INTRODUCTION

Listeria monocytogenes is a Gram-positive, non-spore-forming, rod-shaped, facultative, intracellular bacterium that causes rare but frequently fatal infections, termed listeriosis. Symptoms of systemic listeriosis include meningitis, meningoencephalitis, septicaemia, gastroenteritis, perinatal infections and spontaneous abortion (Schlegel & Acheson, 2000). Despite efficient antibiotic therapy, listeriosis can be fatal in immune-compromised people and pregnant women (Kuhn et al., 2011; Fallah et al., 2003; Lee et al., 2005). Although human listeriosis occurs only sporadically, several outbreaks have been observed. The emergence of antibiotic-resistant L. monocytogenes poses a major and immediate threat to public health (Charpentier & Courvalin, 1999; Walsh et al., 2001; Sakaridis et al., 2011; Fallah et al., 2012).

Transport of proteins across the cell membrane is an important process and often the determines survival and success of a pathogen (Lee & Schweind, 2001). Twin-arginine translocase (Tat), widely observed in plants, bacteria and archaea, is a unique system that transports folded proteins across the cellular membrane (Berks et al., 2003; Lee et al., 2006). Tat target proteins possess a signal peptide with a consensus sequence, SRRXFLK, where there are two signature arginine residues and where X could be any polar amino acid (Yahr & Wickner, 2001; Berks et al., 2003). Tat-substrates include redox-enzymes, multimeric proteins that have to assemble into a complex prior to export, certain membrane proteins and proteins incompatible with Sec-export machinery (Lee et al., 2006). The Tat-pathway is used to different extents in various bacteria (Rose et al., 2002; Dilk et al., 2005; Li et al., 2005). In many pathogenic bacteria the Tat-system is essential for virulence (Ochsner et al., 2002; Barker et al., 2004; McDonough et al., 2005; Posey et al., 2006; Voulhoux et al., 2006). In L. monocytogenes only two proteins, iron-dependent peroxidase (lmo0367) and β-ketoacyl carrier protein synthase II (lmo2201), are predicted to be transported by the Tat-system (Desvaux & Hebrard, 2006).

A Tat-pathway has been reported in L. monocytogenes (Alami et al., 2003; Dilk et al., 2005), though its functionality has yet to be investigated. In contrast to Bacillus subtilis, where some paralogues of tatC and tatA are present, only one copy of each of these genes exists in L. monocytogenes (Jongbloed et al., 2002, 2004). In silico analysis of the listerial genome revealed the presence of tatA (lmo0362) and tatC (lmo0361) genes as a tatAC operon with a Fur-repressor binding sequence, the Fur box, in its promoter (Ledala et al., 2010). The Fur-repressor plays a central role in iron homeostasis by regulating fur-regulons in response to iron availability. Iron is critical for...
bacterial survival through the regulation of key enzymes, haem transporters and siderophore production (Hantke, 2001).

Transcriptomic analysis of a L. monocytogenes fur mutant revealed that the tatC, tatA and lmo0367 genes are highly expressed in response to a fur mutation or upon iron limitation (Ledala et al., 2010). Lmo0367 is homologous to the iron-dependent peroxidase (FepB) in Staphylococcus aureus, which is translocated by the Tat-pathway in this organism (Biswas et al., 2009). In L. monocytogenes, FepB has a putative twin-arginine signal peptide and is predicted to be encoded by the fepB gene, which is a member of the fepCAB operon that also has a Fur box in its promoter. Other genes in this operon encode a putative ‘ferrous-iron’ permease (FepC) and a high-affinity iron-binding lipoprotein (FepA). The proteins encoded from both operons have predicted transmembrane domains. Recently, the importance of the fepCAB operon in L. monocytogenes was established by McLaughlin et al. (2012), who demonstrated that the fepC mutant was severely attenuated in virulence.

