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Role of Osmolytes in Adaptation of Osmotically Stressed and Chill-Stressed *Listeria monocytogenes* Grown in Liquid Media and on Processed Meat Surfaces

LINDA TOMBRAS SMITH*

Department of Agronomy and Range Science, University of California, Davis, California 95616

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***Listeria monocytogenes* is a food-borne pathogen that is widely distributed in nature and is found in many kinds of fresh and processed foods. The pervasiveness of this organism is due, in part, to its ability to tolerate environments with elevated osmolarity and reduced temperatures. Previously, we showed that *L. monocytogenes* adapts to osmotic and chill stress by transporting the osmolyte glycine betaine from the environment and accumulating it intracellularly (R. Ko, L. T. Smith, and G. M. Smith, J. Bacteriol. 176:426–431, 1994). In the present study, the influence of various environmental conditions on the accumulation of glycine betaine and another osmolyte, carnitine, was investigated. Carnitine was shown to confer both chill and osmotic tolerance to the pathogen but was less effective than glycine betaine. The absolute amount of each osmolyte accumulated by the cell was dependent on the temperature, the osmolarity of the medium, and the phase of growth of the culture. *L. monocytogenes* also accumulated high levels of osmolytes when grown on a variety of processed meats at reduced temperatures. However, the contribution of carnitine to the total intracellular osmolyte concentration was much greater in samples grown on meat than in those grown in liquid media. While the amount of each osmolyte in meat was less than 1 nmol/mg (fresh weight), the overall levels of osmolytes in *L. monocytogenes* grown on meat were about the same as those in liquid samples, from about 200 to 1,000 nmol/mg of cell protein for each osmolyte. This finding suggests that the accumulation of osmolytes is as important in the survival of *L. monocytogenes* in meat as it is in liquid media.**

In recent years, listeriosis has come to prominence as a leading cause of death from food-borne illness. Although the responsible organism, *Listeria monocytogenes*, mainly infects immunocompromised and otherwise susceptible individuals, estimates indicate that in the United States alone, 1,092 cases and 248 deaths occurred in 1993 as a result of infection with this pathogen (18). Control of *L. monocytogenes* is difficult for two reasons. The first is its ubiquitous nature. It is commonly found in plant, soil, and surface water samples and has also been isolated from the feces of livestock, in slaughterhouse and food-processing environments, and in the home (5, 7, 12). It has been reported to contaminate 6.2 to more than 60% of fresh meat and poultry (5) and about one-third of processed, ready-to-eat meat products (5, 7). The second reason is its ability to tolerate environmental stresses. Of particular concern is that *L. monocytogenes* is osmotically tolerant and can grow at refrigerator temperatures. For example, it has been reported to grow at more than 10% NaCl (10) and at temperatures as low as −0.1°C (20). *L. monocytogenes* is able to adapt to elevated osmolarity by accumulating compatible solutes (called osmolytes) in the cytosol. These solutes act by counterbalancing the external osmotic strength without destabilizing the cellular protein structure, even at the high concentrations at which they are found in the cell (21). One of the most effective compatible solutes utilized by this and most of the other bacterial species studied thus far is glycine betaine (3, 4). However, since *L. monocytogenes* cannot synthesize glycine betaine, it must be provided in the growth medium and transported into the cell. It was recently reported that *L. monocytogenes* accumulates glycine betaine to adapt to low-temperature stress as well (8). Evidence for this conclusion is that the glycine betaine transport rate increases sharply under either osmotic or chill stress and that addition of glycine betaine to the growth medium confers both enhanced chill and salt tolerance to the cell (8). Although these results provide some insight into the mechanism of adaptation to environmental stresses in the laboratory setting, additional details, such as the identity of osmolytes accumulated under a wide variety of conditions, are missing. Furthermore, the question arises as to the relevance of these findings to the microbiological safety of the food supply. To address these issues, I investigated additional aspects of the mechanism of osmotic and chill stress adaptation of *L. monocytogenes* and also studied the ability of *L. monocytogenes* to utilize these adaptive mechanisms when grown on processed meats at low temperature.

