ELSEVIER

FEMS Microbiology Letters 243 (2005) 373–378

FEMS MICROBIOLOGY Letters

www.fems-microbiology.org

Comparative assessment of acid, alkali and salt tolerance in *Listeria monocytogenes* virulent and avirulent strains

Dongyou Liu *, Mark L. Lawrence, A. Jerald Ainsworth, Frank W. Austin

Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, P.O. Box 6100, Mississippi, MS 39762, USA

Received 24 October 2004; received in revised form 13 December 2004; accepted 21 December 2004

First published online 30 December 2004

Edited by W. Kneifel

Abstract

Listeria monocytogenes is an opportunistic bacterial pathogen of man and animals that has the capacity to survive under extreme environmental conditions. While our knowledge on *L. monocytogenes* and its ability to sustain within wide pH and temperature ranges and salt concentrations has been largely built on the virulent strains of this species, relatively little is known about avirulent strains in this regard. In this study, we extend our analysis on avirulent *L. monocytogenes* strains. By subjecting three virulent (EGD, 874 and ATCC 19196) and three avirulent (ATCC 19114, HCC23 and HCC25) strains to various pH and salt concentrations, it was found that *L. monocytogenes* recovered well after treatment with 100 mM Tris at pH 12.0, and to a lesser extent at pH 3.0. Interestingly, avirulent *L. monocytogenes* strains showed a somewhat higher tolerance to alkali than virulent strains. This unique feature of avirulent *L. monocytogenes* strains may potentially be exploited for the development of a rapid technique for differentiation between avirulent and virulent strains. Furthermore, all *L. monocytogenes* strains tested were resistant to saturated NaCl (about 7 M, or 40% w/v) for a long period of time (20 h and possibly longer). Together, these results highlight that acid, alkali, and/or salt treatments commonly used in food product processing may not be sufficient to eliminate *L. monocytogenes*, and therefore stringent quality control measures at the beginning and end of the food manufacturing process is essential to ensure that such food products are free of listerial contamination.

Keywords: *Listeria monocytogenes*; Acid; Alkali; Salt; Virulence

1. Introduction

Being a Gram-positive facultative intracellular bacterium, *Listeria monocytogenes* has increasingly emerged as an opportunistic human foodborne pathogen. The fact that this bacterium is ubiquitously present in all environments and tolerant to extreme environmental stresses may account for its prevalence. It is well known that *L. monocytogenes* bacteria can sustain wide ranges of pH (4.0–9.5) and temperature (<1–45 °C) and are able to survive in the presence of high salt concentrations (up to 10% NaCl) [1–5]. This renders many of the conventional food manufacturing processes ineffective in eliminating the bacterium. With manufactured, ready-to-eat food products being consumed in increasing quantities nowadays, it is no surprise that the incidence of listeriosis through contaminated foods has become more frequent.

While being pathogenic at the species level, *L. monocytogenes* is actually made up of multiple strains that display varied virulence and pathogenic potential. On the one hand, many *L. monocytogenes* strains are

* Corresponding author. Tel.: +1 662 325 3559; fax: +1 662 325 1031.

E-mail address: liu@cvm.msstate.edu (D. Liu).

0378-1097/$22.00
doi:10.1016/j.femsle.2004.12.025

virulent and capable of causing a deadly disease, particularly in infants, the elderly, pregnant women and immuno-suppressed individuals. On the other, some strains are non-pathogenic, producing no apparent malaise and are rapidly cleared by hosts [6–10]. Through experimental studies on *L. monocytogenes* virulent strains, much is known about their capacity to survive at extreme pH, and salt concentrations and their ability to invade host tissues, with the identification of various virulence-associated, and stress response genes [11–13]. However, we know relatively little about avirulent strains in this regard. For example, in spite of possessing nearly identical virulence gene cluster (comprising *prfA*, *hlyA*, *plcA*, *mpl*, *actA* and *plcB*), and other virulence-associated genes (e.g., *inlA*, *inlB* and *iap*) in comparison with virulent strains, *L. monocytogenes* avirulent strains fail to pass through certain critical stage(s) of the infection cycle.

