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Role of *ctc* from *Listeria monocytogenes* in Osmotolerance

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Listeria monocytogenes **is a food-borne pathogen with the ability to grow under conditions of high osmolarity. In a previous study, we reported the identification of 12 proteins showing high induction after salt stress. One of these proteins is highly similar to the general stress protein Ctc of *Bacillus subtilis*. In this study, induction of Ctc after salt stress was confirmed at the transcriptional level by using RNA slot blot experiments. To explore the role of the *ctc* gene product in resistance to stresses, we constructed a *ctc* insertional mutant. No difference in growth was observed between the wild-type strain LO28 and the *ctc* mutant either in rich medium after osmotic or heat stress or in minimal medium after heat stress. However, in minimal medium after osmotic stress, the growth rate of the mutant was increased by a factor of 2. Moreover, electron microscopy analysis showed impaired morphology of the mutant grown under osmotic stress conditions in minimal medium. Addition of the osmoprotectant glycine betaine to the medium completely abolished the osmotic sensitivity phenotype of the *ctc* mutant. Altogether, these results suggest that the Ctc protein of *L. monocytogenes* is involved in osmotic stress tolerance in the absence of any osmoprotectant in the medium.**

Listeria monocytogenes is a food-borne pathogen widely distributed in the environment. This microorganism is of particular concern in the food industry because of its ability to survive and frequently to grow under a wide range of adverse conditions used to preserve food such as low temperature, low pH, and high osmolarity (8). Growth of *L. monocytogenes* has been reported at NaCl concentrations as high as 10% (21).

Most bacteria cope with elevated osmolarity in the environment by intracellular accumulation of compatible solutes, called osmolytes (32). Among the compatible solutes efficient in *L. monocytogenes*, two quaternary amines, glycine betaine and carnitine, are the most effective (5, 34). The accumulation of these osmoprotectants in *L. monocytogenes* occurs through osmotic activation of their transport from the medium rather than through de novo synthesis. Accumulation of glycine betaine and carnitine occurs via at least two glycine betaine transporters encoded by the *betL* gene and the *gbu* operon (18, 30) and one carnitine transporter encoded by the *opuC* operon (2, 9, 33). A *betL* knockout mutant and a mutant of *gbu* obtained by transposition were significantly affected in their abilities to accumulate glycine betaine and were unable to withstand concentrations of salt as high as the isogenic parent strain can withstand (18, 30). Similarly, a mutant with an insertional inactivation of *opuCA* was defective in the uptake of carnitine and had impaired growth at high osmolarity (9, 33). Proline has been identified as an osmolyte for *L. monocytogenes*. The proline transport mechanism has not been characterized yet. However, the *proBA* operon, coding for the enzymes that catalyze the two first steps of proline biosynthesis, has recently been identified. Disruption of this operon significantly reduced the growth of the corresponding mutant at high salt concentrations (31). However, little information is available concerning other mechanisms that take place in *L. monocytogenes* to enable the organism to cope with osmotic stress, especially when osmolytes are not available in the environment.

In a previous study, by proteomic analysis and mass spectrometry or N-terminal sequencing, 12 proteins showing high induction after a salt stress were identified (7). One of these proteins is similar to the Ctc protein of *Bacillus subtilis*, a general stress protein which belongs to the L25 family of ribosomal proteins (12, 23, 35). In *B. subtilis*, the *ctc* gene is induced in response to osmotic, heat, and oxidative stress and glucose limitation (14, 41). Regulation of the expression of the *ctc* gene of *B. subtilis* occurs via the σ^B RNA polymerase subunit. The *ctc* promoter was one of the first σ^B-dependent promoters identified and for this reason has been extensively studied. It is the best-characterized σ^B-dependent promoter and has become the promoter of choice in nearly all investigations of σ^B regulation (13, 16, 42). In contrast to the wealth of information regarding the *ctc* promoter, the function of the *ctc* product itself in *B. subtilis* is less clear and seems to be dispensable. Only reduced sporulation efficiency at high temperatures has been observed in a *ctc* null mutant (15, 38).

To investigate the function of the Ctc protein in *L. monocytogenes*, especially with regard to the stress resistance of the bacterium, we analyzed the sequence of the corresponding gene and inactivated it by insertional mutation. Physiological studies indicate that the Ctc protein facilitates growth in minimal medium under conditions of high osmolarity and in the absence of an osmoprotectant. This is the first time that a role has been assigned to Ctc, which belongs to a family of unknown proteins.

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MATERIALS AND METHODS

Bacterial strains and plasmids. *L. monocytogenes* LO28, a clinical isolate, was obtained from P. Cossart (Institut Pasteur, Paris, France). Bacterial plasmids were propagated in *Escherichia coli* TG1 (28). Plasmid pHT315 (3) was used as a cloning vector for sequencing, and plasmid pAUL-A (6) was used for gene disruption.

