Activation of PrfA results in overexpression of virulence factors but does not rescue the pathogenicity of *Listeria monocytogenes* M7

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*Listeria monocytogenes* encodes a transcriptional activator, PrfA, to positively regulate the expression of virulence factors. Several mutations in PrfA (PrfA*) have been found to contribute to increased regulatory activity. Here, we describe a strain, M7, containing a PrfA*(G145S) that activates expression of virulence factors but with low pathogenicity. To study this contradictory relationship, we exchanged the prfA genes between strains EGDe and M7 (designated EGDe-prfAM7 and M7-prfAEGDe). The phospholipase B (PlcB) and listeriolysin O (LLO) activities were significantly upregulated in the strain EGDe-prfAM7 (PrfA*). Constitutive activation of PrfA potentiated virulence of the pathogenic strain EGDe, shown as increased adhesion and invasion as well as enhanced cell-to-cell spread in cultured cell lines. However, the strain M7, though PrfA-activated, had significant defects in these virulence-related phenotypes and low pathogenicity in the murine infection model, as compared with EGDe or EGDe-PrfAM7. To further uncover the possible mechanisms, we analysed abundance and distributions of InlA, InlB, LLO and ActA proteins, all regulated by PrfA, in EGDe, M7 and their prfA mutants. Western blotting showed that the PrfA-regulated genes of constitutively activated PrfA strains were overexpressed in vitro, while different distributions were observed. In contrast to the virulent strain EGDe-prfAM7, the majority of InlB in M7 was detected in the culture supernatant and not on the bacterial surface. We suppose that the low virulence of strain M7 is due to its defects in infecting host cells, possibly as a result of failed anchorage on the bacterial cells of surface proteins like InlB, a major protein involved in adhesion and invasion of pathogenic *L. monocytogenes* strains. Further research is warranted to address why InlB detaches from the bacterial cells of this particular strain.

INTRODUCTION

*Listeria monocytogenes* is a food-borne pathogen that can be isolated from a variety of environments, including soil, water, animal farms and food-processing surfaces (Gray & Killinger, 1966; Liu et al., 2006; Velge & Roche, 2010). The bacterium can cause life-threatening infections in pregnant females, newborns and immune-compromised individuals via ingestion of contaminated foods, with high mortality (Farber & Peterkin, 1991). It is considered a severe threat to public health (Hamon et al., 2006).

*L. monocytogenes* encompasses a diversity of strains with varying degrees of pathogenicity, with lineage III strains the most diverse (Chen et al., 2010; Liu et al., 2006). Although lineage I strains (4b and 1/2b) and lineage II strains (1/2a) account for the majority of human listeriosis cases (Chen et al., 2009c; Swaminathan & Gerner-Smidt, 2007), lineage III strains still have potential to threaten human health because of their diversity in virulence.

**Abbreviations:** pi, post-infection; m.o.i., multiplicity of infection

A supplementary table is available with the online Supplementary Material.
The *L. monocytogenes* infection cycle comprises adhesion to and invasion of host cells, escape from phagocytic or internalized vacuoles, intracellular multiplication, and cell-to-cell spread (Vázquez-Boland *et al.*, 2001). The major internalins InlA and InlB mediate bacterial entry into intestinal epithelial cells or other types of cells via E-cadherin and c-Met, respectively (Bergmann *et al.*, 2002). Once inside the host cells, bacterial cells readily escape from the vacuoles upon activation of listeriolysin O (LLO) and phospholipase B (PlcB) under acidic conditions (Bavdek *et al.*, 2012). Following multiplication in the host cytoplasm, *L. monocytogenes* employs its surface protein ActA to recruit actins for spreading to neighbouring cells, thereby initiating a new infection cycle (Suárez *et al.*, 2001). The above virulence factors are delicately controlled by PrfA (Scotti *et al.*, 2007), a 27 kDa protein encoded by *prfA* and structurally related to the enterobacterial regulator Crp (cAMP receptor protein) family (Kreft & Vázquez-Boland, 2001). The roles of PrfA in pathogenicity and environmental adaptation of *L. monocytogenes* have been well studied (de las Heras *et al.*, 2011; Lemon *et al.*, 2010). Substitution of key amino acid residues of PrfA, such as I45S, E77K, L140F, G145S and G155S, can cause constitutive activation of virulence factors, leading to increased pathogenicity (Ripio *et al.*, 1997; Scotti *et al.*, 2007).

