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An ATP-Dependent L-Carnitine Transporter in *Listeria monocytogenes* Scott A Is Involved in Osmoprotection

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***Listeria monocytogenes* is a gram-positive, psychrotrophic, food-borne pathogen which is able to grow in osmotically stressful environments. Carnitine (β-hydroxy-L-τ-*N*-trimethyl aminobutyrate) can contribute significantly to growth of *L. monocytogenes* at high osmolarity (R. R. Beumer, M. C. te Giffel, L. J. Cox, F. M. Rombouts, and T. Abee, Appl. Environ. Microbiol. 60:1359–1363, 1994). Transport of L-[*N-methyl*-^{14}C]carnitine in *L. monocytogenes* was shown to be energy dependent. Analysis of cell extracts revealed that L-carnitine was not further metabolized, which supplies evidence for its role as an osmoprotectant in *L. monocytogenes*. Uptake of L-carnitine proceeds in the absence of a proton motive force and is strongly inhibited in the presence of the phosphate analogs vanadate and arsenate. The L-carnitine permease is therefore most likely driven by ATP. Kinetic analysis of L-carnitine transport in glucose-energized cells revealed the presence of a high-affinity uptake system with a K_m of 10 μM and a maximum rate of transport (V_{max}) of 48 nmol min^{-1} mg of protein^{-1}. L-[^{14}C]carnitine transport in *L. monocytogenes* is significantly inhibited by a 10-fold excess of unlabelled L-carnitine, acetylcarnitine, and τ-butyrobetaine, whereas L-proline and betaine display, even at a 100-fold excess, only a weak inhibitory effect. In conclusion, an ATP-dependent L-carnitine transport system in *L. monocytogenes* is described, and its possible roles in cold adaptation and intracellular growth in mammalian cells are discussed.**

Epidemics of food-borne listeriosis with high fatality rates (about 25%) have resulted in concern about the incidence and control of *Listeria monocytogenes* in the food supply and environment. An important factor enabling the organism to survive and grow in foods is its ability to grow in the presence of salt concentrations up to 10% (16). Understanding of the process of osmotic adaptation in *L. monocytogenes* could indicate mechanisms for controlling growth of this human pathogen in low-water-activity foods. The survival of bacteria at low water activity generally runs parallel with their ability to accumulate compatible solutes (12, 22). These solutes may be taken up from the surrounding medium, or they may be synthesized de novo by the microorganism. Compatible solutes can be accumulated to high concentrations without inhibitory effects on enzymatic activity or adverse effects on macromolecules (51).

The adaptability of *L. monocytogenes* to osmotic stress resembles that of many other bacteria and appears to depend on the ability of the organism to accumulate betaine and amino acids (7, 29, 35). In addition, it was shown that glycine- and proline-containing peptides stimulate growth of this bacterium at high osmotic strength (5). Betaine (*N*,*N*,*N*-trimethylglycine) is present at high concentrations in sugar beets and other foods of plant origin (6), whereas in certain food products increased amounts of amino acids and peptides are available as a consequence of proteolytic activity of other bacteria present in this food. Recently, our laboratory has shown that exogenously supplied carnitine (β-hydroxy-L-τ-*N*-trimethyl aminobutyrate) can contribute significantly to growth of *L. monocytogenes* at high osmolarity (7). L-Carnitine is biosynthesized by mammals and eukaryotic microorganisms from the amino acid L-lysine (28), where it serves as an essential factor in the transport of fatty acids, through the inner mitochondrial membrane (8). Consequently, the carnitine concentration in food of animal origin is relatively high, whereas the carnitine concentration in plant tissue is relatively low (11).

The current study was undertaken to characterize the transport of carnitine in *L. monocytogenes* cells and to establish whether carnitine is accumulated or converted into other compounds after uptake that serve as the actual osmoprotectants. Transport systems for the osmoprotectant betaine in several gram-negative and gram-positive bacteria (3, 12, 17, 20, 32, 37, 42, 45), including *L. monocytogenes* (29, 36), have been described and have been most extensively studied in *Escherichia coli* and *Salmonella typhimurium* (12). Uptake of carnitine has been observed in *Pseudomonas aeruginosa*. This species can use carnitine as the sole source of carbon and nitrogen (27). Members of the family *Enterobacteriaceae* do not assimilate the carbon and nitrogen skeleton of L-carnitine but are able to metabolize carnitine during anaerobic growth, via crotonobetaine which serves as an external electron acceptor, to τ-butyrobetaine in the presence of other substrates which act as carbon and/or nitrogen sources. Recently, Eichler and coworkers (14) characterized the *cai* genes of *E. coli*, which encode the carnitine pathway. The genes belong to the *caiTABCDE* operon, which is only transcribed during anaerobic growth in the presence of carnitine. It was suggested that CaiT is the transport system for carnitine in *E. coli*. CaiT counts 12 or 14 putative hydrophobic membrane-spanning regions being indicative for a secondary transporter (14, 40).

In this study the presence of an ATP-dependent, high-affinity L-carnitine transport system in *L. monocytogenes* is demonstrated, which is distinct from the previously described betaine transport system in this organism (29, 36). Furthermore, it is shown that accumulated L-carnitine is not metabolized in *L.*

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monocytogenes, which presents evidence for its role as an osmoprotectant. Possible roles of the L-carnitine transporter in the cold tolerance of *L. monocytogenes* and in the intracytoplasmic growth of this food-borne human pathogen in mammalian cells are discussed.

MATERIALS AND METHODS

Bacterial strain and media. *L. monocytogenes* Scott A was grown in complex medium, brain heart infusion (BHI), or the defined minimal medium (DM) of Premaratne et al. (44). This medium contains a defined mixture of amino acids and vitamins with glucose as the major carbon source. Media were supplemented with NaCl, KCl, or sucrose to raise the osmotic strength as required.

Transport assays. Cells were inoculated into 100 ml of medium in 300-ml Erlenmeyer flasks as a 1/20 dilution from an overnight culture in identical medium, grown at 30°C with agitation (150 rpm) in a shaker-incubator (Gallenkamp, Griffin Europe, Breda, The Netherlands) to mid-exponential phase, and harvested by centrifugation. Cells were subsequently washed twice in 50 mM potassium phosphate (pH 6.9)–5 mM $MgSO_4$ containing 50 μg of chloramphenicol per ml and stored on ice until use. In experiments in which $HgCl_2$, vanadate, arsenate, *N,N′*-dicyclohexylcarbodiimide (DCCD), or diethylstilbestrol (DES) were used, the potassium phosphate was replaced by 50 mM potassium *N*-2-hydroxyethylpiperazine-*N′*-2-ethanesulfonic acid (HEPES; pH 7.5).

Transport assays were conducted at 30°C in the buffers described above unless indicated otherwise. Cells were incubated (at an optical density at 600 nm [OD_{600}] of approximately 1) in the presence of 0.5% (wt/vol) glucose prior to the addition of L-[^{14}C]carnitine. Samples of 100 μl were withdrawn, and uptake was stopped by addition of 2 ml of cold buffer and immediately filtered over 0.2-μm-pore-size cellulose nitrate filters (Schleicher and Schuell GmbH, Dassell, Germany) under vacuum, and the filters were washed with 2 ml of cold buffer. The filters were inserted into plastic scintillation vials with 4 ml of scintillant, and the radioactivity was subsequently measured with a liquid scintillation counter (model 1600TR; Packard Instruments Co., Downers Grove, Ill.).