Expression of genes encoding virulence factors in L. monocytogenes is influenced by various environmental factors such as carbon source (Freitag et al., 2009), temperature (Shen & Higgins, 2006; McGann et al., 2007) and iron availability (Conte et al., 1996). Several studies have shown that the concentration of intracellular iron influences the transcription rates of over 200 genes in bacteria (Ochsner et al., 2002; Camejo et al., 2009; Ledala et al., 2010). Due to the low availability of iron in the host, bacteria have evolved various iron acquisition systems such as citrate-inducible ferric uptake (Adams et al., 1990; Andrews et al., 2003), a cell surface transferring binding protein (Hartford et al., 1993), an ABC transporter for HN/Hb, ferric hydroxamates (flu), haemin/haemoglobin (hup) (Brown & Holden 2002; Faraldo-Gómez et al., 2003; Jin et al., 2006; Weinberg, 2009) and extracellular and/or surface-associated iron reductases (McLaughlin et al., 2011). Haemin/haemoglobin uptake by HupDGc and ferrichrome siderophore by Flu systems have already been characterized (Jin et al., 2006; Xiao et al., 2011; McLaughlin et al., 2012). Although Listeria does not produce siderophores, it is able to obtain iron using either exogenous siderophores produced by other micro-organisms or natural catechol compounds widespread in the environment (Jin et al., 2006; Xiao et al., 2011). Physiological data suggest the existence of strong surface-associated ferric reductase activity in L. monocytogenes (Deneer et al., 1995; Cowart, 2002), but no genetic determinant for the enzyme has been identified in its genome. The reduction of iron from ferric to ferrous is critical because ferric iron is insoluble and is not possible to mobilize in its unbound state. Recently, fepABC in Staph. aureus was shown to be involved in iron-uptake (Biswas et al., 2009). Furthermore, a ywBN (a fepB homologue) mutant of B. subtilis was unable to grow in the presence of ferric ions but grew in the presence of ferrous, suggesting its possible role as a ferric reductase (van der Ploeg et al., 2011). The homologous EfeB protein in Escherichia coli O157 has been shown to reduce ferric iron (Liu et al., 2011) and transport ferrous iron by the EfeUOB tripartite complex (Cao et al., 2007). In this study we propose and provide evidence to show that fepB encodes a ferric reductase enzyme, which is translocated by the Tat-translocase onto the cell surface, and thus plays an important role in the reductive iron uptake process in L. monocytogenes.

METHODS

Bacterial strains and growth conditions. Bacterial strains and plasmids used in this study are listed in Table 1. L. monocytogenes EDGe strain was used to construct mutations in the tatC and fepB genes. L. monocytogenes was grown in brain heart infusion (BHI) broth (Becton-Dickinson Diagnostic Systems). E. coli strains, Top10, BL21(DE3)/fur-pRSET-A and ET12567/pUZ8002 were grown in Luria–Bertani broth (LB) or agar (LBA). Yeast tryptone (YT) broth, manniitol soy agar and MMC minimal agar medium (Widdick et al. 2006) were used to culture Streptomyces lividans. L. monocytogenes and E. coli were incubated at 37 °C in a shaking incubator (250 r.p.m.) unless otherwise stated. Strep. lividans (tatC mutant and its isogenic parent strain) was grown at 30 °C.

In silico analyses of tat and fep operons. TatAC and FepCAB systems in L. monocytogenes were analysed in silico. The Fur boxes in tatAC and fepCAB operons were aligned to those of the consensus sequence (GATAATGATAATCATTATC; Baichoo & Helmann, 2002) using CLUSTAL W (http://www.genome.jp/tools/clustalw/). The putative signal peptide sequence analysis of the FepB protein was performed using TatP 1.0 Server (http://www.cbs.dtu.dk/services/TatP/) and NCBI BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Structural predictions of the Tat and Fep proteins were carried out using the DAS transmembrane prediction server (http://www.abc.su.se/~milkos/DAS/).

Purification of Fur protein. Fur protein was overexpressed and purified using E. coli BL21(DE3)/fur-pRSET-A as described previously (Ledala et al., 2007).

Electrophoretic mobility shift assay (EMSA). To examine specific interactions of Fur with the Fur box upstream of the tat-operon, EMSSAs were performed as described previously (Singh et al., 1999; Xiong et al., 2000). A set of primers (Table 2), PtatProm-F and PtatProm-R, was designed to amplify the 123 bp region of the tat promoter with 68 bp upstream and 36 bp downstream of the putative Fur box. The radioactive probe was prepared by PCR using [α-32P]dCTP as one of the nucleotide components in the reaction. The Fur protein was added to DNA binding buffer [20 mM Tris- acetate (pH 8.0), 1 mM MgCl2, 50 mM KCl, 5% glycerol, 10 μg salmon sperm DNA ml−1, 2 μg BSA ml−1, 1 mM DTT] in a final volume of 20 μl. After incubating for 15 min, 2 μl purified probe was added to the DNA binding buffer and incubated for an additional 15 min at room temperature. Loading dye (3 μl) was mixed with the reaction mixture and analysed by 7.5% native-PAGE. The effects of unlabelled tat promoter, Mn2+ and EDTA were also determined in other sets of reactions. The gels were subsequently dried and the DNA-protein complexes were visualized by autoradiography.