We attempted to elucidate mechanisms of low virulence of the lineage III strain M7 (Chen *et al.*, 2011b) by exchanging the *prfA* gene between strains M7 and EGDe and examining virulence-related phenotypes and expression of major virulence factors controlled by PrfA.

**METHODS**

**Bacterial strains, plasmids, cells and culture conditions.** *L. monocytogenes* EGDe and M7 (serovar 4a) were used as the wild-type strains. *Escherichia coli* DH5α was employed as the host strain for plasmids pMD18-T and pKSV7. *L. monocytogenes* strains were cultured in brain–heart infusion medium (BHI; Oxoid) and *E. coli* DH5α was grown at 37 °C in Luria–Bertani medium (LB; Oxoid). Stock solutions of ampicillin (50 mg ml⁻¹), gentamicin (50 mg ml⁻¹) and chloramphenicol (10 mg ml⁻¹) (Sangong Biotech) were added to media, where appropriate, at the required concentrations. Cells (macrophages RAW264.7, Caco-2, fibroblast L929 and HepG2) were cultured at 37 °C under 5 % CO₂ in complete RMPI 1640 medium (Gibco) containing 10 % FBS (Gibco).

**DNA manipulations.** Genomic DNA of *L. monocytogenes* was extracted as described previously (Chen *et al.*, 2009b; Jiang *et al.*, 2008). Oligonucleotide primers were synthesized by BGI-tech (China) (Table S1, available in the online Supplementary Material). PCR fragments and plasmids were purified using the DNA Gel Extraction kit and Unit-10 Plasmid Extraction kit (Sangong Biotech), and digested with defined restriction enzymes (Takara) to facilitate their insertion into vectors. Positive clones were then confirmed by PCR and sequencing.

**Construction of prfA replacement mutants.** A homologous recombination strategy was used for construction of replacement mutants of the full *prfA* fragment in *L. monocytogenes* wild-type strains M7 and EGDe according to protocols (Chen *et al.*, 2009a; Monk *et al.*, 2008) using the primer pairs PrfA-fwd and PrfA-rev (Table S1). The products containing EcoRI and HindIII sites were gel-purified and ligated to pMD18-T. After sequencing confirmation, the inserted fragments were digested and subcloned into the temperature-sensitive shuttle vector pKSV7 and transformed into DH5α. Plasmids containing inserts were subsequently extracted and electroporated into *L. monocytogenes* competent cells. Transformants were grown at a non-permissive temperature (41 °C) in BHI containing chloramphenicol (10 μg ml⁻¹) to promote chromosomal integration. The recombinants were passaged, in succession, in BHI without antibiotic at a permissive temperature (30 °C), to enable plasmid excision and curing (Camilli *et al.*, 1993). The recombinants were identified initially by phospholipase activity on yolk plates and confirmed by sequencing. The resulting mutants with *prfA* replacement between EGDe and M7 were designated EGDe-*prfA*M7 and M7-*prfA*EGDe, respectively.

**Transcriptional analysis.** Overnight cultures of *L. monocytogenes* wild-type and *prfA* mutant strains were inoculated into fresh BHI broth and grown to stationary phase (OD₆₀₀ 0.6) at 37 °C. A 1 ml volume of each culture was then pelleted by centrifugation at 4 °C and resuspended in 1 ml BHI broth or fresh whole blood (final bacterial level about 1 × 10⁸ c.f.u. ml⁻¹). The bacterial suspensions were incubated at 37 °C for 2 h. Total RNA was prepared using the Trizol method (Sangon Biotech) and cDNA was synthesized with reverse transcriptase (TOYOBO). Quantitative real-time PCR was performed in 20 μl reaction mixtures containing SYBR green qPCR mix (TOYOBO) to detect the transcriptional levels of several virulence genes on the iCycler iQ5 real-time PCR system (Bio-Rad) with specific primer pairs (Table S1). The housekeeping gene *gyrB* was selected as an internal control for normalization as previously described (Chen *et al.*, 2011a, 2013). The experiment was repeated three times.
Phospholipase and haemolysis assays. Phospholipase activity was performed to detect the activity of PlcB as described elsewhere (Ermolaeva et al., 2003; Jiang et al., 2006). Aliquots of the overnight cultures were plated on BHI agar containing 5% egg yolk suspension, followed by incubation at 37°C for 24 h. Listeriolysin O was tested for haemolytic activity as described (Jiang et al., 2006), using the overnight cultures on BHI agar containing 10% sheep blood.