*Phone: (916) 752-6161. Fax: (916) 752-4361. Electronic mail address: lsmith@ucdavis.edu.

MATERIALS AND METHODS

Materials, bacterial strain, and media. Chloramphenicol-resistant *L. monocytogenes* DP-L1044 (*hly*::Tn*917*-LTV3) (17) was maintained on brain heart infusion (BHI; Difco) agar plates. BHI broth was the rich medium used, and modified Pine's medium (8), which contains no glycine betaine or carnitine, was the defined medium used. Chloramphenicol resistance was determined by using plates containing solidified BHI medium and 10 μg of the antibiotic per ml. Esculin utilization was tested with bile esculin agar (Difco), and the ability to produce acid from carbohydrates was determined with purple agar supplemented with 0.5% maltose or rhamnose or 1.0% mannitol, sucrose, or glucose. Glycine betaine and carnitine were from Sigma, and all other chemicals used were reagent grade or the best grade available. Processed meats were purchased at local markets.

Growth measurements. Cultures were grown in BHI broth for 10 h, subcultured at a dilution of 1:100 into modified Pine's medium, and grown for about 8 h. For growth measurements, 0.05 ml of this culture was transferred to 5.0 ml of modified Pine's medium containing NaCl and 1 mM glycine betaine or carnitine when noted. Growth was monitored by measuring turbidity with a Klett-Summerson colorimeter with a no. 54 filter. Means of at least three separate experiments are reported. The error of quantitation was generally between 2 and 10%.

Inoculation of meat. The processed meats used were bologna (containing pork, chicken, and beef), franks (beef), wieners (containing pork and turkey),

ham, and bratwurst, which have been shown to permit the growth of *L. monocytogenes* (6). Dry salami, which has both low water activity and low pH, served as a control in which the growth of the pathogen is not observed (7). Fresh cultures of *L. monocytogenes* grown on either solidified BHI or modified Pine's medium were used to inoculate meat. Meat samples were inoculated with about 10^{10} to 10^{11} cells on a meat surface of 10 to 30 cm^2 with a sterile loop and were placed in petri dishes, which were then sealed and incubated at 7°C for the indicated time. Cells were harvested by being scraped off the entire inoculated meat surface with a sterile loop and deposited into a microcentrifuge tube. Each sample was subsequently analyzed for osmolyte content and for total cellular protein as described in the following section. The identity of the harvested cells was verified by plating a portion of each sample on solidified BHI medium and then testing for chloramphenicol resistance, catalase activity, esculin utilization, and carbohydrate fermentation (glucose, maltose, rhamnose, sucrose, and mannose).

Uninoculated meat samples were similarly incubated at 7°C for 14 to 21 days, similar areas of the meat surface were scraped, and the resulting material was plated on solidified BHI. By this procedure, no viable colonies were isolated from bologna, wiener, frank, or ham controls. However, colonies were recovered from uninoculated salami and bratwurst. Unlike *L. monocytogenes*, these cells were catalase negative, did not react with esculin, did not produce acid from maltose or rhamnose, and were not resistant to chloramphenicol. To ensure that the samples harvested from inoculated salami and bratwurst were predominantly *L. monocytogenes*, portions of these samples were streaked to single colonies, which were plated on the test plates described above to determine the percentage of contaminants in each sample. Routinely greater than 98% of all CFU tested were chloramphenicol resistant and possessed all of the other characteristics of *L. monocytogenes*.