Fate of intracellular L-[^{14}C]carnitine. Cells grown in DM at 30°C with or without 0.5 M NaCl were harvested at an OD_{600} of 0.6, washed twice, and resuspended (OD_{600}, 20) in 50 mM potassium phosphate (pH 6.9) with 5 mM $MgSO_4$ and 50 μg of chloramphenicol per ml and stored on ice. After preincubation of cells (OD_{600}, 1.0) for 3 min, uptake was started by addition of L-[*N-methyl*-^{14}C]carnitine (final concentration, 24 μM). After 15 min, 300-μl samples were taken and the cells were separated from the external medium by centrifugation (10 min, 8,000 × *g*). The supernatant was stored on ice, and the cell pellets were washed twice with 50 mM potassium phosphate (pH 6.9) containing 5 mM $MgSO_4$ and 50 μg of chloramphenicol per ml. Radioactivity was extracted subsequently with 100 μl of 5% (vol/vol) perchloric acid and 10 mM EDTA on ice for 45 min. The extracts were neutralized with an equal amount of a solution of 1 M KOH and 1 M $KHCO_3$. Aliquots of 10 μl of perchloric acid extracts and supernatant were chromatographed on Silica Gel G (Merck AG, Darmstadt, Germany) thin-layer plates by using a methanol-ammonia (75:25) solvent system (15). Radioautographs were made with Kodak X-ray films.

Metabolism of L-[^{14}C]carnitine by *S. cerevisiae* was examined in the presence of acetyl coenzyme A (acetyl-CoA). Acetyl-CoA is supposed to be involved in the conversion of carnitine during lipid metabolism in eukaryotic cells (8). For this purpose, cell extract (CE) of *S. cerevisiae* was incubated with L-[^{14}C]carnitine (24 μM) in the presence of 250 μM acetyl-CoA for 15 min. For comparison, CE of *L. monocytoges* Scott A grown in DM was obtained by sonification as described (49) and subsequently incubated as was CE of *S. cerevisiae*. Aliquots (7 μl) of these incubations were spotted on Silica Gel G and chromatographed and analyzed as described above.

Measurement of the membrane potential and intracellular ATP concentration. The transmembrane electrical potential ($\Delta\psi$) was determined with an electrode specific for the lipophilic cation tetraphenylphosphonium (final concentration, 4 μM), as described previously (46). Cells of *L. monocytogenes* were prepared for measurements as described above and incubated (OD_{600}, 0.8) at 30°C in 50 mM potassium phosphate (pH 6.9) in the presence of 0.5% (wt/vol) glucose, unless indicated otherwise. By adding the potassium proton exchanger nigericin (2 μM), the pH gradient (alkaline inside) was dissipated such that the proton motive force (PMF) was composed of the $\Delta\psi$ only. The intracellular ATP concentration was determined as described previously (1). A cytoplasmic volume of 3 μl per mg of cell protein was used (35). In experiments in which DCCD, DES, $HgCl_2$, and the phosphate analogs arsenate and vanadate were used, the potassium phosphate was replaced by 50 mM potassium HEPES (pH 7.5).

Protein determination. Protein concentrations were determined by the method of Lowry et al. (31) with bovine serum albumin as a standard.

Chemicals. L-[*N-methyl*-^{14}C]carnitine (1.96 TBq/mol) was obtained from DuPont. BHI was from Difco Laboratories, Detroit, Mich. Other chemicals were reagent grade and obtained from Sigma Chemical Company, St. Louis, Mo., or other commercial sources.

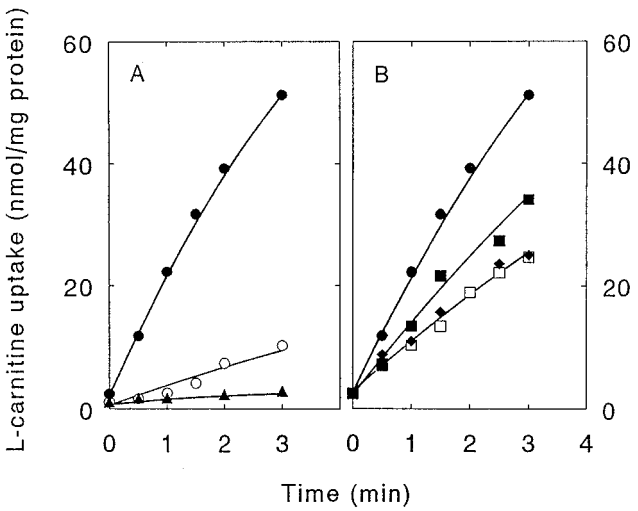

FIG. 1. Transport of L-carnitine in *L. monocytogenes* Scott A. Uptake of L-[*N-methyl*-^{14}C]carnitine (final concentration, 19 μM) was performed in BHI-grown cells at 30°C in 50 mM potassium phosphate (pH 6.9) containing 5 mM $MgSO_4$ and 50 μg of chloramphenicol per ml. Transport was assayed after preincubation for 3 min. (A) Uptake in the absence (○) and presence (●) of 0.5% (wt/vol) glucose and by cells treated with 1% (vol/vol) chloroform (▲). (B) Effect of nigericin (2 μM) (■), valinomycin (1.5 μM) (□), or nigericin plus valinomycin (◆) on glucose-energized L-[^{14}C]carnitine transport (●).

RESULTS

Transport of L-carnitine in *L. monocytogenes*. Uptake of L-[*N-methyl*-^{14}C]carnitine (final concentration, 19 μM) was examined in cells of *L. monocytogenes* grown in BHI. In the absence of an energy source, a low rate of L-carnitine transport was detected whereas a strong stimulation of the uptake was observed in the presence of glucose (Fig. 1A). When the cells were permeabilized with chloroform (1% [vol/vol]), L-carnitine uptake was completely abolished (Fig. 1A). These results suggest that the uptake of L-carnitine is an energy-dependent process. The potassium proton exchanger nigericin (2 μM), which dissipated the transmembrane pH gradient, had no severe effect on L-carnitine transport (Fig. 1B). With the potassium ionophore valinomycin (1.5 μM), which collapsed the membrane potential $\Delta\psi$, L-carnitine transport was only partially inhibited (approximately 50% of the activity of the control). Addition of both valinomycin (1.5 μM) and nigericin (2 μM) resulted in a complete dissipation of the PMF (not shown), whereas uptake of L-carnitine was still functioning (Fig. 1B). The transport of L-[^{14}C]lysine (unpublished observation) and prolyl-[^{14}C]alanine, which are driven by the PMF, was completely abolished in the presence of both valinomycin (1 μM) and nigericin (1 μM) (49). These experiments suggest that L-carnitine transport can proceed in the absence of a PMF.