Culture media and stress conditions. Cells were grown on complex culture media: brain heart infusion (BHI) broth or agar (Difco Laboratories, Detroit, Mich.). A chemically defined minimal medium called Improved Minimal Medium, or IMM (26), was also used, but pyridoxal, which is not necessary for growth, was not added. Different stress conditions used for growth rate or microscopy experiments were induced according to the following procedure. Overnight cultures of strain LO28 or the *ctc* mutant were used to inoculate fresh medium at an initial optical density at 600 nm (OD_{600}) of ~0.05. For heat stress, BHI medium or IMM was inoculated with a preculture grown in the same medium, and the cultures were incubated with shaking at 45°C. For osmotic stress, either BHI medium with or without 5.5% NaCl (final concentration, 6%) was inoculated with a preculture grown in BHI medium, or IMM with or without 3.5% NaCl or with 0.6 M xylose was inoculated with a preculture grown in IMM. Betaine (1 mM) or carnitine (1 mM) was added to IMM containing NaCl when required. The cultures were incubated with shaking at 37°C. Growth rate experiments were performed with a Microbiology Reader Bioscreen C (Labsystems, Helsinki, Finland) in 100-well sterile microplates, each well containing 300 μl of culture medium. The OD_{600} was monitored. Experiments were performed at least in duplicate and were repeated independently, twice for heat stress experiments and three times for osmotic stress experiments.

Antibiotics were used at the following concentrations: ampicillin at 100 μg ml^{-1} for *E. coli*, and erythromycin at 5 μg ml^{-1} and rifampin at 200 μg ml^{-1} for *L. monocytogenes*.

Cloning and sequencing. Plasmids were prepared using the Plasmid Midi kit (Qiagen, S.A., Courtabœuf, France). Bacterial chromosomal DNA was isolated as described previously (22). Restriction endonucleases, T4 DNA ligase, and *Taq* polymerases were used as recommended by the manufacturer (Roche Molecular Biochemicals, Mannheim, Germany). DNA restriction fragments were purified from agarose gels by using the QIAquick gel extraction kit (Qiagen). Oligonucleotides were synthesized by MGW-Biotech. Plasmids were introduced into *E. coli* by standard methods (28), while for *L. monocytogenes*, electroporation was achieved as described previously (25).

DNA sequencing was performed with the BigDye terminator cycle sequencing ready reaction kit (Applied Biosystems, Courtabœuf, France), and sequences were analyzed with an automatic DNA sequencer (ABI Prism 310 genetic sequencer; Applied Biosystems). Searches for sequence homology were performed with the FASTA program (1). Sequencing of the *ctc* gene of *L. monocytogenes* LO28 was performed as follows. A 1,185-bp chromosomal DNA fragment containing the *ctc* gene (Fig. 1) was amplified by PCR using primers OD1 and OD2 with incorporation of two restriction sites, *Hin*dIII and *Bam*HI (Table 1). These primers were designed using the complete nucleotide sequence of *L. monocytogenes* strain EGDe (11). The PCR product was digested with the *Hin*dIII and *Bam*HI restriction enzymes and was cloned into similarly digested plasmid pHT315. Inserts of two plasmids were sequenced.

Construction of a *ctc* insertional mutant. The *ctc* gene was insertionally inactivated by a simple recombination event using the temperature-sensitive suicide vector pAUL-A. A 435-bp internal *ctc* fragment (Fig. 1A) was PCR amplified from chromosomal DNA with primers OD3 and OD4 with the incorporation of two restriction sites, *Hin*dIII and *Bam*HI (Table 1). The purified PCR product was digested with *Hin*dIII and *Bam*HI, ligated into similarly digested plasmid pAUL-A, and then transformed into *E. coli*. The resultant plasmid pAUL-CTC was used to create a knockout in *ctc* by homologous recombination with the *L. monocytogenes* chromosome as described previously (6). In the resulting mutant, 135 bp in the 3′ end of *ctc* was deleted (Fig. 1A). Southern blot analysis and PCR (data not shown) confirmed the authenticity of a single integration of pAUL-CTC in the chromosome of *L. monocytogenes* strain LO28. The stability of the pAUL-A insertion was confirmed by PCR analysis of cultures grown in BHI medium with or without erythromycin selection at 37°C.