HPLC analysis. The levels of osmolytes in *L. monocytogenes* grown either in liquid BHI broth or on meat surfaces were routinely determined by high-pressure liquid chromatography (HPLC). For samples grown in BHI broth, 5 to 10 ml of culture was grown with or without additions to late log phase, unless otherwise noted, and harvested by centrifugation, and the intracellular contents were extracted at room temperature with 0.5 ml of 70% ethanol for 25 min. The extraction procedure was repeated once, and the extracts were combined and filtered through a 0.45-μm-pore-size nylon filter. The pellet and filter were washed with 0.5 ml of extraction solvent, and the extract and wash were combined and lyophilized. Cells grown on meat were similarly extracted after being harvested (as described in the previous section). The lyophilizates were dissolved in 100 μl of distilled water. Aliquots (20 μl) of the samples were chromatographed on an Econosphere NH2 column (250 by 4.6 mm) with 5-μm packing (Alltech Associates, Deerfield, Ill.) An evaporative light-scattering detector (Applied Chromatography Systems, Ltd.) and a Hewlett-Packard 3390A integrator were used for quantitation. The mobile phase was acetonitrile-methanol-water (75:10:15), and the flow rate was 1.2 ml/min. The retention times for glycine betaine and carnitine were 5.1 and 12.7 min, respectively. The protein content of the bacterial samples was determined by the method of Lowry et al. (9).

NMR spectral analysis. Intracellular osmolytes from *L. monocytogenes* and from meat sources were identified and quantified by natural-abundance ^{13}C nuclear magnetic resonance (NMR) spectroscopy. For bacterial samples, cells from 1 liter of culture were harvested and extracted with perchloric acid as described previously (13). Cultures that were grown on meat surfaces were harvested as described above for HPLC analysis and then extracted three times with 70% ethanol (final volume, 10.5 ml). Spectra were obtained at 75.58 MHz with a General Electric Omega-300 spectrometer as described previously (13).

To determine the levels of glycine betaine and carnitine in meat samples, 50 g of meat and 80 ml of 70% ethanol were blended in a Waring blender with 1-min pulses and 3-min rests until the mixture appeared smooth. The blender was chilled in an ice bath during the rests. After being blended, the mixture was incubated at room temperature for 10 min and then centrifuged at 8,700 × *g* for 10 min. The pellet was extracted a second time with 50 ml of extraction solvent and then centrifuged as before. The extracts were combined, the pellet was washed with water, and the wash was added to the extract. The combined extract was centrifuged once more after an overnight incubation at −20°C. Additional water was added to the clarified extract, and the extract was lyophilized. The dry extract was dissolved in 15% D_2O to a final volume of 2.5 to 3.0 ml and subjected to NMR spectroscopy. The concentration of osmolytes was determined by comparison with 50 mM alanine that was added to the samples as internal standard.

RESULTS

Regulation of the osmolyte content of *L. monocytogenes*. Although it has been shown that *L. monocytogenes* accumulates glycine betaine and low levels of carnitine when grown in BHI broth supplemented with 8% NaCl, other NaCl concentrations were not tested in that study (8). Since it is known that the osmolarity of the growth medium is one factor that governs osmolyte preference in bacteria (15, 16), the experiment was repeated at several osmotic strengths (Fig. 1A). In cultures