Fate of translocated L-carnitine. The ability of *L. monocytogenes* to metabolize L-carnitine was investigated by chromatographic analysis of radioactivity accumulated from L-[^{14}C]carnitine uptake in *L. monocytogenes* cells grown in DM. Only a single ^{14}C-labelled compound was detected which moved to the same position as L-[^{14}C]carnitine (Fig. 2, lanes 1 and 2). This indicates that intracellular L-carnitine accumulates as such in the cytoplasm and is not catabolized. This is consistent with the observation that carnitine cannot serve as a carbon or nitrogen source for *L. monocytogenes* (48). Almost all L-[^{14}C]carnitine was taken up by *L. monocytogenes* cells since only very small amounts of radioactivity could be detected in the

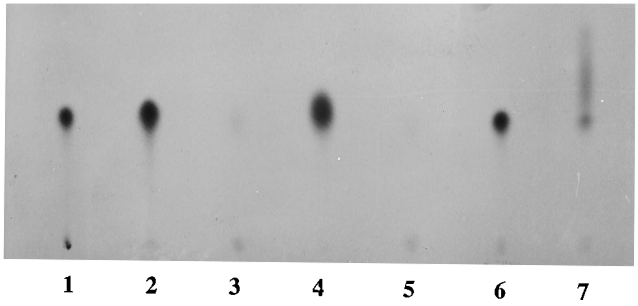

FIG. 2. Fate of intracellular L-carnitine in *L. monocytogenes* Scott A. Uptake of L-[*N-methyl*-^{14}C]carnitine (final concentration, 24 μM), extraction from the cells, and chromatographic analysis were performed as described in Materials and Methods. Lane 1, L-[*N-methyl*-^{14}C]carnitine. Lanes 2 and 3, perchloric acid CE (3 ng of protein) and supernatant of cells grown in DM, respectively. Lanes 4 and 5, perchloric acid CE (3 ng of protein) and supernatant of cells grown in DM with 0.5 M NaCl, respectively. Lanes 6 and 7, sonicated CE of *L. monocytogenes* (2 ng of protein) and *S. cerevisiae* (0.8 ng of protein), respectively, incubated in the presence of acetyl-CoA (250 μM).

supernatant (Fig. 2, lane 3). Results with cells grown in DM with 0.5 M NaCl (Fig. 2, lanes 4 and 5) were indistinguishable from those obtained in the absence of NaCl. Furthermore, L-carnitine metabolism was not observed in CE of *L. monocytogenes* incubated with acetyl-CoA, whereas with CE of *S. cerevisiae* a significant metabolism of L-[^{14}C]carnitine was observed (Fig. 2, lanes 6 and 7). In eukaryotic cells, metabolism involves a reaction in which carnitine is converted into acylcarnitine with the intervention of acetyl-CoA (8). From these results it can be concluded that uptake of carnitine by *L. monocytogenes* in the absence of a PMF (Fig. 1B) is not due to rapid intracellular metabolism.

Energetics of the L-carnitine transport system. The energetics of L-carnitine transport were characterized in detail by analysis of the effects of the phosphate analogs arsenate and vanadate, the H^+-ATPase inhibitor DCCD, and DES on bioenergetic parameters and L-carnitine uptake in *L. monocytogenes* (Table 1). The addition of DCCD resulted in a complete dissipation of the PMF, whereas the intracellular ATP concentration was increased (127% of control). L-Carnitine transport is stimulated under these conditions (146% of control). In the presence of DES, a slight stimulation of the PMF, an increase in the intracellular ATP concentration (133% of control), and an increase in L-carnitine uptake (170% of control) were observed. In the presence of arsenate and vanadate the magnitude of the PMF and the intracellular ATP concentrations were slightly decreased. Transport of L-carnitine was significantly inhibited under these conditions. Combining these results, it can be concluded that ATP or an equivalent energy-rich phosphorylated intermediate supplies the energy for the carnitine translocation process.

The addition of both nigericin (2 μM) and valinomycin (1.5 μM) to *L. monocytogenes* cells which had been preloaded with L-[^{14}C]carnitine (19 μM) did not result in efflux of radioactive label from the cells. Moreover, *L. monocytogenes* did not displace intracellular L-[^{14}C]carnitine for unlabelled L-carnitine when a 40-fold excess of L-carnitine was added to cells preloaded with L-[^{14}C]carnitine (data not shown). These results give evidence for a kinetically irreversible L-carnitine uptake system, which is also characteristic of ATP-dependent transport systems (38).

Feedback regulation of L-carnitine transport. L-[^{14}C]carnitine was taken up at a high rate in cells grown in DM (Fig. 3), reflecting that carnitine uptake is constitutively expressed

TABLE 1. Effect of DCCD, DES, arsenate, and vanadate on bioenergetic parameters and L-[^{14}C]carnitine uptake in *L. monocytogenes*[a]

ATPase inhibitor or phosphate analog added[b]	PMF (mV)[c]	ATP_{in} (mM)[c]	L-[^{14}C]carnitine uptake (nmol min^{-1} mg of $protein^{-1}$)[c]
None	−128 (100)[d]	6.5 (100)	7.6 (100)
DCCD (0.2 mM)	0 (0)	8.3 (127)	11.1 (146)
DES (0.15 mM)	−130 (108)	8.8 (133)	12.9 (170)
Arsenate (0.5 mM)	−119 (93)	5.0 (76)	1.0 (13)
Vanadate (0.5 mM)	−119 (93)	5.6 (85)	1.1 (14)

[a] Uptake of L-[^{14}C]carnitine (final concentration, 19 μM) was determined in BHI-grown cells incubated at 30°C in 50 mM potassium HEPES (pH 7.5) containing 0.5% (wt/vol) glucose, 5 mM $MgSO_4$, and 50 μg of chloramphenicol per ml. The potassium-proton exchanger nigericin was added (final concentration, 2 μM) to dissipate the pH gradient (ΔpH) such that the PMF was composed of the membrane potential only (PMF = Δψ). Uptake was started after preincubation for 5 min by addition of L-[^{14}C]carnitine. The Δψ was determined in parallel experiments, and samples were taken for determination of intracellular ATP concentrations. The rate of uptake of L-[^{14}C]carnitine was determined between 5 and 10 min, since the effect of the phosphate analogs arsenate and vanadate became evident after about 5 min.

[b] DCCD, DES, arsenate, and vanadate were added to the indicated final concentrations, after preincubation for 5 min. After incubation for another 5 min, uptake was started by the addition of L-[^{14}C]carnitine.