RNA isolation and analysis. RNA samples were prepared from 10 ml of mid-logarithmic-phase cells (OD_{600}, ~0.5) grown in BHI medium or IMM, 30 min after the addition (or not) of NaCl (3.5% in IMM and 5.5% in BHI medium). After the shock, cells were harvested by centrifugation at 7,000 × *g* for 4 min at 4°C. Cells were treated with rifampin and RNAprotect Bacteria reagent (Qiagen) and centrifuged at 7,000 × *g* for 10 min at 4°C. The pellet was resuspended in 400 μl of buffer S (10% glucose, 12.5 mM Tris [pH 7.6], 66 mM EDTA, 0.5% sodium dodecyl sulfate) containing 10 mg of lysozyme ml^{-1}, 200 μg of proteinase K ml^{-1}, and 200 μg of rifampin ml^{-1}. After the addition of glass beads, cells were subjected to mechanical disruption. RNA was purified by a Trizol extraction, two chloroform-isoamyl alcohol extractions, and an isopropanol precipitation. The final RNA pellet was dissolved in water, treated with DNase, and quantified spectrophotometrically before storage at −70°C. The integrity and the relative concentrations of RNA samples were checked by agarose gel electrophoresis and ethidium bromide staining. The mRNA of *ctc* was semiquantitatively analyzed by a slot blot technique as follows. RNA samples were treated at 65°C in 20% formaldehyde for 15 min. The samples were then vacuum blotted with a Bio-Rad slot blot apparatus onto positively charged nylon membranes (Amersham Biosciences, Saday, France). RNA was cross-linked to the membrane by UV radiation. Transcription of *ctc* was monitored using an intragenic digoxigenin (DIG)-labeled probe generated by PCR with primers OD3 and OD4 (Table 1). Detection of the labeled probe was mediated by addition of an anti-DIG alkaline phosphatase-conjugated enzyme and CDP-star substrate (Roche Molecular Biochemicals). Light emission was captured by standard autoradiography (Hyperfilm; Amersham Biosciences). A 16S rRNA probe was used to control the loading uniformity of RNA extracted under different conditions of stress (data not shown).

Electron microscopy. Stationary-phase-grown bacteria were processed for observation by scanning electron microscopy (SEM). Bacteria were fixed for 1 h with 4% glutaraldehyde in a 0.2 M cacodylate buffer. Cells were dehydrated using a graded ethanol series (70, 95, and 100% ethanol, three times for 10 min each time) and subjected to an acetone dehydration series of 30, 50, 70, and 100% acetone for 10 min each. One drop was spread on a microcover, coated with gold in an Emscope SC500, and observed with the Philips SEM 505.

RESULTS

Sequence analysis of the *ctc* gene of *L. monocytogenes* LO28. Primers designed by using the sequences surrounding the *ctc* gene (lmo00211) of *L. monocytogenes* strain EGDe, whose genome has been entirely sequenced (11), were used to amplify the *ctc* gene of strain LO28 by PCR. The PCR yielded one band, and the nucleotide sequence of this DNA fragment (Fig. 1A) revealed 100% identity between the *ctc* sequences of strains LO28 and EGDe.

The *ctc* gene starts at an ATG codon, 9 nucleotides downstream of a potential ribosome binding site (5′ GGAG 3′), and ends with a TAA stop codon. The deduced polypeptide contains 207 residues with a calculated molecular weight of 22,654 (pI 4.14). Only one amino acid differs between the Ctc sequences of *L. monocytogenes* LO28 and *Listeria innocua* CLIP 11262 (11) (Lys201→Asn substitution in *L. innocua* Ctc protein). Homology searches revealed a significant degree of similarity with the Ctc protein of *B. subtilis* (42% identity) (23), the equivalent protein, TL5, from *Thermus thermophilus* (36% identity) (12), and all members of the Ctc protein family identified during different genome-sequencing projects. Like those of other Ctc proteins, the N-terminal part of the *L. monocytogenes* Ctc protein presents similarities with members of the 50S ribosomal protein L25 family, for instance, 28% identity with the L25 protein of *E. coli*, RplY (39).

A putative σ^B-dependent promoter was found 45 bp upstream from the ATG start codon (5′ GTTT-N15-GGGTAG 3′) based on a comparison with the σ^B-dependent consensus promoter (5′ GTTT[15/16 nt]GGGTAA3′) (4). A stem-and-loop structure (ATGGAAGATTCGCA TTTGTT TGCGAT ATCTTCCAT; $\Delta G = -77$ kJ mol^{-1}) followed by a T track is located 16 bp downstream from the TAA stop codon of the *ctc* gene. This palindromic structure is a putative transcriptional terminator. Analysis of the regions surrounding the *ctc* gene in strain EGDe revealed an upstream gene (lmo00210) transcribed in the opposite direction and encoding a putative lactate dehydrogenase (67% identity with *Lactobacillus casei* lac-