Reporter assay for Tat signal peptide on FepB. To evaluate the role of the Tat-transporter in translocation of FepB, an agarose translocation assay was performed as described by Widdick et al. (2008). Briefly, a forward primer (FepBsp-F) with an Ndel restriction site and a reverse primer (FepBsp-R) with a BamHI restriction site were used to amplify the first 189 nt of the fepB gene by PCR. The PCR fragment was ligated into the pGEM-T plasmid and transformed into the E. coli Top10 strain. The Ndel–BamHI fragment was purified
from the recombinant plasmid and subcloned into pTDW46. The resulting construct (fepBsp-pTDW46) was transferred into E. coli Top10 cells and transformants were selected on LBA containing 100 μg apramycin ml⁻¹. The spliced fepB in-frame with the agarase (dagA) gene that lacks its native signal peptide encoding sequence was identified by PCR using P_fepBsp-F and P_dagA-R primers.

Intergeneric conjugation was carried out to transfer the fepBsp-pTDW46 construct from E. coli ET12567/pUZ8002 into the wild-type and tatC mutant strain of Strep. lividans as described by Widdick et al. (2008). The transconjugant colonies were inoculated onto MMC minimal agar plates and incubated for 5 days at 30 °C. Lugol’s iodine solution (1 %) was added to the plates and incubated for 45 min to visualize agarase activity of the transconjugant Streptomyces isolates.

<table>
<thead>
<tr>
<th>Table 1. Bacterial strains and plasmids</th>
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<tr>
<td><strong>Strains</strong></td>
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<tr>
<td><em>L. monocytogenes</em></td>
</tr>
<tr>
<td>EDGe</td>
</tr>
<tr>
<td>EDGe ΔtatC</td>
</tr>
<tr>
<td>EDGe ΔfepB</td>
</tr>
<tr>
<td>E. coli Top10</td>
</tr>
<tr>
<td>BL21(DE3)/fur-pRSET-A</td>
</tr>
<tr>
<td>ET12567/pUZ8002</td>
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<tr>
<td><em>Strep. lividans</em></td>
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<tr>
<td>Parent strain</td>
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<tr>
<td>ΔtatC</td>
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</tbody>
</table>

| **Plasmids** | | |
| pGEM-T | PCR TA-cloning vector; CarbR | Promega |
| pAUL-A | ts-Vector; ErmR | Chakraborty et al. (1992) |
| tatCint-pAUL-A | pAUL-A with 280 bp amplicon from internal sequence of tatC gene | This study |
| fepBint-pAUL-A | pAUL-A with 284 bp amplicon from internal sequence of fepB gene | This study |
| pTDW46 | A derivative of [aac3(IV) bla lacZ rep63 oriT] | Widdick et al. (2006) |
| fepBsp-pTDW46 | pTDW46 with fepB signal peptide encoding sequence ligated in-frame with agarase gene | This study |

CarbR, carbenicillin resistance; ErmR, erythromycin resistance.