Adhesion and invasion assays in Caco-2 and HepG2 cells. Overnight cultures were harvested by centrifugation (5000 g for 10 min), resuspended in 10 mM PBS (pH 7.4) and adjusted to OD600 0.25. The Caco-2 and HepG2 cells were grown at 37°C with 5% CO2 for 18–24 h, to confluence (about 2 × 105), in 12-well plates (Corning) and infected with L. monocytogenes at m.o.i. 10:1 for 1 h. For adhesion, cells were lysed after being washed three times with PBS. For estimation of invasion, cells were washed with PBS after 1 h incubation and incubated for an additional hour in RPMI 1640 medium containing 10% FBS and 200 μg ml⁻¹ gentamicin. At the indicated times, the cells were lysed and 10-fold diluted for plating on BHI agar. The agar plates were incubated overnight at 37°C for colony counting. Adhesion was expressed as the ratio of recovered colonies to colonies inoculated, while invasion was calculated as the ratio of colonies recovered after gentamicin treatment to colonies inoculated.

Survival in macrophage RAW264.7. Cells were infected with resuspended bacteria as above (m.o.i. 10:1) at 37°C with 5% CO2 for 1 h. Extracellular bacteria were killed with additional 1 h incubation in the presence of 200 μg ml⁻¹ gentamicin. Cells were lysed at 0, 1.5 and 3 h with ice-cold distilled water and diluted appropriately for plating on BHI agar plates and colony counting. Survival index was calculated for each strain by dividing the colony counts recovered at indicated time points with those at time zero (initial intracellular counts).

Plaque formation. Plaque-forming assay was performed on mouse fibroblast L929 cell monolayers in six-well plates (Corning) according to the method described previously (Gracieux et al., 2003; Sun et al., 1990). Briefly, overnight cultures were added to cell monolayers (approx. 80% confluence) at m.o.i. < 1:10 at 37°C with 5% CO2 for 1 h. Extracellular bacteria were then killed with 200 μg ml⁻¹ gentamicin as above. The cells were washed three times with 10 mM PBS and overlaid with 3 ml complete RPMI 1640 medium (without phenol red) containing 0.7% agarose and 20 μg ml⁻¹ gentamicin. After 3 days of incubation, plaques were visualized by adding an additional 2 ml RPMI 1640 medium containing 0.7% agarose and 0.01% filter-sterilized neutral red (Sigma). The diameter of plaques was measured by Adobe Photoshop software (version 6) for each strain. The plaque size of strain EGDe was set as 100% and data are shown as mean ± SD.

Actin-tail formation in Caco-2 and macrophage cells. The Caco-2 and RAW264.7 cells were cultured in complete RPMI 1640 medium for 12 h. Overnight bacterial cultures were treated as above, with OD600 adjusted to 0.25. Cells were infected at m.o.i. 10:1 at 37°C with 5% CO2 for 1 h. Extracellular bacteria were then killed with 200 μg ml⁻¹ gentamicin for 1 h and incubated for an additional 5 h. Cells were washed gently with 10 mM PBS, fixed with 4% paraformaldehyde and then permeabilized with 0.5% Triton X-100. The bacterial cells were stained with polyclonal antibodies to L. monocytogenes for 1 h at 37°C, washed twice with PBS and probed with Alexa Fluor 488-conjugated donkey anti-rabbit antibody (Abcam) for 1 h at 37°C. F-actin was then stained with 6.6 μM phalloidin-Alexa Fluor 568 (Abcam). DAPI was stained for 5 min.

Fig. 2. Virulence analysis of L. monocytogenes M7 and EGDe in ICR mice by intragastric infection. Bacterial load in liver (a) and spleen (b) were enumerated at 24 and 48 h post-infection. Survival (c) was recorded daily for 7 days post-infection. Values were expressed as mean ± SD (n=8); *P < 0.05, **P < 0.01.

Fig. 3. Phospholipase activity on 5% yolk BHI agar plate (a) and haemolytic activity on 10% sheep blood BHI agar of L. monocytogenes M7, EGDe, and their prfA mutant strains.
(4’,6-diamidino-2-phenylindole; Invitrogen) was used to stain the nuclei.
Actin tails recruited by the bacteria were visualized using a confocal microscope (FLV 1000; Olympus, Japan).