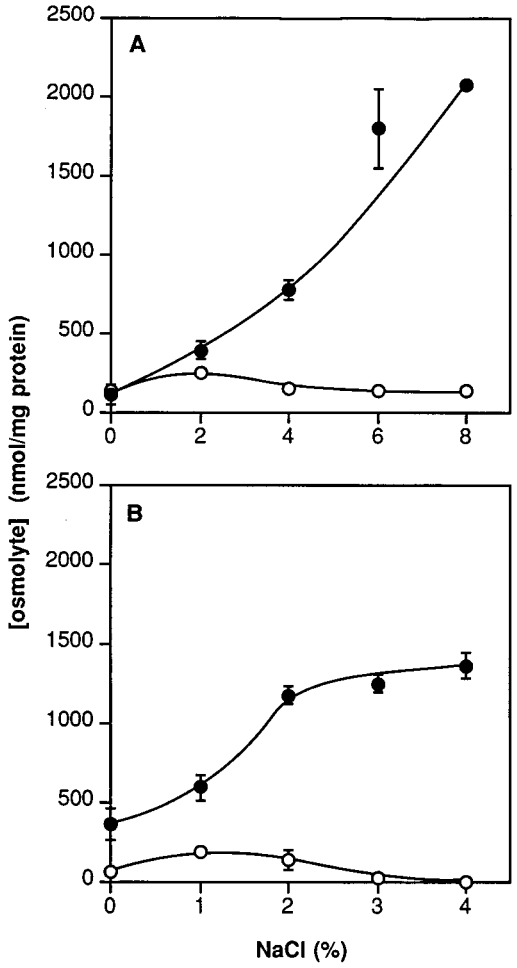

FIG. 1. Dependence of the intracellular osmolyte composition of *L. monocytogenes* on the osmolarity of the growth medium. Cultures were grown in BHI broth with NaCl added where indicated, at either 30°C (A) or 7°C (B). Samples were harvested at late log to early stationary phase and extracted with 70% ethanol. Intracellular glycine betaine (●) and carnitine (○) concentrations were quantified by HPLC analysis. Cultures incubated at 30°C were grown with 0 to 8% NaCl added to the growth medium, and those incubated at 7°C were grown with 0 to 4% NaCl. Means of duplicate trials are shown.

grown at 30°C, the glycine betaine content increased with increasing concentrations of NaCl in the growth medium up to the maximum NaCl concentration tested. The carnitine concentration peaked at about 2% NaCl, but since it remained low and relatively constant, the ratio of glycine betaine to carnitine steadily increased as the osmolarity increased.

The osmolyte content in cultures grown under both osmotic and chill stress was also determined, since low temperature also stimulates the accumulation of osmolytes in *L. monocytogenes* (Fig. 1B). Just as with cultures grown at 30°C, the level of carnitine in cultures at 7°C was comparatively low and peaked in media containing 1 to 2% NaCl, but the decrease in the level of carnitine at higher NaCl concentrations was more pronounced in cultures grown at 7°C. In fact, carnitine was undetectable in chilled cells grown in only 4% NaCl. On the other hand, the glycine betaine content rose with less NaCl in the medium of chilled cultures than in 30°C cultures. For example, at 4% NaCl, there was almost twice as much glycine betaine in cells grown at 7°C than in those grown at 30°C. Hence, although similar trends were found at the two temperatures