Table 2. Primers used in the study

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence* (5’ to 3’)</th>
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<tbody>
<tr>
<td>PtatProm-F</td>
<td>GAAGCGCTTAAGTTTAA</td>
</tr>
<tr>
<td>PtatProm-R</td>
<td>TGATGCAGATCATTTTGG</td>
</tr>
<tr>
<td>PtatCint-F</td>
<td>GATCGGATCCCGAGCTTTTCAAATCTATAC (BamHI)</td>
</tr>
<tr>
<td>PtatCint-R</td>
<td>GATCGAATTCAATGAAAATAATCTCGAGCAGAC (EcoRI)</td>
</tr>
<tr>
<td>PtatC-F</td>
<td>GTGCGAAGAGTCTCATGAG</td>
</tr>
<tr>
<td>PtatC-R</td>
<td>TTAGAATCTTGTTTCCATTTCCA</td>
</tr>
<tr>
<td>PfepBint-F</td>
<td>GATCGGATCCCGAGCGCTTTTCAAATCTATAC (BamHI)</td>
</tr>
<tr>
<td>PfepBint-R</td>
<td>GATCGGATCCCGAGCGCTTTTCAAATCTATAC (KpnI)</td>
</tr>
<tr>
<td>PfepB-F</td>
<td>GATCGGATCCCGAGCGCTTTTCAAATCTATAC (BamHI)</td>
</tr>
<tr>
<td>PfepB-R</td>
<td>GATCGGATCCCGAGCGCTTTTCAAATCTATAC (KpnI)</td>
</tr>
<tr>
<td>PfepBsp-F</td>
<td>GATCGGATCCCGAGCGCTTTTCAAATCTATAC (NdeI)</td>
</tr>
<tr>
<td>PfepBsp-R</td>
<td>GATCGGATCCCGAGCGCTTTTCAAATCTATAC (BamHI)</td>
</tr>
<tr>
<td>PdagA-R</td>
<td>CTACACGCAGCTGATACGTCC</td>
</tr>
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*Underlined nucleotides represent the restriction endonuclease sites for the corresponding restriction enzymes shown in parentheses.
vector, pAUL-A (Chakraborty et al., 1992), as described by Ledala et al. (2010). An internal 280 bp portion of tatC was PCR amplified using a forward primer (PtatCint-F) with a BamHI restriction site and a reverse primer (PtatCint-R) with an EcoRI restriction site. The PCR fragment was ligated into the pGEM-T vector and transformed into competent E. coli Top10 strain. The recombinant plasmid was extracted from a transformant, digested with BamHI/EcoRI and electrophoresed. The fragment was gel-purified and then ligated into the pAUL-A vector. The ligated construct (ptatC-pAUL-A) was transformed into E. coli Top10 and transformants were selected on an LB plate containing 100 μg erythromycin ml⁻¹. Recombinant plasmid (tatC-pAUL-A) was isolated and transformed into L. monocytogenes by electroporation as described by Park & Stewart (1990). The transformants were inoculated into 2 ml BHI broth containing 3 μg erythromycin ml⁻¹ and incubated at 30 °C for 6 h. The culture was diluted in BHI and plated on BHA containing 3 μg erythromycin ml⁻¹. The plates were incubated at 42 °C to allow the bacteria to integrate pAUL-A into the genome at the tatC gene by homologous recombination. A few selected colonies were serially passaged by restreaking on BHA plates and incubating at 42 °C. Insertional disruption of the tatC gene in the mutant was confirmed by checking the absence of the 735 bp fragment by PCR using PtatC-F and PtatC-R primers. Similarly, a L. monocytogenes fepB mutant was constructed. Briefly, primers PfepBint-F with a BamHI restriction site and PfepBint-R with a KpnI restriction site were used to amplify 284 bp within the fepB gene. Thus, fepB-pAUL-A was constructed and transformed into wild-type Listeria. The mutation was confirmed by PCR using PfepB-F and PfepB-R primers to check for the absence of 1266 bp from fepB in the genome of the mutant.

**RESULTS AND DISCUSSION**

### In silico analysis of Tat and Fep systems

In silico analyses revealed that the tatAC and fepCAB operons are close to each other but on complementary strands in the genome of L. monocytogenes (Fig. 1). Both operons possess a characteristic 19 bp Fur box in their promoters. Only two nucleotides in the tat promoter and one in the fep promoter are different from that of the consensus sequence. The difference in all cases is either TA or AT pairs. Similar organizations of operons are predicted in Listeria innocua, with identical Fur boxes in the promoters and signal peptide in FepB to that of L. monocytogenes. However, tatAC and fepCAB are not predicted in other Listeria species (Listeria grayi, L. ivanovii, L. sedligeri, L. murrayi, L. bulgarica, L. perluemolytica, L. fleischmannii and L. welshimeri). The putative amino acid sequences from the operons are 50 to 70 % homologous to those of B. subtilis, Staph. aureus and E. coli. All members of the two operons are predicted to encode transmembrane proteins. Based on sequence homologies, TatC is predicted to be a 28 kDa protein exhibiting six transmembrane helices and bears the site of recognition for the twin arginine motif, whereas tatA is predicted to encode a 6.3 kDa protein with a single transmembrane helix. FepC is predicted to be a 54 kDa iron permease; FepA a 43 kDa lipoprotein; and FepB a 47 kDa peroxidase protein possessing a tat signal peptide (Fig. 1).

