. welshimeri* suşunun bakteriyolitik derecesini saptamak amacıyla gerçekleştirildi. Bakteriyoliz, 650 nm dalga boyunda spektrofotometre ile belirlendi. Bu amaçla, logaritmik fazın son aşamalarında elde edilen hücreler potasyum fosfat tampon solusyonuna (100 mM, pH 7.0) transfer edildi ve 37 °C'de inkübasyona bırakıldı. 48 saatlik inkübasyon sonunda *L. innocua* suşlarının bakteriyoliz oranı % 48-76 arasında belirlenirken; *L. welshimeri* izolatları, % 10-67 arasında değişen oranlarda olmak üzere, daha geniş bir bakteriyolitik değişkenlik gösterdi. *L. innocua* ve *L. welshimeri*'nin bakteriyolizine ilişkin bu ön çalışmanın, *Listeria* cinsi içerisinde bakteriyolitik sistemin ayrıntılı olarak değerlendirilmesinde yararlı olabileceği düşünülmektedir.

Anahtar Sözcükler: *Listeria innocua*, *Listeria welshimeri*, bakteriyoliz

Introduction

The genus *Listeria* consists of a group of Gram-positive, non-spore forming coccobacilli, within which 6 closely related species are recognized taxonomically: *L. monocytogenes*, *L. ivanovii*, *L. seeligeri*, *L. innocua*, *L. welshimeri* and *L. grayi*. Of these 6 species, only the intracellular pathogen *L. monocytogenes* is pathogenic in humans, causing gastroenteritis, abortions, meningitis, encephalitis and sepsis, and it is a significant cause of foodborne infections in humans (1,2).

Although it is non-pathogenic for mice (3), *L. innocua* is isolated from foods and in food processing plants, often in conjunction with *L. monocytogenes* (4-6). *L. innocua*

could be an ideal and safe marker organism for *L. monocytogenes* in the food industry since it shares morphological, biochemical and molecular characteristics with the pathogenic species *L. monocytogenes* except for hemolysin production, i.e. pathogenicity (7).

The autolysins produced by bacteria are endogenous enzymes that can break covalent bonds in the peptidoglycan of their own cell walls. These molecules have been implicated in various biological functions, such as cell wall turnover, cell separation, cell division flagella formation and antibiotic-induced autolysis (8-11). Bacterial autolysis can be measured by the direct transfer of bacteria in a nutritional starvation environment in which enzymatic activity is induced.

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In the present paper, we describe the bacteriolysis of *L. innocua* and *L. welshimeri* strains isolated from foods using an efficient, less laborious and rapid technique.

Materials and Methods

Bacterial strains and growth characteristics. All the strains used in the present study were obtained from a UVF collection (Uludağ University, Faculty of Veterinary Medicine, Bursa) and are listed in the Table. They were originally isolated from foods and maintained at -80 °C in Brain Heart Infusion Broth (Oxoid, CM225) supplemented with 20% glycerol. Prior to each experiment, isolates were grown in Brain Heart Infusion Broth and incubated at 35 °C for 12 h.

Bacteriolysis measurements. Spectrophotometric measurement of bacteriolytic activity in buffered solutions as described by Cibik and Chapot-Chartier (12) was used to determine bacteriolysis. For this purpose, late exponential phase bacterial cells (15 h of growth) grown in Brain Heart Infusion Broth were harvested, washed twice in cold water and resuspended at room temperature in 100 mM potassium phosphate buffer (pH 7.0) to have an initial optical density (O.D.) of 0.6 and 0.8 at 650 nm in a spectrophotometer (Shimatzu UV-Visible, UV-1601, Japan). Cell suspensions were incubated at 37 °C and the percentage bacteriolysis was defined as follows:

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

where A1 is the lowest O.D. and A2 is the maximum O.D. measured. The extent of bacteriolysis was expressed as the percentage decrease in the O.D.₆₅₀ after 48 h of incubation.

Statistical Analysis. The results of triple independent replications are represented. Statistical calculations were processed by Mann-Whitney test using the MINITAB statistics program (release 13.20, Minitab Inc.).

Results

When the bacteria were transferred from the culture media into the buffer media, the bacteriolytic process as described by the turbidimetric decrease, measured by spectrophotometer, was triggered immediately. For that reason, to prevent measuring mistakes the buffer solutions used in the study were kept at refrigeration temperature to limit the enzymatic activity. Furthermore, the washing of whole cells twice in cold water was a crucial step in terms of obtaining accurate results. Bacteriolysis ranging from 48% to 76% was observed with the strains belonging to *L. innocua* after 48 h of incubation. Meanwhile, the bacteriolytic extent of *L. welshimeri* strains exhibited broader variability, ranging from 10% to 67% (Table). There was no significant difference between bacteriolysis levels of *L. innocua* and

Table. Strains of *L. innocua* and *L. welshimeri* and their bacteriolysis in 100 mM potassium phosphate buffer at 37 °C (pH 7.0).

