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Processing plant persistent strains of *Listeria monocytogenes* appear to have a lower virulence potential than clinical strains in selected virulence models

Anne Jensen [a,*], Line E. Thomsen [b], Rikke L. Jørgensen [b], Marianne H. Larsen [b], Bent B. Roldgaard [c], Bjarke B. Christensen [c], Birte F. Vogel [a], Lone Gram [a], Hanne Ingmer [b]

[a] *Department of Seafood Research, National Institute of Aquatic Resources, Technical University of Denmark, Søltofts Plads bldg. 221, DK-2800 Kgs. Lyngby, Denmark*
[b] *Department of Veterinary Pathobiology, Faculty of Life Sciences, University of Copenhagen, Stigbøjlen 4, DK-1870 Frederiksberg C, Denmark*
[c] *Department of Microbiology and Risk Assessment, National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, DK-2860 Søborg, Denmark*

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Abstract

Listeria monocytogenes is an important foodborne bacterial pathogen that can colonize food processing equipment. One group of genetically similar *L. monocytogenes* strains (RAPD type 9) was recently shown to reside in several independent fish processing plants. Persistent strains are likely to contaminate food products, and it is important to determine their virulence potential to evaluate risk to consumers. We compared the behaviour of food processing persistent and clinical *L. monocytogenes* strains in four virulence models: Adhesion, invasion and intracellular growth was studied in an epithelial cell line, Caco-2; time to death in a nematode model, *Caenorhabditis elegans* and in a fruit fly model, *Drosophila melanogaster* and fecal shedding in a guinea pig model. All strains adhered to and grew in Caco-2 cells in similar levels. When exposed to 10^6 CFU/ml, two strains representing the persistent RAPD type 9 invaded Caco-2 cells in lower numbers (10^2–10^3 CFU/ml) as compared to the four other strains (10^4–10^6 CFU/ml), including food and human clinical strains. In the *D. melanogaster* model, the two RAPD type 9 strains were among the slowest to kill. Similarly, the time to reach 50% killed *C. elegans* worms was longer (110 h) for the RAPD type 9 strains than for the other four strains (80 h). The Scott A strain and one RAPD type 9 strain were suspended in whipping cream before being fed to guinea pigs and the persistent RAPD type 9 strain was isolated from feces in a lower level (approximately 10^2 CFU/g) than the Scott A strain (approximately 10^5 CFU/g) ($P<0.05$). The addition of NaCl has been shown to cause autoaggregation and increases adhesion of *L. monocytogenes* to plastic. However, growth in the presence of NaCl did not alter the behaviour of the tested *L. monocytogenes* strains in the virulence models.

Overall, the two strains representing a very common fish processing plant persistent group (RAPD type 9) appear to have a lower virulence potential in all four virulence models than Scott A and a strain isolated from a clinical case of listeriosis.

Keywords: *Listeria monocytogenes*; Caco-2 cells; *Drosophila melanogaster*; *Caenorhabditis elegans*; Guinea pig; Persistence

1. Introduction

Listeria monocytogenes is a gram-positive pathogenic bacterium, which can cause listeriosis (meningitis, septicaemia) in humans. The fatality rate is very high at approximately 25–30% (Farber and Peterkin, 1991), but this foodborne disease is rare and affects primarily immunosuppressed people, or during pregnancy, the developing fetus. The vehicles of infection are typically ready-to-eat food products (Rocourt, 1996; Vazquez-Boland et al., 2001), in which the organism can grow to high numbers.

L. monocytogenes has a remarkable ability to reside in the food processing environment (Autio et al., 1999; Norton et al., 2001b; Rørvik et al., 1995; Vogel et al., 2001a), and specific molecular subtypes can repeatedly be isolated from the

* Corresponding author. Tel.: +45 45 25 49 09; fax: +45 45 88 47 74.
E-mail address: aje@aqua.dtu.dk (A. Jensen).

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processing environment (Wulff et al., 2006). We recently demonstrated that a particular Random Amplified Polymorphic DNA (RAPD) type (RAPD type 9) was found as a persistent type in several fish processing facilities (Wulff et al., 2006), although, this RAPD type is not common in the outside environment (Hansen et al., 2006). The reason for the persistence of this particular subtype is not known.