**Cytotoxic assays based on LDH release and Live/Dead kit.**
Overnight cultures were treated as above and adjusted to OD_{600} 0.6. Caco-2 cells were cultured in RPMI 1640 medium (no phenol red) in 96-well plates and infected with *L. monocytogenes* at m.o.i. values 10 : 1, 100 : 1 and 1000 : 1 for 2 h at 37 °C with 5 % CO_{2}. Positive and mock controls were included by adding cell lysis agent and culture medium, respectively. Relative cytotoxicity (%) was determined using the formula \(\frac{(OD_{test} - OD_{blank})}{(OD_{positive} - OD_{blanks})} \times 100\%\), according to the manufacturer’s instructions (CytoTox 96; Promega).

Caco-2 cells were infected at m.o.i. values 10 : 1, 100 : 1 and 1000 : 1 at 37 °C with 5 % CO_{2} for 1 h, and then the extracellular bacteria were killed with 200 µg ml^{-1} gentamicin for 1 h and incubated for an additional 12 h. Live cells were stained with the SYTO 9 and propidium iodide mixture from the Live/Dead kit (Invitrogen) according to the manufacturer’s instructions. Live cells with intact membranes were green, while dead cells with damaged membranes were red.

**Virulence in mouse model.** The assay was conducted as previously reported (Liu, 2004), with modifications. Female ICR mice weighing 20–22 g (Zhejiang College of Traditional Chinese Medicine), eight per group, were acclimatized for 3 days in a standard class II laboratory...
animal facility. Overnight cultures were treated as above, with OD600 adjusted to 0.6. Mice were inoculated intragastrically with $2 \times 10^8 \text{ c.f.u.}$ bacteria. At 24 and 48 h post-infection (pi), mice were euthanized, and liver and spleen samples were homogenized and diluted appropriately for enumeration on BHI agar plates. Mice were observed for mortalities twice a day for 7 days. For estimation of LD$_{50}$ in mice, strains M7, EDGe, EGDe-prfA M7 and M7-prfA EGDe were inoculated intraperitoneally into groups of mice as described elsewhere (Jiang et al., 2006), and inoculated mice were observed for 7 days. LD$_{50}$ values were calculated by the trimmed Spearman–Karber method. For in vivo competition assay between strains or mutants, mice were infected with similar amounts of bacteria (sublethal dose) by intraperitoneal injection. Mice were euthanized at 48 or 72 h pi. Liver samples were homogenized and serially diluted for enumeration on BHI agar with 5% glycoyl. PrfA* strains with constitutive PrfA expression (M7 and EGDe-prfAEGDe) could be differentiated from their non-constitutive expression counterparts (EGDe and M7-prfA EGDe) by phospholipase activity in the total counts recovered from the liver samples. The recovered counts of lipase-positive and lipase-negative colonies at indicated time points were first normalized with respect to their initial inocula, and the relative percentage of each colony type (representing the population of corresponding strain or mutant) was then calculated for the total counts (total population), which was set as 100%.

Statistical analysis. All results are presented as mean $\pm$ SD of triplicate experiments and were subjected to one-way ANOVA. Differences were considered statistically significant at $P < 0.05$ or $P < 0.01$.

RESULTS

PrfA of the low-virulence strain M7 was constitutively activated

Sequence analysis has shown that PrfA of the serovar 4a strain M7 from milk has three amino acids (Ser145, Ala165 and Asn197) different from those of EGDe (Gly145, Thr165 and Lys197) (Chen et al., 2011b), indicating that the PrfA of M7 could be constitutively active for transcriptional regulation, seen as strong phospholipase and haemolytic activities (Ripio et al., 1997; Vega et al., 2004; Wong & Freitag, 2004). Transcriptional analysis of PrfA-regulated genes in BHI medium and whole sheep

Surface proteins analysed by SDS-PAGE and Western blotting.