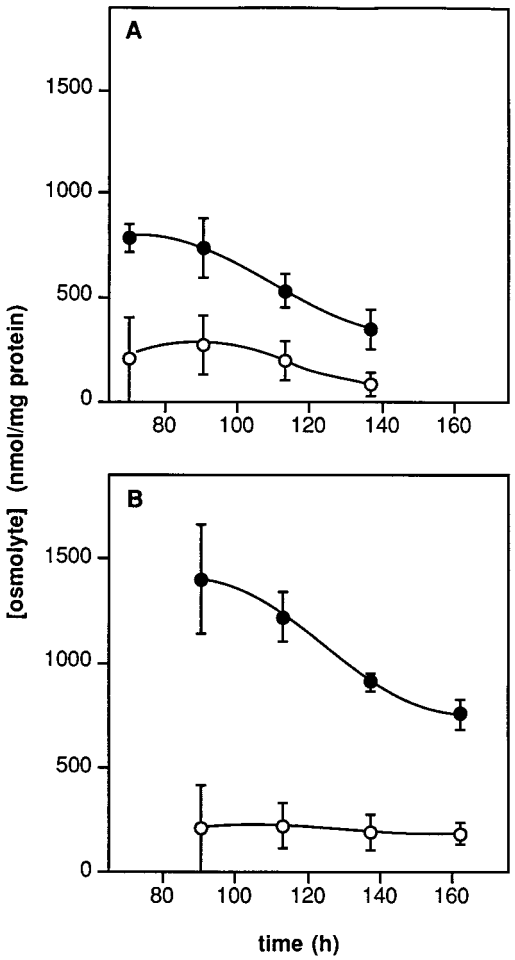

FIG. 2. Dependence of the intracellular osmolyte composition on the phase of growth of *L. monocytogenes*. Inocula were grown in BHI broth, and at the start of the experiment, grown cultures were diluted 100-fold into BHI broth containing no addition (A) or 2% NaCl (B) and incubated at 7°C. Aliquots were harvested at the indicated times corresponding to early log phase through stationary phase for each culture. Samples were extracted with ethanol, and the osmolytes glycine betaine (●) and carnitine (○) were quantified by HPLC. The two cultures were not sampled at identical times because of a difference in the length of their respective lag phases. Means of duplicate trials are shown.

tested, quantitative differences in absolute concentration and relative ratios of glycine betaine and carnitine were apparent.

The phase of growth of the culture can also affect the relative levels of intracellular solutes. While it has been shown that for salt-stressed gram-negative bacteria the osmolyte profiles during rapid growth can be quite different from those during the stationary phase (15), the effect on chill stress-stimulated osmolyte accumulation has not been investigated. To determine the effect of the growth phase, the osmolyte content was determined as a function of the age of the culture for chill-stressed (Fig. 2A) and chill-stressed and osmotically stressed (Fig. 2B) cultures. In this experiment, cultures were grown at 7°C either with or without 2% NaCl added to the medium. Under both conditions, the concentration of glycine betaine in the cells was higher in younger cultures. On the other hand, the carnitine level remained low over the entire course of the experiment. The change in the ratio of osmolyte levels during the stationary phase is not due to limiting amounts of available glycine betaine, since about 2 mM glycine betaine was present in the growth medium.

TABLE 1. Effects of exogenously added osmolytes on the growth of osmotically stressed and chill-stressed *L. monocytogenes*

Temp (°C)	NaCl concn (%)	Growth rate (h/generation) with following addition[a]:			
		None	GB	CAR	GB + CAR
30	0	3.0	3.2	3.1	3.6
	4	9.7	4.8	5.5	4.3
	8	>150[b]	7.4	15	7.2
7	0	51	31	38	33
	2	59	29	30	30
	4	NG[c]	40	48	38

[a] Cultures were grown in modified Pine's medium plus NaCl and 1 mM glycine betaine (GB) or carnitine (CAR) where indicated.
[b] Although growth was observed under these conditions, it was too slow to quantify accurately.
[c] NG, no growth was observed over a 1-month period.

Osmotic and cryoprotection conferred by carnitine. Considering that carnitine seems to be a constant component of the osmolyte profile of *L. monocytogenes*, it was of interest to determine if this compound protects the cell against osmotic and chill stress in a manner similar to that of glycine betaine. To address this issue, growth experiments were conducted under a variety of osmotic and chill stress conditions (Table 1). The results obtained with cultures grown at 30°C with and without NaCl and osmolytes demonstrated the ability of carnitine to protect the culture against osmotic stress. For example, when the culture was grown with 8% NaCl, almost no growth was observed in the absence of added osmolytes. In the presence of 1 mM glycine betaine or carnitine, the doubling time was 7.4 and 15 h per generation, respectively. Hence, carnitine protected the cell against osmotic stress but only half as well as glycine betaine did. The inclusion of 0.1 mM carnitine resulted in nearly the same growth rate as that with 1.0 mM carnitine, and concentrations greater than 1.0 mM did not improve the growth rate further (data not shown).

Carnitine also protected *L. monocytogenes* against chill stress (Table 1). However, just as was found with osmotic stress protection, carnitine was somewhat less effective than glycine betaine in enhancing chill tolerance. With either chill-stressed or osmotically stressed cultures, the addition of both osmolytes to the growth medium did not cause growth to improve more than that observed with glycine betaine alone. Examination of ^{13}C NMR spectra of *L. monocytogenes* cellular extracts (data not shown) revealed that carnitine was not accumulated in the cytosol of unstressed cells or if carnitine was omitted from the growth medium. In contrast, when cultures were grown in NaCl plus carnitine, the intracellular carnitine concentration was close to 1000 nmol/mg of protein. Hence, it appears that carnitine behaves just as glycine betaine in that the two osmolytes alleviate osmotic and chill stress by similar mechanisms.