[c] The values for PMF, intracellular ATP concentration (ATP_{in}), and initial rate of uptake of L-[^{14}C]carnitine in the control experiment (no addition) were set at 100%. Relative percentages in the presence of inhibitors are given in parentheses.

rather than induced during growth in the carnitine-containing BHI (29). Uptake rates in DM-grown cells, however, appeared to be four- to fivefold higher than those in BHI-grown cells. To investigate whether the observed lower L-[^{14}C]carnitine uptake rates in BHI-grown cells are caused by the presence of preaccumulated unlabelled carnitine in the cell, L-[^{14}C]carnitine transport was studied in *L. monocytogenes* cells grown in DM

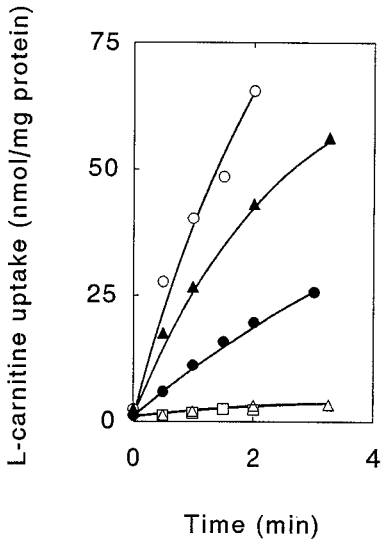

FIG. 3. Feedback regulation of L-carnitine transport in *L. monocytogenes* Scott A. The influence of the presence of L-carnitine during growth was determined. Transport assays were performed in 50 mM potassium phosphate (pH 6.9) containing 5 mM $MgSO_4$ and 50 μg of chloramphenicol per ml. Uptake was started after preincubation with 0.5% (wt/vol) glucose by the addition of L-[^{14}C]carnitine (final concentration, 19 μM). ●, cells grown in BHI; ○, cells grown in DM; ▲, cells grown in DM plus 0.02 mM L-carnitine; △, cells grown in DM plus 0.2 mM L-carnitine; □, cells grown in DM plus 2 mM L-carnitine.

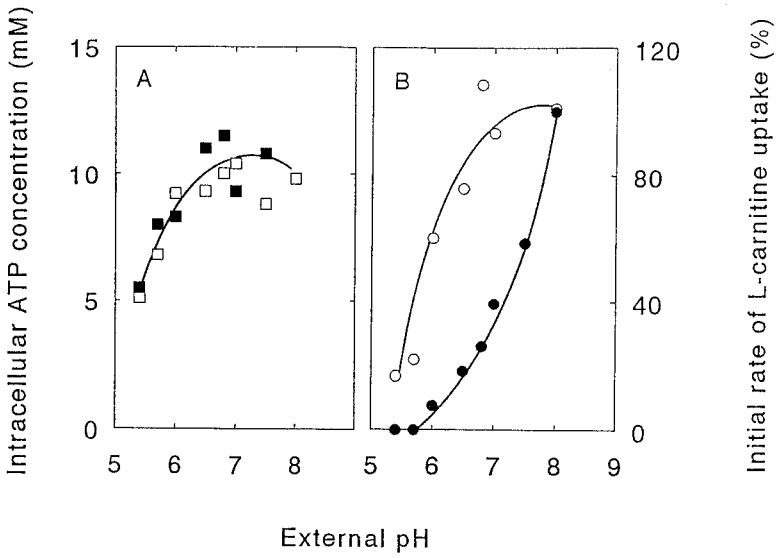

FIG. 4. Effect of nigericin on ATP levels and L-carnitine transport in *L. monocytogenes* Scott A at different external pH values. (A) Intracellular ATP concentrations were measured in BHI-grown cells in 50 mM potassium phosphate (pH 6.9) containing 0.5% glucose (wt/vol), 5 mM $MgSO_4$, and 50 μg of chloramphenicol per ml at different external pH values in the absence (□) and presence (■) of 2 μM nigericin. (B) Initial uptake rates of L-[^{14}C]carnitine (final concentration, 19 μM) were determined over the first 2 min in parallel experiments with these cells in the absence (○) or presence (●) of 2 μM nigericin at the indicated pH values. In the presence of nigericin the internal pH was equal to the external pH. Cells were preincubated for 3 min before the addition of L-[^{14}C]carnitine.

in the presence of different L-carnitine concentrations. Growth in DM containing 0.02 mM L-carnitine resulted in a markedly lower rate of L-carnitine transport in these cells than in cells grown in DM (control). Growth in DM in the presence of higher L-carnitine concentrations (0.2 mM and 2 mM) had even a more pronounced effect on the rate of L-carnitine transport (Fig. 3). To exclude a possible repression of the synthesis of the transporter by L-carnitine, the following experiment was devised. *L. monocytogenes* was grown in DM, harvested at mid-exponential phase, and washed twice in 50 mM potassium phosphate (pH 6.9)–5 mM $MgSO_4$ containing 50 μg of chloramphenicol per ml. Cells were subsequently incubated for 45 min with different L-carnitine concentrations in the presence of chloramphenicol, which inhibits de novo protein synthesis. The cells were washed three times and finally subjected to L-[^{14}C]carnitine as described in Materials and Methods. Relative uptake rates were comparable (not shown) to those shown in Fig. 3. These data indicate that the L-carnitine transport system is feedback inhibited by intracellular L-carnitine.

Kinetics of L-carnitine transport. Initial rates of transport were determined over a wide range of L-carnitine concentrations (1 to 500 μM) with cells grown in DM. An Eadie-Hofstee plot of the data was monophasic, suggesting the presence of a single carnitine transport system, and revealed a K_m value of 10 μM and a V_{max} value of 48 nmol of carnitine transported per min per mg of cell protein (data not shown). The same K_m value was found for BHI-grown *L. monocytogenes*, indicating that no additional L-carnitine uptake system is induced during growth in this carnitine-containing medium. A V_{max} value of 18 nmol min^{-1} mg of protein^{-1} was obtained in BHI-grown cells. The lower V_{max} in BHI-grown cells can be explained by feedback regulation of the transport system (see above).

pH dependence of L-carnitine transport. The effect of the external pH (pH_{ext}) on the rate of L-[^{14}C]carnitine uptake was studied in the absence and presence of the potassium proton exchanger nigericin (2 μM). Without nigericin, a decrease in medium pH led to a decrease in the initial rate of uptake, which was accompanied by a decrease in the intracellular ATP concentrations (Fig. 4). Over the pH_{ext} range from 5.5 to 8, *L. monocytogenes* Scott A maintains its internal pH (pH_{in}) relatively constant; the pH_{in} increases from 7.2 at pH_{ext} 5.5 to 8.1 at pH_{ext} 8 (9). The addition of nigericin, which dissipates the pH gradient across the cytoplasmic membrane, did not affect intracellular ATP levels (Fig. 4A). Transport of L-carnitine was completely inhibited in the presence of nigericin at acidic pH values (Fig. 4B). Apparently, transport of L-carnitine is dependent on the intracellular pH with optimum activity at alkaline pH values.

Osmotic effects. Osmoprotection by L-carnitine at different L-carnitine concentrations was determined. A concentration as low as 10 μM appeared to be sufficient to stimulate growth of *L. monocytogenes* in high osmolarity DM (data not shown). This suggests that the high-affinity L-carnitine transporter can play an important role in the adaptation of *L. monocytogenes* to growth at high osmolarity. Initial rates of L-carnitine (19 μM) uptake in *L. monocytogenes* cells grown in BHI containing 0.5 M NaCl were found to be indistinguishable from those in cells grown in the absence of NaCl (data not shown). Furthermore, the initial uptake rate of L-carnitine (19 μM) was demonstrated to be similar in assay buffers in which the osmolarity was increased by addition of 0.4 M NaCl, 0.4 M KCl, or 0.6 M sucrose (not shown). The activity of the L-carnitine transporter is consequently not stimulated by a rise in the extracellular osmolarity.