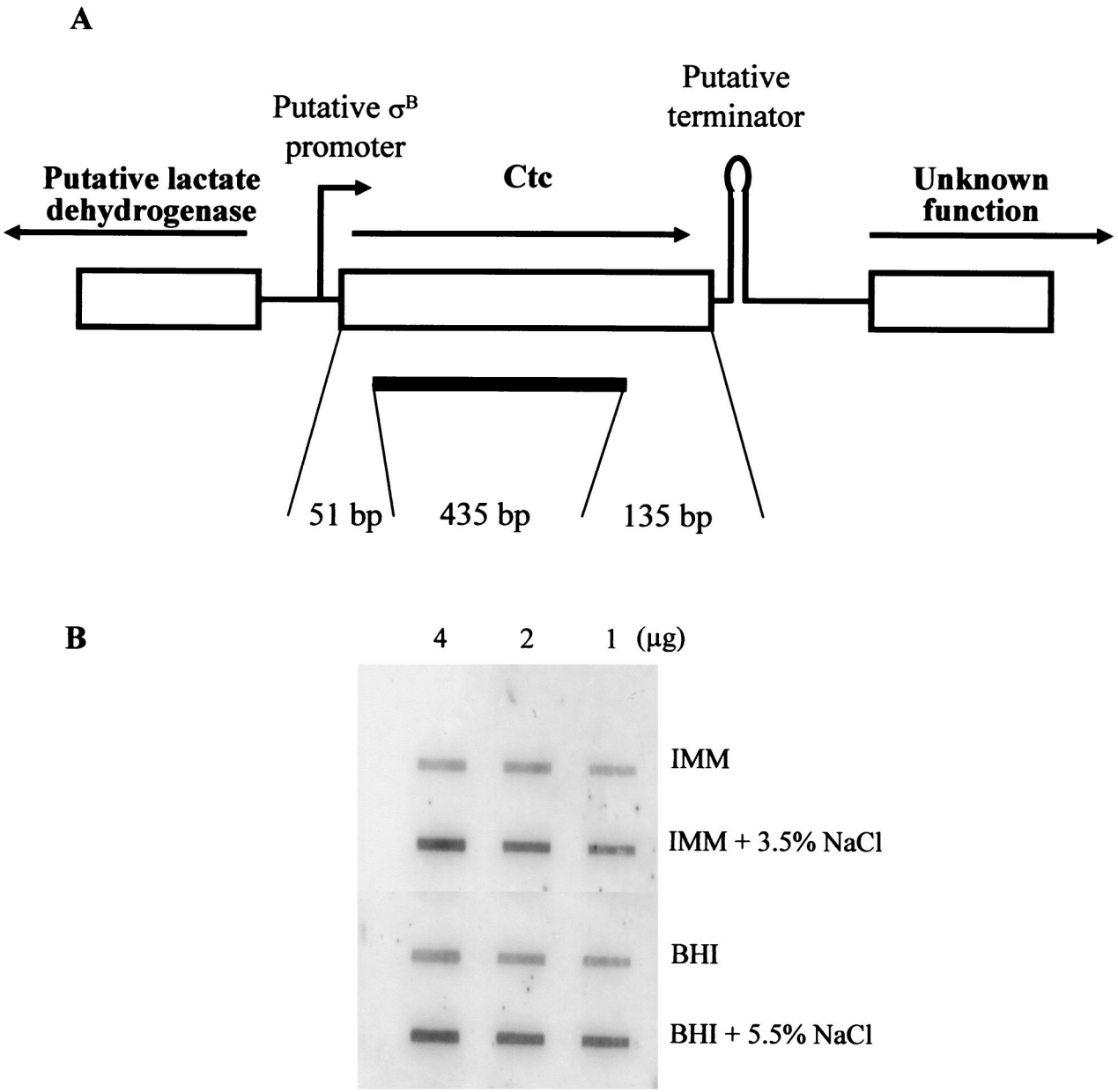

FIG. 1. (A) Schematic representation of the organization of the DNA fragment amplified by PCR with primers OD1 and OD2 in order to sequence the *ctc* gene of *L. monocytogenes* LO28. The solid bar represents the DNA fragment amplified by PCR with primers OD3 and OD4 and subsequently cloned into the pAUL-A plasmid in order to interrupt the *ctc* gene. Arrows indicate the orientations of the genes. (B) RNA slot blot transcription analysis of the *ctc* gene.

tate dehydrogenase [17]). A gene (lmo00212) encoding a protein with no significant similarities with any of the proteins recorded in the databases is located downstream of the *ctc* gene and in the same direction of transcription. This gene is separated from the *ctc* gene by the putative terminator mentioned above.

Transcriptional analysis of the *ctc* gene. In a previous study (7), the Ctc protein was identified as a protein showing high induction after salt stress. Moreover, as described above, we found a putative σ^B promoter in front of the *ctc* gene, and σ^B transcription is strongly induced after an osmotic upshift (4). Therefore, semiquantitative analysis of the *ctc* gene transcription was carried out using slot blots with RNA extracted from cultures of strain LO28 grown in rich or minimal medium before or after an osmotic shock. Similar results were obtained in both media (Fig. 1B). Under growth conditions without added NaCl, constitutive expression of *ctc* was observed. Thirty minutes of exposure to NaCl (3.5% in IMM and 5.5% in BHI medium [final concentration, 6%]) resulted in an increase in the level of transcription.

Role of *ctc* in osmotic and high-temperature adaptation. To investigate the function of the *ctc* gene of *L. monocytogenes*

TABLE 1. Primers used in PCR experiments

Primer	Sequence[a] (5′ → 3′)	Restriction site
OD1	GCCGAAGCTTACCAACTGCTCCGTCGCC	*Hind*III
OD2	CGCGGATCCCACCAGTCTTCTTCCAACC	*Bam*HI
OD3	GCCGAAGCTTGTAACGAGATTGCGTAGTG	*Hind*III
OD4	CGCGGATCCGTTTTCCGGAAGGTCGTTC	*Bam*HI

[a] Restriction sites are underlined.