Stress adaptation of *L. monocytogenes* grown on bologna. The results thus far presented suggest that glycine betaine and carnitine enhance the osmotic and chill tolerance of *L. monocytogenes*. However, the question of the relevance of these findings to the safety of the food supply arises. Since it is known that a significant source of *L. monocytogenes* is ready-to-eat and other processed meats (5, 7), this question was addressed by growing *L. monocytogenes* on bologna at 7°C and identifying and quantifying the levels of osmolytes accumulated (if any) in the cytoplasm of the bacterial cells. Initially, natural-abundance ^{13}C NMR spectroscopy was used to ob-

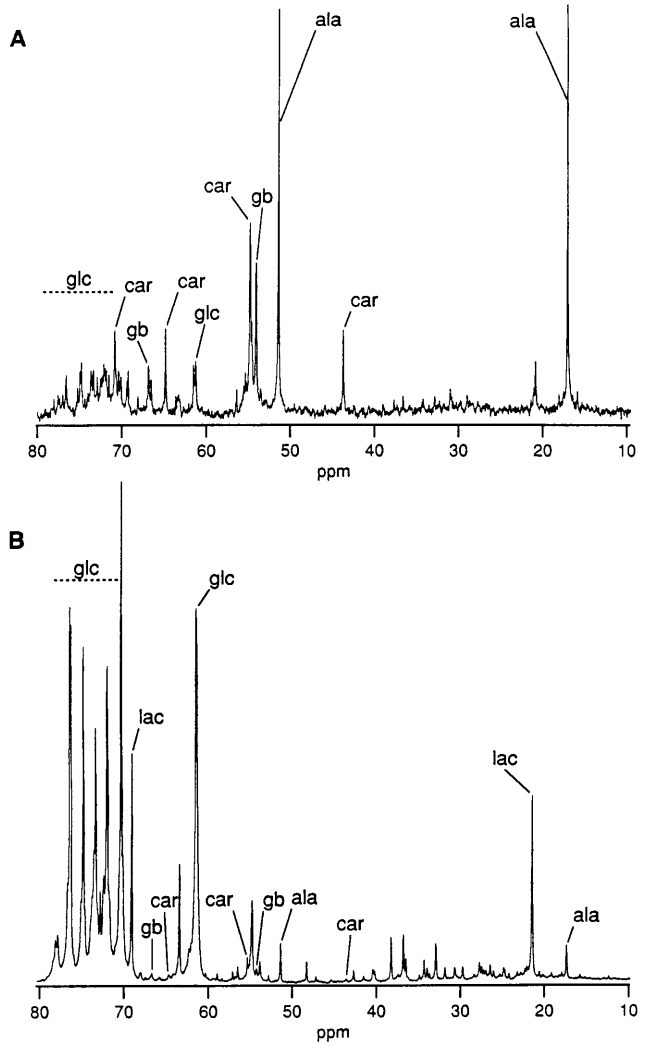

FIG. 3. Natural-abundance ^{13}C NMR spectra of extracts of *L. monocytogenes* and bologna. (A) An ethanol extract of a sample of *L. monocytogenes* grown on bologna for 14 days at 7°C was subjected to analysis by NMR spectroscopy. (B) NMR spectrum of an ethanol extract of uninoculated bologna. Resonances arising from glycine betaine (gb), carnitine (car), and glucose (glc) were observed in both samples. Resonances from lactate (lac) were also present in the uninoculated bologna sample. Alanine (50 mM) (ala) was added to each sample as an internal standard.