Temperature dependence of L-carnitine transport. Considering the ability of *L. monocytogenes* to grow at refrigeration temperature, it was of interest to determine the temperature dependence of L-carnitine uptake. The initial rate of L-carnitine transport of *L. monocytogenes* cells grown at 30°C decreased with decreasing assay temperature (Fig. 5). The uptake of L-carnitine at 30°C was approximately 30 nmol min^{-1} mg of protein^{-1} and was decreased to 3 nmol min^{-1} mg of protein^{-1} at 5°C. It should be stressed that the uptake of L-carnitine at 5°C is still significant. Strikingly, cells grown at 7°C transported L-carnitine at a rate of approximately 3 nmol min^{-1} mg of protein^{-1} over the whole temperature range assayed (Fig. 5).

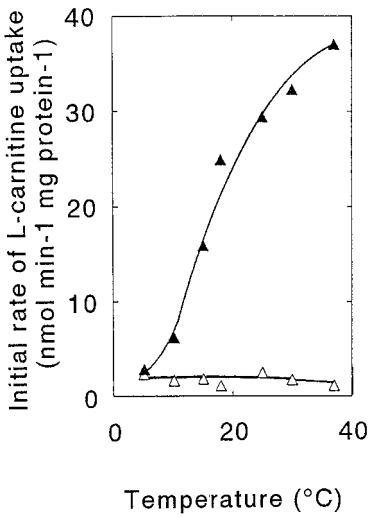

FIG. 5. Effect of temperature on L-carnitine transport in *L. monocytogenes* Scott A. Initial uptake rates of L-[^{14}C]carnitine were determined at different temperatures in cells grown at 30°C (▲) and 7°C (△). Transport assays were performed in 50 mM potassium phosphate (pH 6.9) containing 5 mM $MgSO_4$ and 50 μg of chloramphenicol per ml. Uptake was started after preincubation with 0.5% (wt/vol) glucose by the addition of L-[^{14}C]carnitine (final concentration, 19 μM).

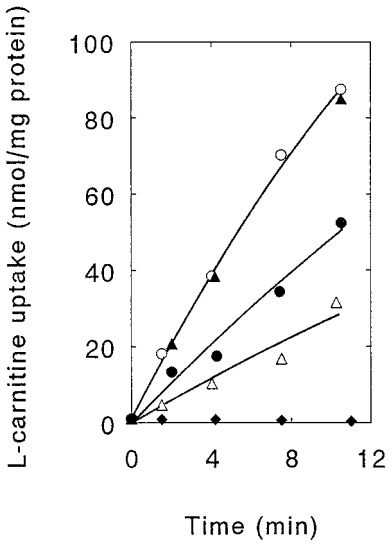

FIG. 6. Effect of SH reagents on the uptake of L-carnitine by *L. monocytogenes* Scott A. Cells were incubated in 50 mM potassium phosphate (pH 6.9) containing 2 μM nigericin, 5 mM $MgSO_4$ and 50 μg of chloramphenicol per ml. Experiments with $HgCl_2$ were performed in 50 mM potassium HEPES (pH 7.5). After 5 min of preincubation, SH reagents were added, and after incubation for another 5 min, uptake was started by the addition of L-[^{14}C]carnitine (final concentration, 19 μM). Symbols: ▲, no additions; ●, NEM (500 μM); △, *p*CMBS (100 μM); ○, *O*-[3-hydroxymercuri-2-methoxypropyl]carbamylphenoxyacetate (mersalyl) (200 μM), and ◆, $HgCl_2$ (50 μM).

Specificity of the L-carnitine transport system. The specificity of the carnitine transport system was investigated by studying the initial rates of L-[^{14}C]carnitine uptake in assay mixtures in which structural analogs were introduced at a 10- and 100-fold excess of L-carnitine (19 μM, final concentration). The carnitine transport system was found to be highly specific for L-carnitine, since D-carnitine was not an efficient inhibitor (Table 2). L-Proline and betaine displayed even at a 100-fold excess only a weak inhibitory effect. Of the other analogs investigated, only butyrobetaine and acetylcarnitine competed efficiently with L-carnitine for uptake.

TABLE 2. Uptake of L-[^{14}C]carnitine by *L. monocytogenes* in the presence of excess unlabelled analog

Analog	Uptake of L-[^{14}C]carnitine (% activity)[a]	
	10-fold unlabelled analog	100-fold unlabelled analog
Choline	100	86
Betaine	100	70
τ-Aminobutyric acid	100	100
Acetylcholine	102	100
Pivalic acid	102	92
L-Proline	100	80
D-Carnitine	51	33
DL-Carnitine	17	0
L-Carnitine	8	0
Acetylcarnitine	15	0
Octanoylcarnitine	100	55
Butyrobetaine	13	0

[a] Uptake of L-[^{14}C]carnitine (final concentration, 19 μM) was determined in the presence of 10- and 100-fold excess of simultaneously added unlabelled analog. The initial rate of uptake was determined over the first 2 min in BHI-grown cells after preincubation for 3 min in 50 mM potassium phosphate (pH 6.9) containing 0.5% (wt/vol) glucose, 5 mM $MgSO_4$, and 50 μg of chloramphenicol per ml. The initial rate of L-[^{14}C]carnitine uptake (final concentration, 19 μM) at 30°C (100% activity) was 18 nmol min^{-1} mg of $protein^{-1}$. Data represent the mean of at least two experiments. Absolute deviations in the data were less than 10%.

Effect of sulfhydryl group (SH) reagents on the L-carnitine transport system. The effect of various SH-modifying reagents on the activity of the L-carnitine transporter was examined in the presence of nigericin (2 μM). L-Carnitine uptake was not affected by mersalyl at a concentration of 200 μM. *N*-Ethylmaleimide (NEM) and *p*-chloromercuribenzene sulfonate (*p*CMBS) inhibited L-carnitine uptake to a certain extent at the concentrations indicated, whereas $HgCl_2$ blocked transport completely (Fig. 6). Parallel to the transport assays, $\Delta\psi$ was determined and samples were taken for determination of intracellular ATP concentrations. In control cells the $\Delta\psi$ was −130 mV and the intracellular ATP concentration was approximately 6.8 mM. The presence of NEM, *p*CMBS, or mersalyl did not influence the magnitude of the PMF (data not shown). The intracellular ATP concentration was not altered by NEM, whereas with *p*CMBS or mersalyl the intracellular ATP concentrations increased (210% and 300% of control, respectively; data not shown). The addition of $HgCl_2$ resulted in a complete dissipation of the PMF and the intracellular ATP concentration decreased drastically (15% of control) (data not shown). These results indicate that inactivation of the L-carnitine transport system by NEM and *p*CMBS is due to specific reactions with SH groups of the transporter.