Overnight cultures were incubated in 100 ml of fresh BHI medium (1:100 ratio) for 6 h at 37°C and harvested by centrifugation (15 000 g, 10 min). The supernatant samples were filtered through 0.22 μm filters (Millipore) and proteins were precipitated with trichloroacetic acid (final concentration 10%) at 4°C. Cell-wall-associated proteins were extracted from bacterial pellet with 1% SDS [30 mg wet weight (ml 1% SDS)$^{-1}$] at 37°C for 1 h. Protein samples were analysed by 12% SDS-PAGE. The secreted and surface-associated proteins InlA, InlB, LLO, Mpl and ActA were blotted and probed with respective polyclonal antibodies produced in our laboratory or with monoclonal antibody (ActA, a gift from Dr Xin’an Jian, Yangzhou University, China).

Statistical analysis. All results are presented as mean $\pm$ SD of triplicate experiments and were subjected to one-way ANOVA. Differences were considered statistically significant at $P < 0.05$ or $P < 0.01$.
blood showed that M7 did have a significantly higher expression than EGDe ($P<0.01$) (Fig. 1a,b). The intragastric infection model showed that the bacterial load in liver and spleen samples of M7-infected mice was significantly less than in their EGDe-infected counterparts at 24 and 48 h pi ($P<0.01$) (Fig. 2a,b). All mice infected with strain M7 remained alive during the observation period, while those with EGDe died within 1 week (Fig. 2c), suggesting that strain M7 is of low pathogenicity.

**PrfA constitutive activation-dependent phenotypes in prfA exchange mutants**

To reveal why M7 had low virulence with an activated PrfA, we exchanged prfA between M7 and EGDe. Phospholipase and haemolytic activities of EGDe-prfA$_{M7}$ were apparent as compared with its parent strain EGDe and the reverse was seen with M7-prfA$_{EGDe}$ (Fig. 3). The strains M7 and EGDe-prfA$_{M7}$ presented significantly higher cytotoxic activity than EGDe and M7-prfA$_{EGDe}$ (Fig. 4). These
results indicate apparent activation of plcB and hly by the M7 PrfA due to G145S substitution (Ripio et al., 1997; Vega et al., 2004; Wong & Freitag, 2004). At similar inoculum levels in mice simultaneously infected with the two competition strains, the M7 load in liver samples was about 19.1 and 0.2 % of the total bacterial counts at 48 and 72 h pi, respectively, far less than the EGDe strain (P < 0.01) (Fig. 5a). Mutant EGDe-prfAM7 (PrfA constitutive activation) had a higher bacterial load than that of EGDe at 72 h pi (P < 0.05) (Fig. 5b). However, replacement of the M7 strain PrfA with PrfAEGDe did not change the ratio between the mutant and its parent strain M7 (Fig. 5c). The pathogenicity of strain M7 remained low irrespective of prfA exchange, while strain EGDe-prfAM7 became even more pathogenic than its parent strain (Fig. 5d). These data suggest that constitutive activation of PrfA in M7 did not play a role in its low pathogenicity.

**Defects of the M7 strain in virulence-related phenotypes**

The epithelial cell line Caco-2 and hepatocytes HepG2 were used to compare M7 and EGDe as well as their mutants in terms of adhesion, invasion, intracellular survival and intercellular spread. EGDe generally had more efficient adhesion and invasion than M7 in Caco-2 but not in HepG2 cells (Fig. 6). The EGDe-prfAM7 mutant, but not the M7-prfAEGDe strain, was significantly more adhesive and invasive than the parent strain in both cell lines (Fig. 6a–d). There was no significant difference in intracellular survival as a result of the prfA exchange (Fig. 6e). Exchange of the prfA gene between the strains did not change the plaque sizes in the cell-to-cell spread assay (Fig. 7a), while M7 showed smaller plaque sizes than EGDe. This was further confirmed in the actin-tail formation assay where EGDe and EGDe-prfAM7 were able to form longer actin tails both in epithelial cells and in macrophages than the M7 and M7-prfAEGDe strains (Fig. 7b, c). These results might suggest that infection defects of the M7 strain could be due to its low efficiency in entering the host cells and subsequent cell-to-cell spread.

**Overexpressed InlB of strain M7 failed to anchor on the bacterial surface**

Since InlA, InlB and ActA are responsible for adhesion, invasion and intercellular spread, we compared the distribution of these proteins on the bacterial surface and in
culture supernatants. Fig. 8(a) shows that these proteins from M7 and M7-prfA EGDe were generally fewer in number than those of EGDe and EGDe-prfA M7. InlA, InlB, LLO, Mpl and ActA were all overexpressed in M7 and EGDe-prfA M7 (Fig. 8b). However, the majority of InlB in M7 was present in the supernatant instead of anchored on the cell wall (Fig. 8c,d), a clear contrast with EGDe-prfA M7, which showed an elevated amount of InlB in the surface-associated fraction though the supernatant also contained a higher level.