Strains	Bacteriolysis (%)			Strains	Bacteriolysis (%)		
	<i>L. innocua</i> ^a				<i>L. welshimeri</i> ^b		
	2 h	5 h	48 h		2 h	5 h	48 h
UVF 100	16 ± 0.60	25 ± 0.25	68 ± 0.25	UVF 125	10 ± 0.60	16 ± 0.35	50 ± 1.20
UVF 101	25 ± 0.30	33 ± 0.15	60 ± 0.70	UVF 134	8 ± 1.28	16 ± 0.20	51 ± 0.70
UVF 102	15 ± 0.60	23 ± 0.60	59 ± 0.55	UVF 138	10 ± 0.05	17 ± 0.95	61 ± 0.50
UVF 103	16 ± 0.35	21 ± 0.70	59 ± 0.10	UVF 139	18 ± 0.00	24 ± 1.60	45 ± 8.70
UVF 104	14 ± 0.35	19 ± 0.35	48 ± 0.35	UVF 140	45 ± 2.30	65 ± 3.10	59 ± 1.55
UVF 105	15 ± 0.05	23 ± 0.15	65 ± 0.55	UVF 141	7 ± 0.00	9 ± 2.45	10 ± 1.65
UVF 108	22 ± 0.80	29 ± 0.10	65 ± 0.65	UVF 143	33 ± 1.60	48 ± 0.35	64 ± 3.20
UVF 117	11 ± 0.40	16 ± 0.15	76 ± 0.25	UVF 145	37 ± 0.90	54 ± 1.70	55 ± 1.75
UVF 120	11 ± 0.35	23 ± 0.45	75 ± 0.53	UVF 146	33 ± 1.90	62 ± 0.30	67 ± 1.35
UVF 121	15 ± 1.60	29 ± 4.90	74 ± 11.40				

^{a,b}: Means only at 48 h are statistically different (P < 0.05)

L. welshimeri strains at 2 h or 5 h but there was at 48 h ($P < 0.05$). It should be noted that there were no remarkable turbidimetric changes when longer incubation periods were applied. Representative results of 2 strains of *L. innocua* and *L. welshimeri* are represented in the Figure.

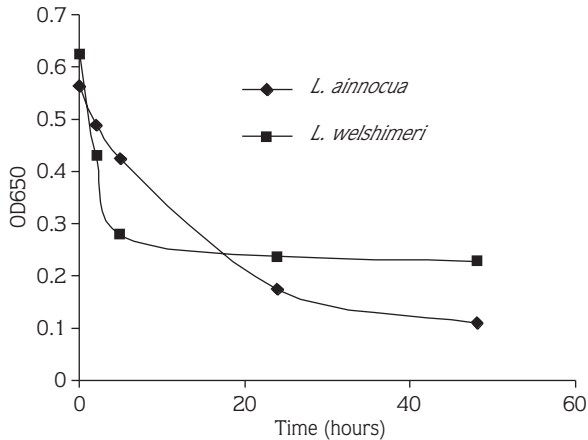


Figure. Bacteriolysis in potassium phosphate buffer measured at 650 nm.

Discussion

In the present study, the bacteriolytic activity of 2 members of the genus *Listeria* was analyzed in a buffer system. To our knowledge, no studies regarding the bacteriolytic activities of these 2 species have been

published to date. Both of the tested species presented quite variable levels of bacteriolysis, suggesting that bacteriolysis in *L. innocua* and *L. welshimeri* strains is strain dependent rather than species dependent. This phenotype was observed with several bacteria (13, 14). In our recent studies we obtained the same results with *L. monocytogenes*, the best known member of the genus *Listeria* (data not shown). In a study performed on leuconostocs a high level of variability in terms of the autolytic activities of the tested strains was demonstrated (12).

In a functional manner, bacterial lysis of dairy lactic acid bacteria is of technological interest. The release of intracellular content into cheese curd as a consequence of bacteriolysis is in fact a crucial step in the formation of aromatic compounds from di or tri peptides, which generally have a bitter taste (15). In addition, the time required for storage can be reduced by the acceleration of ripening. On the other hand, several authors have reported that for pathogenic bacteria autolysis is one of the factors playing an important role in the pathogenesis. In *Streptococcus pneumoniae* it was reported that inactivation of an autolysin encoding gene had an important effect on the reduction of the pathogenicity (16). Carvin et al. (17) observed the same phenomenon in animal studies with infected mice. These preliminary results obtained in the present study might be a basis for the selection of candidate strains to better understand bacteriolysis in the genus *Listeria*.

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