From a risk analysis perspective, it is important to assess the virulence potential of strains that are very likely contaminants of food products, such as strains persisting in the food processing environment. Several *in vitro* models and animal models have been used to investigate pathogenicity and virulence of *L. monocytogenes*. *In vitro* models using tissue culture cell lines such as the epithelial cell line Caco-2 have simplified the study of particular virulence functions and are widely used to compare adhesion, invasion and intracellular growth of different strains. A more complete analysis of virulence is obtained using animal models, and for the study of *L. monocytogenes*, the mouse and the guinea pig models have been used (Andersen et al., 2007; Bakardjiev et al., 2004; Dustoor et al., 1977; Garner et al., 2006a; Lecuit et al., 2001b; Takeuchi et al., 2006; Williams et al., 2007). In contrast to the guinea pig, the mouse model has some limitations for oral infections as the E-cadherin receptor is different from the human and guinea pig E-cadherin (Lecuit et al., 2001a) and *L. monocytogenes* Internalin A does not bind properly to the mouse E-cadherin.

To avoid the ethically controversial animal models, simpler eukaryotic models have recently been developed to study host–pathogen interactions. The fruit fly model, *Drosophila melanogaster*, was introduced by Mansfield et al. (2003) for *L. monocytogenes* and the nematode *Caenorhabditis elegans* appears to be an appropriate host for *L. monocytogenes* infection (Thomsen et al., 2006), since non-virulent mutants kill the worms more slowly than the wild-types.

The purpose of the present study was to determine if strains representing the common persistent RAPD type isolated from fish processing environments have a higher or lower virulence potential than clinical strains. This was done using an array of virulence model assays and, hence, the study also serves as an intercomparison of these different models. Further, the role of NaCl in the bacterial growth medium for the adhesion, invasion and virulence potential of *L. monocytogenes* was investigated, as we previously have observed that addition of 2–5% NaCl to the growth medium dramatically changed cell aggregation and adhesion to plastic surfaces of *L. monocytogenes* (Jensen et al., 2007a).

2. Materials and methods

2.1. Bacterial stains and growth conditions

The behaviour of six strains of *L. monocytogenes* belonging to different serotypes, lineages and RAPD types in four virulence models was compared in this study (Table 1). N53-1 and La111 belongs to a group of genetically similar strains, which frequently dominates and persists in fish processing environments (Wulff et al., 2006). This group of strains appear phenotypically similar (Jensen et al., 2007a) and N53-1 and La111 were chosen as representatives for RAPD type 9. *L. monocytogens* EGD was obtained from Werner Goebel (1999) and Scott A was obtained from Campden Food and Drink Association (1989) and was both used as reference strains. Strain 7418 was isolated from spreadable sausages, and strain 4446 was isolated from a human case of listeriosis (Larsen et al., 2002). The strains also represent genetic lineage 1 (Scott A, 7418, 4446) and lineage 2 (N53-1, La111, EGD) and the three serotypes (1/2a, 1/2b, 4b) typically involved in disease.

Escherichia coli strain OP50 was used as food for the *C. elegans* nematodes, and as a negative control in both the *D. melanogaster* and the *C. elegans* models. *Listeria innocua* strain Div-A8 (culture collection, Department of Veterinary Pathobiology, University of Copenhagen) was used as a non-virulent *Listeria* control in the *D. melanogaster* model. For the sequencing of *inlA*, *L. monocytogenes* strains H13-1 and M103-1 belonging to RAPD type 9 (Jensen et al., 2007a) and strain LO28 were included.

Stock cultures were stored in −80 °C in a medium containing 4% (wt/vol) glycerol, 2% (wt/vol) skim milk powder and 3% (wt/vol) Tryptone Soya Broth (TSB) (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) and grown in Brain Heart Infusion (BHI) broth (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom), or TSB supplemented with glucose to a final concentration of 1% (wt/vol) with or without 5% NaCl (wt/wt) (Jensen et al., 2007a). The Caco-2 cell adhesion and invasion as well as the virulence potential of strains were studied with and without the addition of NaCl to the growth medium. Numbers of *L. monocytogenes* were determined by spread plating onto Palcam agar (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) or BHI agar (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) followed by two days of incubation at 37 °C. LB agar (Difco, Becton, Dickinson, Sparks, Md, USA) was used as grow medium for all bacterial strains in the *C. elegans* trials.

Table 1
Origin and subtype of *Listeria monocytogenes* strains used in the present study

Strain	Origin	Serotype	Lineage	RAPD-type	Reference
N53-1	Smoke house equipment	1/2a	2	9	(Wulff et al., 2006)
La111	Cold-smoked salmon	1/2a	2	9	(Vogel et al., 2001b)
EGD	EGD	1/2a	2	68	A
Scott A	Human, clinical	4b	1	72	B
7418	Spreadable sausage	1/2b	1	14	(Larsen et al., 2002)
4446	Human, clinical	4b	1	71	(Larsen et al., 2002)

A: The strain was kindly provided by Werner Goebel, University of Würzburg, Germany.
B: The strain was kindly provided by Campden Food and Drink Association, UK.