DISCUSSION

Carnitine has been shown to stimulate growth of *L. monocytogenes* at high osmotic strength (7). The results presented here demonstrate that accumulation of L-carnitine is mediated via a specific transport system with a high affinity and a high capacity for L-carnitine (K_m = 10 μM; V_{max} = 48 nmol min^{-1} mg of $protein^{-1}$). L-Carnitine was detected in an unmodified form inside the cells (Fig. 2), which is in agreement with the observation that it cannot serve as a carbon or nitrogen source for *L. monocytogenes* (48). These observations unequivocally

show that L-carnitine is metabolically inert in *L. monocytogenes* and that the L-carnitine transporter has a crucial role in the supply of this bacterium with L-carnitine.

The accumulation of L-carnitine in *L. monocytogenes* was found to require metabolic energy since the omission of glucose abolished uptake. It was deduced that the driving force for uptake is supplied by ATP or another energy-rich phosphorylated intermediate, on the basis of the following properties of the system. (i) The uptake of L-carnitine was only partially inhibited in the presence of nigericin, an electroneutral K^+/H^+ ionophore, and valinomycin, a K^+-specific ionophore (Fig. 1B), indicating that transport can proceed in the absence of an electrochemical gradient for protons. Extra evidence for the exclusion of the involvement of the PMF came from the observation that in the presence of the ATPase inhibitor DCCD, the PMF was completely abolished, whereas the uptake of L-carnitine was stimulated twofold, concomitant with a rise in the intracellular ATP concentration (Table 1). DCCD most likely blocks conversion of the glycolytically generated ATP into a PMF, owing to the absence of a proton-translocating electron transport system. (ii) Exchange of intracellular L-[^{14}C] carnitine against a high concentration of extracellular nonlabelled L-carnitine did not occur. Efflux of L-[^{14}C]carnitine upon addition of the ionophores valinomycin and nigericin was also not observed (data not shown). (iii) The L-carnitine accumulation ratio (in/out) can reach a value of about 10^5, which exceeds the thermodynamic limits set by the PMF for secondary transport systems (40). (iv) L-Carnitine transport was susceptible to arsenate and vanadate, which interact with reactions involving high-energy phosphate bonds, whereas these compounds had a small effect on the PMF and caused only a slight decrease in the amount of intracellular ATP (Table 1). These findings indicate that inhibition of L-carnitine transport by arsenate and vanadate might be due to inhibition of the transport system itself. The L-carnitine transport system most likely belongs to the bacterial ATP-driven binding-protein-dependent transport systems of the superfamily of ABC transporters which are widely distributed among all living organisms (4, 18). Many of these ATP-driven transport systems are sensitive to vanadate (30, 41). In general, an ABC transporter comprises two transmembrane and two cytoplasmic domains. Bacteria possess an extra binding subunit for the import of substrates, which is periplasmic in gram-negative bacteria, whereas in gram-positive bacteria it is a lipoprotein anchored to the cytoplasmic membrane (18). Recent genetic studies revealed that transport of arginine in *L. monocytogenes* is also mediated by an ABC transporter, which synthesis is induced during intracellular growth in infected mammalian cells (26).

Variations in the magnitude of the ΔpH influenced the activity of the transport (Fig. 4B) despite the dependence of the L-carnitine transport on phosphate bond energy. *L. monocytogenes* Scott A maintains its pH_{in} relatively constant over a pH_{ext} range of 5 to 8 (9, 10). These results might indicate that the pH_{ext} alters the affinity of the transport system by affecting the available concentration of transported solute via protonation and deprotonation or that interactions of protons with the transporter alter its catalytic activity. The apparent relationship between L-carnitine transport and pH_{ext}, however, can also be attributed to the decline in intracellular ATP concentration with decreasing pH (Fig. 4B). The dependence of the initial rate of L-carnitine transport on the intracellular pH showed that L-carnitine transport requires a neutral or alkaline cytoplasm for optimum activity. Regulatory intracellular pH effects on ATP-dependent transport systems can be classified as allosteric rather than catalytic since protons are not directly involved in the energy coupling mechanism. ATP-dependent transport systems, including the L-carnitine transport system described here, apparently all function optimally at slightly alkaline pH values (2, 24, 39).

The different L-carnitine uptake rates obtained in cells grown in BHI and DM can be explained by feedback regulation of the transport system by intracellular L-carnitine (Fig. 3). Feedback (*trans*) inhibition acts as a regulatory device to prevent solute accumulation to unacceptable high intracellular levels. This type of regulation of transport is typical for ATP-dependent transport systems, since these systems, like the L-carnitine transporter in *L. monocytogenes*, are unidirectional and can catalyze the uptake of solutes to high accumulation levels. Regulation of the activity of a compatible solute transport system has not been described until recently. Pourkomailian and Booth (43) and Stimeling et al. (47) established that betaine transport in *Staphylococcus aureus* is subject to feedback inhibition by preaccumulated betaine. Similarly, *L. monocytogenes* closes down its L-carnitine uptake system once sufficient L-carnitine has been accumulated. In addition, we observed that the presence of betaine in the cultivation medium, which was shown not to be a substrate for the L-carnitine transporter (Table 2), also reduced the activity of the L-carnitine uptake system (not shown). The L-carnitine transporter might possibly be capable of sensing overall intracellular osmolality. Further detailed studies should lead to an understanding of the mechanisms by which the activity of the L-carnitine transport system is regulated.

Uptake studies with structural analogs of L-carnitine (Table 2) revealed that the transport system exhibits a high degree of substrate specificity. The system prefers the naturally occuring L-carnitine to its enantiomer D-carnitine. L-Proline, which has been shown to confer osmotic tolerance in *L. monocytogenes* (7), was not recognized by the L-carnitine permease. Other amino acids were also not able to compete with L-carnitine uptake (data not shown) suggesting that L-carnitine transport is not mediated by any amino acid permease. Betaine did not compete for the uptake of L-carnitine, indicating that the L-carnitine transporter in *L. monocytogenes* differs from the recently described betaine transport system in this organism (29, 36). The finding that butyrobetaine, a carnitine precursor, hindered L-carnitine uptake while betaine was not inhibitory might indicate that the length of the alkylchain between the *N*-trimethyl group on the one end and the carboxyl group on the other end is crucial in recognition. Furthermore, the substrate specificity of the L-carnitine transporter of *L. monocytogenes* is distinct from the system that transports L-carnitine in *E. coli*, which is induced during anaerobic growth in the presence of carnitine. D-Carnitine, betaine, butyrobetaine, and crotonobetaine were shown to be potent competitors for carnitine transport in *E. coli* (14, 25). Surprisingly, L-carnitine transport in *L. monocytogenes* was strongly inhibited by a 10-fold excess of acetylcarnitine. The carnitine carrier in eukaryotic cells also mediates translocation of L-carnitine and acetylcarnitine in addition to other acylcarnitines (21, 33). The substrate specificity of the L-carnitine transport system in *L. monocytogenes* therefore shows some resemblance to that of the eukaryotic carnitine:acylcarnitine antiporter. SH reagents were applied to investigate whether the substrate binding site of the L-carnitine permease of *L. monocytogenes* resembles that of the eukaryotic transporter. The L-carnitine permease of *L. monocytogenes* is inhibited by the SH reagent NEM but was not sensitive to mersalyl (Fig. 6), whereas the eukaryotic carnitine:acylcarnitine antiporter is inhibited by NEM and mersalyl (34). Thus, in both systems sulfhydryl groups are involved in transport, but the dissimilarity in the ability of these SH reagents to block the two L-carnitine transporters suggests that reactive SH

groups, near or in the substrate binding sites of the two systems, are oriented differently. The interaction of the L-carnitine transporter in *L. monocytogenes* with the organomercurial *p*CMBS (Fig. 6) might however be advantageous in its purification for further characterization. Interaction of organomercurials with transport carriers was suggested as a feasible purification method for transport systems (13). In conclusion, the L-carnitine transporter in *L. monocytogenes* is distinct from any transport system previously described and therefore represents a novel transport system for the translocation of L-carnitine.