### Interaction between tatAC promoter and Fur

To determine whether Fur binds to the Fur box sequences in the promoter of the tat gene, EMSAs were performed. The [z-³²P]dCTP-labelled tat promoter was run in a native-PAGE with or without varying concentration of Fur protein (Fig. 2a). As shown in Fig. 2(a), the probe was shifted in the presence of Fur protein as observed in an earlier study (Ledala et al., 2007). The lowest concentration...
of Fur to shift the probe was found to be 190 nM. To determine the specificity of this binding, a competition assay was performed in the presence of unlabelled probe DNA. As shown in Fig. 2(b) (lanes 3 and 5), the addition of unlabelled promoter rescued the shift in mobility of the probe, indicating that Fur binding to the promoter is sequence specific. The interaction between the protein and the probe was not affected by the presence of Mn\(^{2+}\), salmon sperm DNA or EDTA, as observed by Ledala et al. (2007).

**Tat-signal peptide in FepB**

FepB is one of the two predicted Tat-translocase targets in *L. monocytogenes* (Desvaux and Hebrard, 2006). *In silico* analysis revealed the FepB protein to possess twin-arginine signature sequences with a Tat-recognizable signal peptide. Though a putative position is located in between the fortieth and forty-first amino acids, there is no definitive signal cleavage site and listerial FepB is unlikely to be a secretory protein. To demonstrate functionality of the tat-signal peptide in FepB, a heterologous agarase reporter assay was performed as described by Widdick et al. (2006, 2008). The Tat signal peptide encoding nucleotides of listerial *fepB* was ligated in-frame with an agarase gene and the construct mobilized into the *tatC* mutant of *Strep. lividans* and its isogenic parent strain. The strains were then tested for their agar-hydrolysing ability on minimal agar. As expected, the parent *Strep. lividans*, but not its *tatC* mutant, was able to hydrolyse agar as observed by halo formation not stained with iodine around the colony on minimal agar plate (Fig. 3). These results suggest that the signal peptide from *Listeria* was recognized by the Tat-translocase system in *Strep. lividans* and the agarase gene was successfully translocated through the membrane. Thus, the reporter system clearly demonstrated the presence of a functional Tat-signal peptide in FepB of *L. monocytogenes*.

**Ferric reductase activity in the tatC and fepB mutants**

*L. monocytogenes* has been shown to have a strong surface-associated ferric reductase enzyme (Deneer et al., 1995; Cowart, 2002). However, genome searches for homologous sequences encoding ferric reductase enzymes in other Gram-positive bacteria did not show any significant homology. To determine the role of the Tat-system and FepB in reductive iron uptake in *L. monocytogenes*, the *tatAC* and *fepCAB* operons were inactivated by mutating *tatC* and *fepB* genes. A significant reduction in surface-associated ferric reductase activity was observed in both mutants when compared with the isogenic parent strain (Fig. 4), suggesting their involvement in reduction of ferric to ferrous ion. As observed in other Gram-positive bacteria (Jongbloed et al., 2002, 2004; Biswas et al., 2009; van der Ploeg et al., 2011), TatC in *L. monocytogenes* is supposed to be a substrate-docking protein recognizing the twin-arginine motif of FepB that forms a TatC–FepB complex, which is translocated across the membrane through TatA.

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**Fig. 2. Mobility shift of the Fur-bound tat promoter.** (a) Effect of Fur concentrations on mobility of the probe. Lane 1, probe; lanes 2 to 6, twofold increment in Fur protein (23.75 to 380 nM) along the lanes to the same level of the probe. (b) Effect of unlabelled DNA, Mn\(^{2+}\) or Mn\(^{2+}\) and EDTA on binding of Fur to the probe. Lane 1, probe; lanes 2 to 7, probe and 190 nM Fur with or without unlabelled DNA or Mn\(^{2+}\) or EDTA.