LO28, it was mutated by insertional mutation by using the pAUL-A plasmid, a temperature-sensitive suicide vector, as described in Materials and Methods. Phenotypic analysis did not show any difference between the *ctc* mutant and the wild-type strain with respect to the aspect of colonies, catalase, hemolysis on blood agar, and metabolic profiles, as determined by using API-CH50 microplate assays.

The stress tolerance of the *ctc* mutant was compared with that of strain LO28. Because the Ctc protein was primarily identified as a protein induced by an osmotic upshift, we first examined whether the absence of Ctc would have any effect on the ability of *L. monocytogenes* to grow under conditions of high osmotic strength in a rich (BHI) or minimal (IMM) medium. Growth rates (doubling times) were found identical for the wild-type strain and the *ctc* mutant in BHI medium (55 ± 5 min) (Fig. 2). No significant difference was found between the growth rates of the two strains after the addition of NaCl to BHI medium (85 ± 10 min for the wild-type strain and 110 ± 20 min for the *ctc* mutant) (Fig. 2) or in IMM without added NaCl (100 ± 15 min for the wild-type strain and 125 ± 15 min for the *ctc* mutant). However, in IMM supplemented with 3.5% NaCl, the growth of the *ctc* mutant was significantly impaired (Fig. 3). The growth rate reached 620 ± 140 min, whereas it was 270 ± 15 min for the wild-type strain. In order to test if the sensitivity of the *ctc* mutant was linked to salt stress or osmotic stress, growth was performed in IMM supplemented with 0.6 M xylose. An average increase of 95% for the growth rate of the *ctc* mutant was obtained (data not shown). The ability of *L. monocytogenes* to survive high salt concentrations is attributed mainly to the accumulation of compatible solutes such as glycine betaine or carnitine. In order to test if the *ctc* mutation still had an effect in the presence of osmoprotectants, we added 1 mM glycine betaine or carnitine to IMM supplemented with 3.5% NaCl. Addition of glycine betaine nearly allowed strain LO28 and the *ctc* mutant to recover the growth rate of strain LO28 cultivated in IMM without NaCl (Fig. 3). The effect of the addition of carnitine was intermediate, but no significant difference between the growth rates of the two strains was observed in this situation (Fig. 3).

In order to test if the Ctc protein was involved in general stress tolerance, the growth of the *ctc* mutant versus LO28 was measured under heat stress conditions at 45°C in BHI medium and IMM. Under these conditions, the growth of the mutant was similar to that of the wild-type strain (Fig. 4). This suggests that the role of Ctc in stress tolerance is restricted to osmotic tolerance.

The morphology of the *ctc* mutant is impaired during stationary phase in minimal medium containing NaCl. The wild-type strain LO28 and the *ctc* mutant were subsequently examined using photonic microscopy during the exponential and stationary phases of growth at 37°C in BHI medium and IMM with or without NaCl. No difference in morphology was observed between the two strains during growth in BHI medium

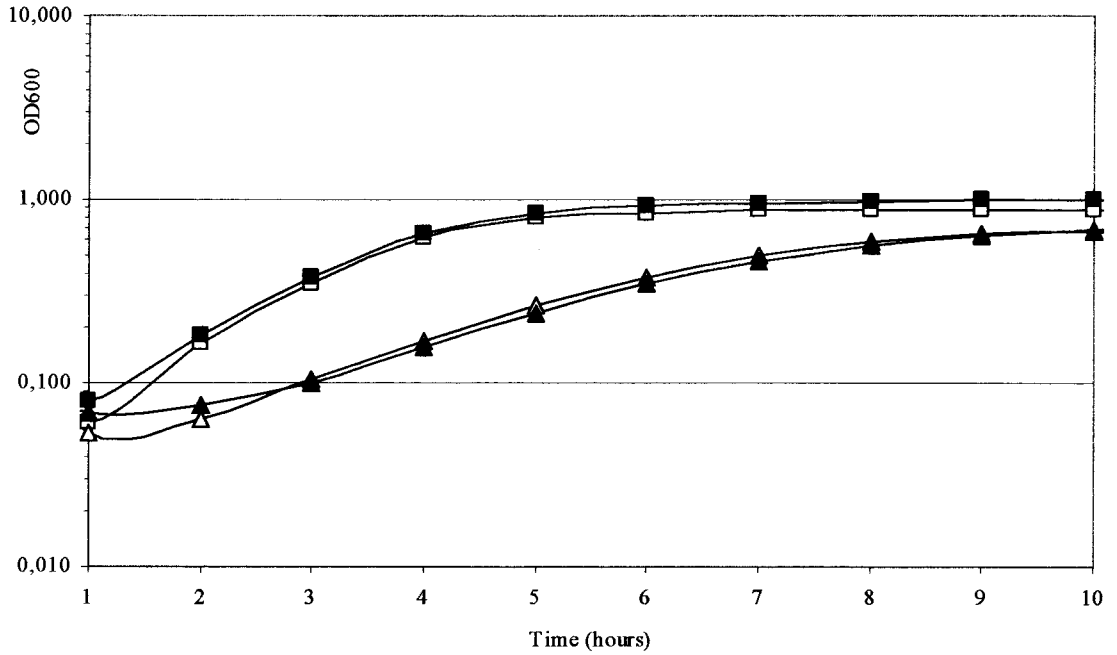

FIG. 2. Growth of the wild-type *L. monocytogenes* strain LO28 and its *ctc* mutant in BHI medium with or without 5.5% NaCl. □, strain LO28 grown in BHI medium; ■, *ctc* mutant grown in BHI medium; △, strain LO28 grown in BHI medium with NaCl; ▲, *ctc* mutant grown in BHI medium with NaCl.