The study reported here shows that *L. monocytogenes* is in the possession of a constitutive high-affinity uptake system for L-carnitine that enables the bacterium to scavenge L-carnitine when it is available at trace levels in foods (11). It was found that the transport capacity of the L-carnitine permease was very high in the absence of salt and that this activity could not be stimulated upon imposition of an osmotic stress. In contrast, betaine transport in *L. monocytogenes* is highly stimulated in the presence of high concentrations of NaCl (29, 36). This implicates that carnitine accumulation in *L. monocytogenes* can occur even under conditions of low osmolarity. The physiological role of this feature of the L-carnitine transporter is intriguing. Recently, it was demonstrated that betaine combats both osmotic and chill stress in *L. monocytogenes* (29). It is conceivable that chill tolerance in *L. monocytogenes* can also be conferred by the accumulation of L-carnitine. It was shown that *L. monocytogenes* cells grown at 7°C are able to take up L-carnitine at a rate of about 3 nmol min^{-1} mg of $protein^{-1}$, and in addition it was observed that L-carnitine can be accumulated to high concentrations under these conditions (data not shown). The possible role of the L-carnitine transport system in adaptation of *L. monocytogenes* to growth at low temperature is being further investigated.

It can also be speculated that the L-carnitine transporter is a means for *L. monocytogenes* to maintain its turgor pressure in the absence of stress. Gram-positive bacteria generally maintain a higher turgor than gram-negative bacteria, arising from their higher cytoplasmic concentrations of solutes at equivalent osmotic pressures (19, 23, 35, 50). Thus, *L. monocytogenes* would benefit from the uptake of compatible solutes even in a low-osmolarity environment. Moreover, the L-carnitine transporter may facilitate the intracellular growth and survival of *L. monocytogenes* in mammalian cells, which is an essential component of the pathogenesis of this organism. Both, carnitine and acetylcarnitine are present in the cytosol at concentrations high above the K_m values of the L-carnitine transport system in *L. monocytogenes* for these compounds (21). Considering that (i) the replication of *L. monocytogenes* in mammalian cells occurs in the cytoplasm, (ii) the osmolarity of the cytoplasm is probably relatively high, and (iii) both carnitine and acetylcarnitine (unpublished data) are effective osmoprotectants in *L. monocytogenes*, the availability of these osmolytes might increase the capacity of this human pathogen to grow in the host cell.

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REFERENCES

1. **Abee, T., T. R. Klaenhammer, and L. Letellier.** 1994. Kinetic studies of the action of lactacin F, a bacteriocin produced by *Lactobacillus johnsonii* that forms poration complexes in the cytoplasmic membrane. Appl. Environ. Microbiol. **60:**1006–1013.
2. **Abee, T., and W. N. Konings.** 1990. The important role of internal pH in energy transduction in bacteria. Trends Biomembr. Bioenerg. **1:**75–92.
3. **Abee, T., R. Palmen, K. J. Hellingwerf, and W. N. Konings.** 1990. Osmoregulation in *Rhodobacter sphaeroides*. J. Bacteriol. **172:**149–154.
4. **Ames, G. F.-L., C. S. Mimura, and V. Shyamala.** 1990. Bacterial periplasmic permeases belong to a family of transport proteins operating from *Escherichia coli* to human: traffic ATPases. FEMS Microbiol. Rev. **75:**429–446.
5. **Amezaga, M.-R., I. Davidson, D. McLaggan, A. Verheul, T. Abee, and I. R. Booth.** 1995. The role of peptide metabolism in the growth of *Listeria monocytogenes* ATCC 23074 at high osmolarity. Microbiology **141:**41–49.
6. **Belitz, H.-D., and W. Grosch.** 1986. Food chemistry, p. 549–562. Springer-Verlag, New York.
7. **Beumer, R. R., M. C. te Giffel, L. J. Cox, F. M. Rombouts, and T. Abee.** 1994. Effect of exogenous proline, betaine, and carnitine on growth of *Listeria monocytogenes* in a minimal medium. Appl. Environ. Microbiol. **60:**1359–1363.
8. **Bieber, L. L.** 1988. Carnitine. Annu. Rev. Biochem. **57:**261–283.
9. **Breeuwer, P., and T. Abee.** Unpublished data.
10. **Bruno, M. E. C., A. Kaiser, and T. J. Montville.** 1992. Depletion of proton motive force by nisin in *Listeria monocytogenes* cells. Appl. Environ. Microbiol. **58:**2255–2259.
11. **Combs, G. F.** 1992. The vitamins. fundamental aspects in nutrition and health, p. 403–411. Academic Press. San Diego.
12. **Csonka, L. N.** 1989. Physiological and genetic response of bacteria to osmotic stress. Microbiol. Rev. **53:**121–147.
13. **Driessen, A. J. M., and W. N. Konings.** 1990. Reactive exofacial sulfhydryl-groups on the arginine-ornithine antiporter of *Lactococcus lactis*. Biochim. Biophys. Acta **1015:**87–95.
14. **Eichler, K., F. Bourgis, A. Buchet, H.-P. Kleber, and M.-A. Mandrand-Berthelot.** 1994. Molecular characterization of the *cai* operon necessary for carnitine metabolism in *Escherichia coli*. Mol. Microbiol. **13:**775–786.
15. **Eneroth, P., and G. Lindstedt.** 1965. Thin-layer chromatography of betaines and other compounds related to carnitine. Anal. Biochem. **10:**479–485.
16. **Farber, J. M., and P. I. Peterkin.** 1991. *Listeria monocytogenes*, a food-borne pathogen. Microbiol. Rev. **55:**476–511.
17. **Fougère, F., and D. Le Rudelier.** 1990. Uptake of glycine betaine and its analogs by bacteroides of *Rhizobium meliloti*. J. Gen. Microbiol. **136:**157–163.
18. **Gilson, E., G. Alloing, T. Schmidt, J.-P. Claverys, R. Dudler, and M. Hofnung.** 1988. Evidence for high-affinity binding-protein dependent transport systems in Gram-positive bacteria and in *Mycoplasma*. EMBO J. **7:**3971–3974.
19. **Graham, J. E., and B. J. Wilkinson.** 1992. *Staphylococcus aureus* osmoregulation: roles for choline, glycine betaine, proline, and taurine. J. Bacteriol. **174:**2711–2716.
20. **Hutkins, R. W., W. L. Ellefson, and E. R. Kashket.** 1987. Betaine transport imparts osmotolerance on a strain of *Lactobacillus acidophilus*. Appl. Environ. Microbiol. **53:**2275–2281.
21. **Idell-Wenger, J. A.** 1981. Carnitine:acylcarnitine translocase of rat heart mitochondria. J. Biol. Chem. **256:**5597–5603.
22. **Imhoff, J. F.** 1986. Osmoregulation and compatible solutes in bacteria. FEMS Microbiol. Rev. **39:**57–66.
23. **Ingraham, J.** 1987. Effect of temperature, pH, water activity, and pressure on growth, p. 1543–1554. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.). *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
24. **Joshi, A. K., S. Ahmed, and G. F.-L. Ames.** 1989. Energy coupling in bacterial periplasmic transport systems. J. Biol. Chem. **264:**2126–2133.
25. **Jung, H., K. Jung, and H.-P. Kleber.** 1990. L-Carnitine uptake by *Escherichia coli*. J. Basic Microbiol. **30:**507–514.
26. **Klarsfeld, A. D., P. L. Goossens, and P. Cossart.** 1994. Five *Listeria monocytogenes* genes preferentially expressed in infected mammalian cells: *plcA*, *purH*, *purD*, *pyrE*, and an arginine transporter gene, *arpJ*. Mol. Microbiol. **13:**585–597.
27. **Kleber, H.-P., and H. Aurich.** 1967. Evidence for an inducible active transport of carnitine in *Pseudomonas aeruginosa*. Biochem. Biophys. Res. Commun. **26:**255–260.
28. **Kleber, H.-P., and H. Aurich.** 1985. Stoffwechsel des carnitins bei mikroorganismen. Wiss. Z. Karl-Marx-Univ. Leipzig. Math.-Naturwiss. Reihe **34:**224–237.
29. **Ko, R., L. T. Smith, and G. M. Smith.** 1994. Glycine betaine confers enhanced osmotolerance and cryotolerance on *Listeria monocytogenes*. J. Bacteriol. **176:**426–431.
30. **Kunji, E. R. S., E. J. Smid, R. Plapp, B. Poolman, and W. N. Konings.** 1993. Di-tripeptides and oligopeptides are taken up via distinct transport mechanisms in *Lactococcus lactis*. J. Bacteriol. **175:**2052–2059.
31. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
32. **Molenaar, D., A. Hagting, H. Alkema, A. J. M. Driessen, and W. N. Konings.**