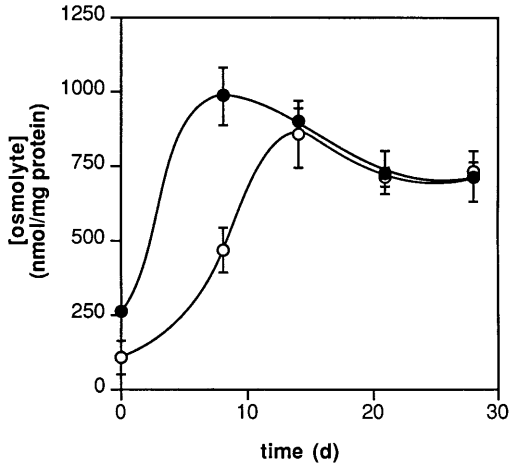

FIG. 4. Intracellular accumulation of osmolytes in *L. monocytogenes* grown on bologna. Bologna samples were inoculated with *L. monocytogenes* and incubated at 7°C. At the indicated times, bacterial samples were harvested and the cytosolic contents were extracted for HPLC analysis as described in Materials and Methods. Both glycine betaine (●) and carnitine (○) were observed. Means of duplicate trials are shown.

serve all classes of organic osmolytes that might be accumulated regardless of their physical or chemical properties. The NMR spectrum of an extract of *L. monocytogenes* grown on bologna showed resonances corresponding to carnitine and glycine betaine (Fig. 3A), which was confirmed by HPLC (data not shown). These results demonstrate that *L. monocytogenes* utilizes its osmoregulatory mechanism of adaptation on meat surfaces. Less intense resonances corresponding to glucose, which may have arisen from the cytosol of the bacterium, were also observed. Alternatively, the glucose may be a contaminant in the meat, considering the intense glucose resonances observed in the spectrum of the bologna extract (Fig. 3B). An NMR spectrum of bologna extracted with perchloric acid (data not shown) exhibited essentially the same results as those in Fig. 3B, indicating the adequacy of the ethanol extraction procedure.

To determine if the osmolyte profile is significantly altered as the culture ages, the intracellular osmolyte content of *L. monocytogenes* grown on bologna was sampled over a 4-week period (Fig. 4). While the level of glycine betaine approached that in cultures grown in BHI broth at 7°C (Fig. 1 and 2), the carnitine concentration was as much as 27-fold higher in cultures grown on bologna than those in BHI broth. Consequently, the intracellular carnitine level was nearly identical to that of glycine betaine in cultures that were at least 14 days old (Fig. 4). These results suggest that the osmolyte of choice was glycine betaine early during the course of the experiment but that carnitine played an increasingly important role in osmoregulation as the culture grew.

Stress adaptation of *L. monocytogenes* on other processed meat surfaces. The ability of *L. monocytogenes* to osmoregulate on other processed meat surfaces was also determined (Table 2). While both glycine betaine and carnitine were found to accumulate in *L. monocytogenes* grown on favorable meat surfaces, the relative ratios of osmolytes accumulated in the pathogen varied considerably. For example, the carnitine concentration was about twofold higher than that of glycine betaine in cells grown on bratwurst, but the glycine betaine concentration was twofold higher than that of carnitine in cells grown on ham. Values for the other samples fell between these

TABLE 2. Osmolytes accumulated in *L. monocytogenes* grown on processed meat surfaces[a]

Meat substrate	Concn of accumulated osmolyte (nmol/mg of cell protein)	
	Glycine betaine	Carnitine
Bologna	720	710
Franks	970	580
Wieners	920	550
Ham	830	380
Bratwurst	200	420
Salami	260	T[b]

[a] Meat surfaces were inoculated with *L. monocytogenes* and incubated at 7°C for 3 weeks. The cultures were then harvested, and the cytosolic osmolytes were extracted and quantified by HPLC as described in Materials and Methods.
[b] T, trace.