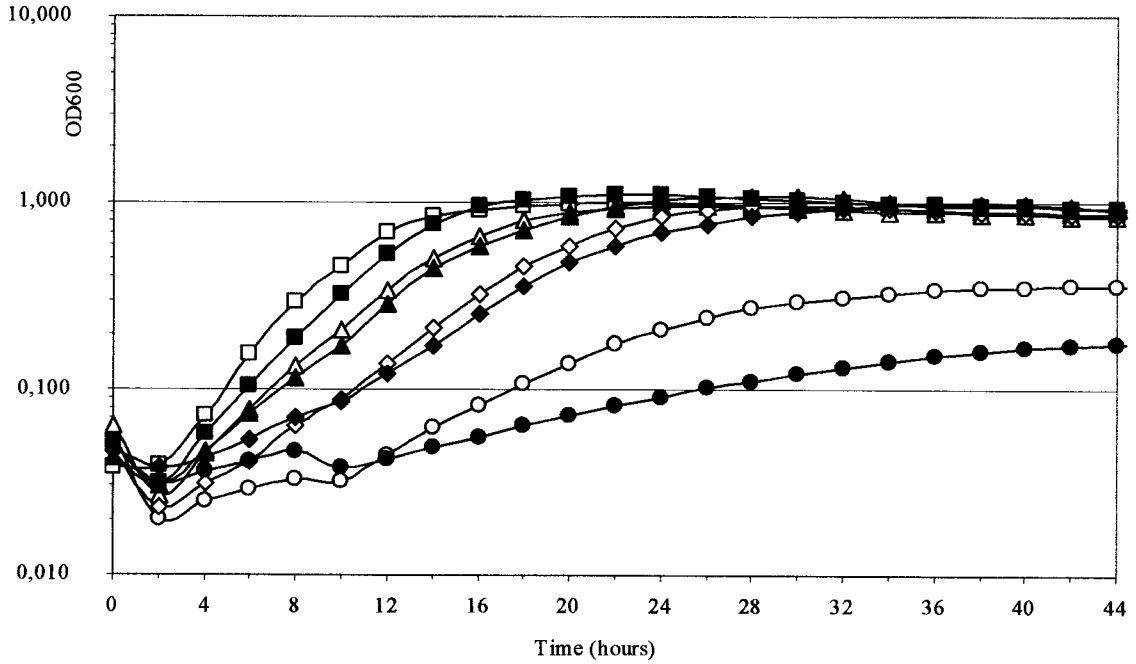

FIG. 3. Growth of the wild-type *L. monocytogenes* strain LO28 and its *ctc* mutant in IMM with or without 3.5% NaCl and with or without 1 mM glycine betaine or carnitine. □, strain LO28 grown in IMM; ■, *ctc* mutant grown in IMM; ○, strain LO28 grown in IMM with NaCl; ●, *ctc* mutant grown in IMM with NaCl; △, strain LO28 grown in IMM with NaCl and glycine betaine; ▲, *ctc* mutant grown in IMM with NaCl and glycine betaine; ◇, strain LO28 grown in IMM with NaCl and carnitine; ◆, *ctc* mutant grown in IMM with NaCl and carnitine.

with or without NaCl or in IMM without NaCl (data not shown). This was confirmed by transmission electron microscopy (TEM) using negative staining and by SEM by examining 48-h-grown cultures (data not shown). In these three different media, bacteria appeared as small rods measuring 1 to 2 μm in length. In contrast, in IMM containing 3.5% NaCl, the *ctc* mutant displayed a different morphology than the wild-type strain as observed by photonic microscopy, TEM (data not