1993. Characteristics and osmoregulatory roles of uptake systems for proline and glycine betaine in *Lactococcus lactis*. J. Bacteriol. **175:**5438–5444.
33. **Murthy, M. S. R., and S. V. Pande.** 1984. Mechanism of carnitine acylcarnitine translocase-catalyzed import of acylcarnitines into mitochondria. J. Biol. Chem. **259:**9082–9089.
34. **Noël, H., T. Goswami, and S. V. Pande.** 1985. Solubilization and reconstitution of rat liver mitochondrial carnitine acylcarnitine translocase. Biochemistry **24:**4504–4509.
35. **Patchett, R. A., A. F. Kelly, and R. G. Kroll.** 1992. Effect of sodium chloride on the intracellular solute pools of *Listeria monocytogenes*. Appl. Environ. Microbiol. **58:**3959–3963.
36. **Patchett, R. A., A. F. Kelly, and R. G. Kroll.** 1994. Transport of glycine-betaine by *Listeria monocytogenes*. Arch. Microbiol. **162:**205–210.
37. **Peters, P., E. Tel-Or, and H. G. Trüper.** 1992. Transport of glycine betaine in the extreme haloalkaliphilic sulphur bacterium *Ectothiorhodospira halochloris*. J. Gen. Microbiol. **138:**1993–1998.
38. **Poolman, B.** 1993. Energy transduction in lactic acid bacteria. FEMS Microbiol. Rev. **12:**125–148.
39. **Poolman, B., A. J. M. Driessen, and W. N. Konings.** 1987. Regulation of solute transport in streptococci by external and internal pH values. Microbiol. Rev. **51:**498–508.
40. **Poolman, B., and W. N. Konings.** 1993. Secondary solute transport in bacteria. Biochim. Biophys. Acta **1183:**5–39.
41. **Poolman, B., D. Moolenaar, and W. N. Konings.** 1992. Diversity of transport mechanisms in bacteria, p. 1–50. *In* M. Shinitzky (ed.). Handbook of biomembranes, vol. 2. Balaban Publishers, Rehovot, Israel.
42. **Pourkomailian, B., and I. R. Booth.** 1992. Glycine betaine transport by *Staphylococcus aureus*: evidence for two transport systems and their possible roles in osmoregulation. J. Gen. Microbiol. **138:**2515–2518.
43. **Pourkomailian, B., and I. R. Booth.** 1994. Glycine betaine transport by *Staphylococcus aureus*: evidence for feedback regulation of the activity of two transport systems. Microbiology **140:**3131–3138.
44. **Premaratne, R. J., W.-J. Lin, and E. A. Johnson.** 1991. Development of an improved chemically defined medium for *Listeria monocytogenes*. Appl. Environ. Microbiol. **57:**3046–3048.
45. **Riou, N., M. C. Poggi, and D. Le Rudelier.** 1991. Characterization of an osmoregulated periplasmic glycine betaine-binding protein in *Azospirillum brasilense* sp7. Biochimie **73:**1187–1193.
46. **Shinbo, T., N. Kama, K. Kurihara, and Y. Kobataka.** 1978. PVC-based electrode sensitive to DDA^+ as a device to monitor the membrane potential in biological systems. Arch. Biochem. Biophys. **187:**414–422.
47. **Stimeling, K. W., J. E. Graham, A. Kaenjak, and B. J. Wilkinson.** 1994. Evidence for feedback (*trans*) regulation of, and two systems for, glycine betaine transport by *Staphylococcus aureus*. Microbiology **140:**3139–3144.
48. **te Giffel, M. C., and T. Abee.** Unpublished data.
49. **Verheul, A., A. Hagting, M.-R. Amezaga, I. R. Booth, F. M. Rombouts, and T. Abee.** 1995. A di- and tripeptide transport system can supply *Listeria monocytogenes* Scott A with amino acids essential for growth. Appl. Environ. Microbiol. **61:**226–233.
50. **Whatmore, A. M., and R. H. Reed.** 1990. Determination of turgor pressure in *Bacillus subtilis*: a possible role for K^+ in turgor regulation. J. Gen. Microbiol. **136:**2521–2526.
51. **Yancey, P. H., M. E. Clark, S. C. Hand, R. D. Bowlus, and G. N. Somero.** 1982. Living with water stress: evolution of osmolyte systems. Science **217:** 1214–1222.