In this report, we attempt to address this imbalance by examining the ability of *L. monocytogenes* avirulent strains to recover after exposure to a range of pH and salt concentrations. A detailed understanding on *L. monocytogenes* avirulent strains and the molecular mechanisms behind their avirulence will help provide a more comprehensive picture on listerial virulence and pathogenicity. This in turn may lead to the development of novel, and more effective treatment and prevention strategies for listeriosis.

2. Materials and methods

2.1. Bacteria and culture conditions

Six *L. monocytogenes* strains of known virulence (i.e., virulent strains EGD, 874 and ATCC 19116; and avirulent strains ATCC 19114, HCC23 and HCC25) were examined in this study (Table 1). These strains came either from the American Type Culture Collection (ATCC) and the National Collection of Type Culture (NCTC), or from clinical or catfish specimens [6,8]. They were previously characterized by PCR targeting *L. monocytogenes* putative transcriptional regulator and internalin genes and by mouse virulence assay [6,8,9]. *L. monocytogenes* was initially cultivated on 5% sheep blood agar plates (TSA II, Becton Dickinson Microbiology Systems, Cockeysville, MD), from which several colonies of each strain were used to inoculate 50 ml of brain heart infusion (BHI) broth (Difco Laboratories, Detroit, MI) in flasks and incubated at 37 °C for 18 h with aeration before undergoing acid, alkaline or salt treatments.

2.2. Acid and alkaline treatments

Cell densities of *L. monocytogenes* BHI broth cultures were measured at $OD_{540\ nm}$, *L. monocytogenes* bacteria were then centrifuged at 2500*g* for 10 min, washed once with 20 ml of sterile physiologic saline (0.9% NaCl) and resuspended in about 10 ml saline (the volume was adjusted to one fifth of that at an $OD_{540\ nm}$ value of 1.35 for each strain). In the first experiment, 100 mM Tris solutions with pH ranging from 2.0 to 5.0, and 9.0 to 12.0 were prepared by adding appropriate amounts of 1 M HCl or 1 M NaOH. In a 15 ml tube containing 5 ml of 100 mM Tris solution, 0.25 ml of each *L. monocytogenes* strain was added, mixed by inversion and left at room temperature for 1 h. Then 40 μl of each treated sample was transferred into an eppendorf tube containing 960 μl of saline and serially diluted to 10^{-6} and 10^{-8}, from which 25 μl was used to inoculate one half of a BHI agar (Difco Laboratories, Detroit, MI) plate (11 cm in diameter). After overnight incubation at 37 °C, the colony forming unit (CFU) of each treated sample was enumerated in accordance with a conventional plate count method (Bacteriological Analytical Manual, FDA). The experiment was performed in duplicate, so that the CFU for a test strain was averaged from the two separate plate counts each with 25 μl inoculon of treated *L. monocytogenes*. Therefore, the final CFU/ml values would be the products of these averaged CFU numbers and the dilution factors (40 and 10^8). As a control, individual tubes containing 5 ml of sterile saline were added with 0.25 ml of *L. monocytogenes* strains, incubated and emunerated as treated samples.

In the second experiment, 10 mM HCl and 100 mM NaOH were prepared in sterile saline. In a 15 ml tube

Table 1
L. monocytogenes strains of known virulence used in the study

Strain	Serovar	Source	*lmo2821* PCR[a]	Pathogenicity[b]
EGD (NCTC7973)	1/2a	Guinea pig	+	V
874	4c	Cow brain	+	V
ATCC 19116	4c	Chicken	+	V
ATCC 19114	4a	Human	–	A
HCC23	4a	Catfish brain	–	A
HCC25	4a	Catfish kidney	–	A

[a] *lmo2821* is a *L. monocytogenes* putative internalin gene that has proven valuable for determination of listerial virulence [8,10].
[b] The pathogenicity of these strains was previously determined by mouse virulence assay, with V being virulent and A being avirulent [6,8,9].