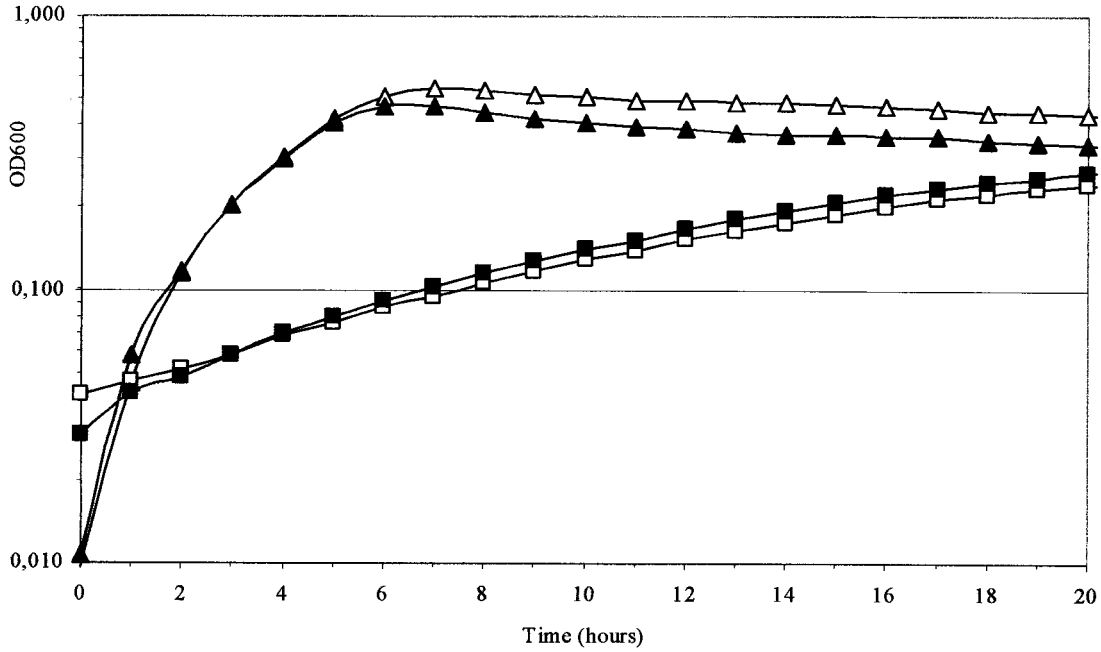

FIG. 4. Growth of the wild-type *L. monocytogenes* strain LO28 and its *ctc* mutant after a heat stress at 45°C in BHI medium and IMM. △, strain LO28 grown in BHI medium; ▲, *ctc* mutant grown in BHI medium; □, LO28 grown in IMM; ■, *ctc* mutant grown in IMM.

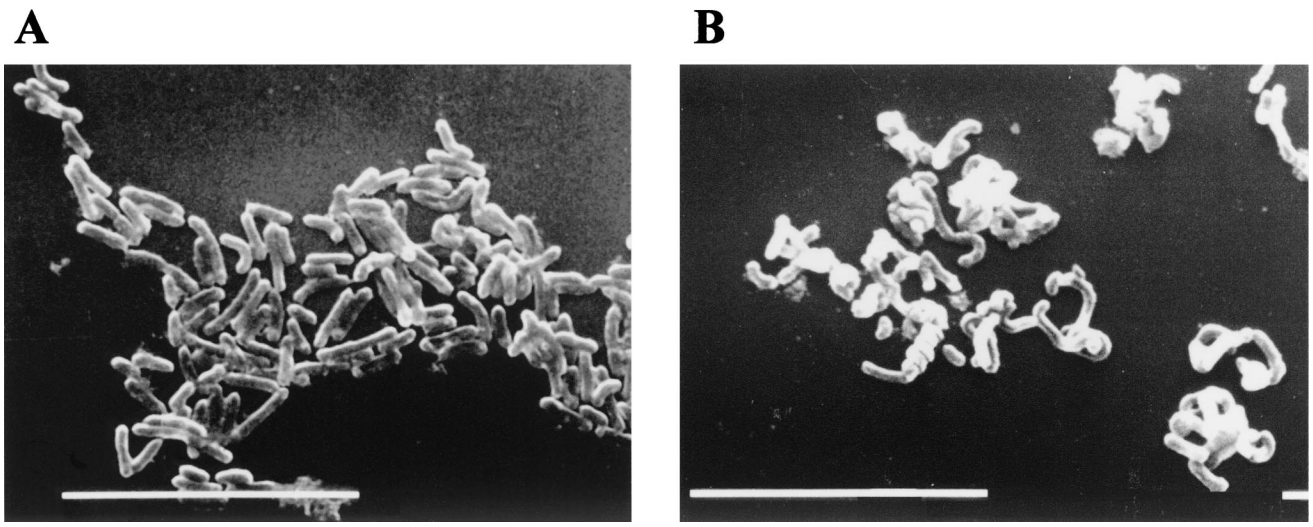

FIG. 5. Scanning electron microscopy observations of *L. monocytogenes* strain LO28 (A) and its *ctc* mutant (B) during the stationary phase of growth in IMM supplemented with 3.5% NaCl. Bar, 10 μm.

shown), and SEM (Fig. 5). After 48 h of growth in this medium, the morphology of strain LO28 was characterized by a rod shape with a variable size ranging between 1 and 4 μm. Approximately 1% of the cells displayed a bent rod shape. The *ctc* mutant also displayed a rod shape with a variable size ranging between 1 and 6 μm, but 80% of the cells had a bent or twisted rod shape. The morphology of the *ctc* mutant did not differ significantly from that of the LO28 strain when 1 mM glycine betaine was added to the medium (data not shown).