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**Fig. 3. Reporter assay.** Agarase activities of isogenic parent strain (a) and *tatC* mutant (b) *Strep. lividans* transconjugants containing recombinant vector (*fepBsp*-pTDW46) were investigated by growing the transconjugants on a minimal agar plate and visualization with 1% Lugol’s iodine. The parent strain hydrolysed the agar around its colony and formed a halo, whereas the *tatC* mutant was unable to hydrolyse agar around its colony.
A mutation in \(\text{tatC}\) most likely results in the inability of TatC to translocate FepB, hence a lower ferric reductase activity; whereas a mutation in \(\text{fepB}\), which we propose encodes a ferric reductase, results in loss of ferric reductase activity. The inability to detect ferric reductase activity in spent medium by Deener \textit{et al.} (1995) was probably due to the lack of a cleavage site in the FepB protein of \(L.\) \textit{monocytogenes} as predicted by \textit{in silico} analyses. The presence of internal integral transmembrane domains in FepB thus makes it a functional membrane-associated enzyme.

As mentioned above, \textit{Listeria} exhibits ferric reductase activity but lacks the gene encoding ferric reductase. Ferric reductase activity is indispensable and is required for organisms to generate ferrous ion, which is critical for the regulation of the Fur-repressor protein, a global iron regulator that maintains the intracellular iron concentration (Bagg & Neilands, 1987; Hantke, 2001; Lee & Helmann, 2006). Accumulation of iron above physiological levels causes the production of toxic free radicals by the Fenton reaction and is deleterious for cells. The \(\text{fepB}\) gene in \(L.\) \textit{monocytogenes} has been shown to be derepressed fourfold under iron-limiting conditions (Ledala \textit{et al.}, 2010). If \(\text{fepB}\) encodes the peroxidase then it should be theoretically repressed rather than de-repressed under iron-limiting conditions and induced under excess iron and oxidative stress due to iron toxicity (Bagg & Neilands, 1987). Therefore, we propose that FepB is likely to exhibit ferric reductase activity rather than peroxidase activity in \(L.\) \textit{monocytogenes}. Although Biswas \textit{et al.} (2009) observed peroxidase activity of the homologous FepB in \textit{Staph. aureus}, the importance of this redundant peroxidase activity is not understood.

**Fig. 4.** Ferric reductase activities of \(L.\) \textit{monocytogenes} strains. Reaction progress for the isogenic parent strain (triangles), \(\text{tatC}\) (squares) and \(\text{fepB}\) (diamonds) mutants. Error bars represent SD values based on three independent experiments.

**Fig. 5.** Proposed model for reductive iron uptake in \(L.\) \textit{monocytogenes} under iron-limiting conditions. Under iron-limiting conditions, the Fur-repressor is unable to repress the \(\text{tatAC}\) and \(\text{fepCAB}\) operons. Thus, the expression of \(\text{tatAC}\) forms a TatAC complex which inserts into the membrane. Following the expression of \(\text{fepCAB}\), FepB is translocated across the membrane by TatAC onto the cell membrane. FepC and FepA form a transport channel. As proposed, FepB acts as a ferric reductase enzyme, reducing ferric to ferrous ions, which subsequently bind to the iron-binding protein FepA and are internalized by the ferrous transporter, FepC. Since Fri is also overexpressed under iron-limiting conditions, this helps to stabilize ferrous iron in the cytosol. When ferrous iron is at sufficient concentrations in the cell, it binds to Fur and represses the \(\text{tatAC}\) and \(\text{fepCAB}\) operons to control iron uptake.
The *L. monocytogenes* genome possesses a putative gene, *lmo0983*, for a peroxidase enzyme with a consensus Per box in its promoter similar to that of *B. subtilis* and *Staph. aureus*. However, not much is known about its regulation. Fur and PerR negatively regulate the fur gene in *L. monocytogenes* (Ledala et al., 2010). The presence of a Fur box but not a Per box in the promoter of the *fepCAB* operon, *FepB* in *L. monocytogenes*, is likely to be associated with iron homeostasis. The TatC-mediated homologous FepABC system in *Staph. aureus* has been proposed to be involved in the iron uptake process (Biswa et al., 2009). It has been reported that the *fri* gene was also induced under iron-limiting conditions in *Listeria* (Polidoro et al., 2002; Ledala et al., 2010). The *fri* gene in *L. monocytogenes* is similar to the ferritin-like Dps protein in other bacteria, which binds to excess iron in the cytoplasm and protects bacteria from oxidative stress (Olsen et al., 2005). Based on our current understanding and together with the finding of *fur* bacteria from oxidative stress (Olsen et al., 2005),Coordinate regulation of virulence genes in *Listeria monocytogenes* requires the product of the prfA gene. *J Bacteriol* 174, 568–574.

**ACKNOWLEDGEMENTS**

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Tat system in iron uptake of Listeria monocytogenes


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