**DISCUSSION**

*L. monocytogenes* is an environmental saprotroph that becomes a pathogen following ingestion by mammalian hosts. Yet, not all *L. monocytogenes* strains have the same pathogenicity, because transition from environmental organism to pathogen requires significant changes in expression of virulence factors, especially for PrfA-regulated genes (de las Heras et al., 2011; Freitag et al., 2009). Constitutive activation of PrfA results in overexpression of virulence factors, including the LIPI1 cluster gene products and major internalins (InlA and InlB) (Bruno & Freitag, 2010). However, the relationship between virulence and PrfA activation remains unclear (Lauer et al., 2008; Ripio et al., 1997; Vega et al., 2004; Wong & Freitag, 2004).

Glomski et al. (2003) demonstrated that mutants failing to compartmentalize LLO activities were cytotoxic and of low virulence. In a more recent study by Mariscotti et al. (2012), an activated prfA mutant was found to be hyper-virulent in animal infection.

We found that the M7 strain had apparent phospholipase activity due to high-level expression of PlcB, probably as a result of constitutive activation of PrfA (Chen et al., 2011b). Transcription levels of major PrfA-regulated genes in M7 were higher than in EGDe, both in broth media and in whole blood, while M7 did not cause any lethality to mice and its load in the liver and spleen was far lower than for EGDe. Intraperitoneal infection with a mixture of the two strains showed a significant difference in virulence.
strains (M7 and EGDe) also showed lower recovery of M7 from the liver than of EGDe. This prompted us to postulate that the low virulence of M7 might result from exposure of the bacterial cells to the host immune system owing to high cytotoxicity from LLO and PkB overexpression.

With M7 as a low-virulence model strain, we attempted to define the relationship between PrfA activation and virulence by exchanging the prfA gene between M7 and EGDe. The pathogenicity of the M7-prfAM7EGDe mutant remained low in the murine infection model and in cultured cells, while the EGDe-prfAM7 mutant became more cytotoxic, adhesive and invasive in cultured cell lines and was more virulent than its parent strain in mice as well (Figs. 4–6). These results suggest that constitutive expression of PrfA exacerbates the virulence of pathogenic strains, as was seen in the strain 10403S by Bruno & Freitag (2010). With M7, there should be other mechanisms for its low virulence because the strain had apparent defects in adhesion, invasion and cell-to-cell spread irrespective of overexpression of major virulence factors in the LIP11 cluster.

Analysis of surface proteins and culture supernatants showed that PrfA activation (EGDe-prfAM7 or M7) did result in overexpression of PrfA-regulated genes, including InlA, InlB, LLO, Mpl and ActA (Fig. 8a,b). As InlA is covalently bound on the cell wall via its LPXTG motif (Cabanes et al., 2002; Mariscotti et al., 2012), only a trace amount was present in the supernatants regardless of PrfA activation. InlB mediates entry into several kinds of host cells via c-Met or Gc1q-R (Bierne & Cossart, 2002; Braun et al., 2000; Dramsi et al., 1995; Gründler et al., 2013); it plays a key role in Listeria entry into epithelial cells, where junctional endocytosis could be accelerated by activating c-Met at multicellular junctions (Pentecost et al., 2010). However, the majority of InlB in M7 was present in the culture supernatant instead of anchored on the bacterial cell wall, which was quite different from EGDe-prfAM7, where overexpressed InlB was still predominantly on the cell wall (Fig. 8c,d). This might be the reason for low adhesion to and invasion of both Caco-2 and HepG2 cell lines by strain M7. Further research is needed to address why InlB detaches from the bacterial cells of M7. One possibility could be aberrant binding interaction with cell-wall components such as lipoteichoic acid via its GW motif (Jonquières et al., 1999).

In summary, the low virulence of strain M7 is due to its defects in infecting host cells, probably as a result of failed anchorage on the bacterial cell walls of surface proteins like InlB, a major protein involved in adhesion and invasion of pathogenic L. monocytogenes strains. With the virulent strain EGDe, overexpression of virulence factors owing to PrfA activation enhances its pathogenicity.

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