DISCUSSION

We have shown that the *ctc* gene is involved in the resistance of *L. monocytogenes* to high osmolarity in the absence of osmoprotectants such as glycine betaine and carnitine in the medium. Thus, a *ctc* insertional mutant grew twice as slowly as the wild-type strain LO28 under conditions of high osmolarity (0.6 M NaCl or xylose) in minimal medium. Moreover, the morphology of the *ctc* mutant was impaired in this growth condition. Whereas the morphology of the wild-type strain LO28 was characterized by a rod shape, the *ctc* mutant morphology was characterized by a bent or twisted rod shape under osmotic stress conditions. When glycine betaine or carnitine, known to be the most efficient osmoprotectants in *L. monocytogenes* (5, 19), was added to this medium, the growth of the mutant became identical to the growth of its isogenic parent strain. This can explain why the growth rates of the mutant and the wild-type strain were identical in rich medium (BHI) supplemented with 5.5% NaCl. The BHI medium contains carnitine, which is relatively abundant in some mammalian tissues. The role of *ctc* in stress tolerance seems to be restricted to osmotic stress tolerance, since no difference between the wild-type strain and the *ctc* mutant was observed under conditions of growth at high temperatures in rich or minimal medium.

Few genes involved in salt stress tolerance have been identified in *L. monocytogenes* until now. Survival of *L. monocytogenes* at high salt concentrations is attributed mainly to the accumulation of three osmoprotectants, glycine betaine, carnitine, and proline (5). Independently of genes involved in the transport or biosynthesis of osmoprotectants, two genes encoding proteins of the Clp family have been identified, *clpC* (27) and *clpP* (10). Inactivation of these genes confers a general stress sensitivity phenotype, including salt stress sensitivity, on the corresponding mutants. These genes are known to encode general stress proteins, chaperones assisting the proper folding, refolding, or assembly of proteins and proteases processing those that cannot be refolded. A recent study identified *relA*, a gene encoding a (p)ppGpp synthetase, as a gene involved in osmotolerance (24). The authors showed that (p)ppGpp is involved in the growth of *L. monocytogenes* under high osmotic pressure and that the intracellular accumulation of (p)ppGpp is probably controlled by mechanisms distinct from accumulation of compatible solutes. The last gene which has clearly been associated with osmotolerance in *L. monocytogenes* is σ^B. The absence of σ^B impaired the ability of *L. monocytogenes* to use glycine betaine or carnitine as an osmoprotectant and impaired the transport of glycine betaine (4). The transport of carnitine has not been studied. A potential σ^B-dependent promoter has been identified upstream of the *betL* gene and the *opuC* operon (9, 30). This suggests that σ^B plays a key role in osmotolerance of *L. monocytogenes* via regulation of the expression of two major osmoprotectant transport systems. We have identified a putative σ^B-dependent promoter upstream of the *ctc* gene. In contrast to that of *B. subtilis* (15), the *L. monocytogenes ctc* gene does not seem to belong to an operon. Moreover, expression of the *ctc* gene is strongly induced by an osmotic upshift, like that of the σ^B gene. Taken together, these observations suggest that the *ctc* gene may be regulated, at least in part, at the transcriptional level by σ^B. This emphasizes the role of σ^B, which is probably a key regulator of osmotolerance in *L. monocytogenes* in the presence or absence of compatible solutes in the environment.

Currently, the function of the Ctc proteins is unknown. Our results suggest that the Ctc protein of *L. monocytogenes* belongs to a novel system utilized by this bacterium to adapt to an

osmotic upshift in the absence of an osmoprotectant. This is the first time that a role has been assigned to the Ctc protein, whose gene is widely distributed in bacterial genomes. The product of the *ctc* gene of *L. monocytogenes*, like other *ctc* gene products, presents similarities in its N-terminal part with the 50S ribosomal L25 protein and consequently belongs to the L25 ribosomal protein family. According to the COG database, which compares the protein sequences encoded in 43 complete genomes, representing 30 major phylogenetic lineages (37), no L25 homologue is present in the archaeal genomes, but L25 homologues are present in nearly all eubacterial genomes. L25 homologues are found in all gram-negative bacteria and in all gram-positive bacteria except *Lactococcus lactis*, *Streptococcus pyogenes*, *Mycoplasma pneumoniae*, and *Mycoplasma genitalium*. The sequence homologies observed between the Ctc proteins and the L25 proteins include many conserved residues, which the 5S rRNA-L25 structure confirmed to be involved in the rRNA-protein binding interaction, thereby confirming that these two groups of proteins are strongly related (12, 20, 36). It is highly probable that the Ctc proteins are associated at least in their N-terminal parts with the ribosome and bind the 5S rRNA. Ribosomes have been implicated as sensors of heat and cold shock in *E. coli* (40). Recent results implicated the ribosome as a possible mediator of the activity of Obg, an essential GTP-binding protein, and the stress induction of σ^B, suggesting that ribosomes are also general sensors in *B. subtilis* (29). We can hypothesize that the ribosome is also a sensor of salt stress, at least in *L. monocytogenes*, through the activity of Ctc. Further investigations will be required to clarify the function of Ctc in *L. monocytogenes* and in other bacteria.

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