TABLE 3. Effect of inoculum on the intracellular osmolyte concentration of *L. monocytogenes* cultures grown on meat surfaces[a]

Meat substrate	Concn of accumulated osmolyte (nmol/mg of cell protein)	
	Glycine betaine	Carnitine
Bologna	990	870
Franks	560	140
Wieners	630	300

[a] Methods and experimental conditions were identical to those in the experiment in Table 2, except that the inocula used here were from cultures grown on solidified modified Pine's medium.

two extremes. On the other hand, cells incubated on salami, which did not support good growth of the pathogen, did not seem to accumulate glycine betaine or carnitine, even though adequate levels of these osmolytes were found in salami (see below). It should be noted that to maintain inoculum quality and experimental reproducibility, the inocula used for these experiments were from cultures grown on BHI plates. Hence, the inoculum itself contained low levels of glycine betaine and carnitine (Fig. 1). Therefore, additional experiments with processed meats that produced the most abundant growth of *L. monocytogenes* were carried out with inocula that were grown on modified Pine's medium, which contains neither glycine betaine nor carnitine. The levels of osmolytes in these samples (Table 3) were similar to those in which BHI-grown inocula were used (Table 2), with the exception of the carnitine level in cells grown on franks, which was fourfold higher in BHI-grown inocula. To show that the glycine betaine and carnitine observed were not due to contamination of the bacterial sample with the meat substrate, the concentrations of osmolytes in all meats used in this investigation were measured (Table 4). The results indicate that the levels of osmolytes in the processed meats used here are at least 100 times lower than in the corresponding bacterial samples.

DISCUSSION

Evidence presented in this report sheds light on the roles of glycine betaine and carnitine in the mechanism of osmotic and chill adaptation of *L. monocytogenes* in two types of environments. Previously, it was shown that glycine betaine (8, 11) and carnitine (2) protect *L. monocytogenes* against osmotic stress and that each behaves as a classic osmolyte by accumulating in the cytosol (8, 19). Glycine betaine is a more effective osmotic stress protectant than carnitine (2), and when both osmolytes are present in the growth medium, no further improvement in growth rate is found above that observed with glycine betaine alone (Table 1). One reason for this result is that the presence of glycine betaine appears to suppress the level of carnitine in the cell. NMR spectral results for osmotically stressed cultures grown with carnitine showed that the capacity of the cell to accumulate carnitine was about as high as its capacity to accumulate glycine betaine. When both osmolytes are supplied in the medium, the level of carnitine in the cell is markedly lower than that of glycine betaine, as shown in Fig. 1 and 2. In contrast, the internal concentration of glycine betaine is high regardless of the presence of carnitine in the growth medium; therefore, a synergistic effect of both osmolytes would not have been expected. The ability of glycine betaine to suppress the accumulation of other osmolytes, both exogenously and endogenously produced, is typical in those bacterial species that can accumulate glycine betaine along with other osmolytes (13–15) and is even more apparent in young cultures than in stationary-phase cultures (15).

TABLE 4. Levels of osmolytes in processed meats[a]

Meat	Concn of accumulated osmolyte (nmol/mg [fresh wt])	
	Glycine betaine	Carnitine
Bologna	0.48	0.23
Franks	0.34	0.84
Wieners	0.39	0.31
Ham	0.42	0.60
Bratwurst	0.36	0.74
Salami	0.43	0.95

[a] Meat samples were extracted with 70% ethanol as described in Materials and Methods, and the levels of osmolytes were quantified by ^{13}C NMR spectroscopy.

Just as glycine betaine and carnitine both confer osmotic tolerance, they also both confer cryotolerance. The mechanistic details of the accumulation of carnitine are somewhat different from those of the accumulation of glycine betaine. While the rate of glycine betaine uptake actually increases at low temperatures to effect a net accumulation (8), the rate of carnitine uptake does not (19). Perhaps the rate of carnitine efflux is decreased so that the uptake rate is high enough at 7°C for an effective level of carnitine accumulation to result. The preference of glycine betaine over carnitine in *L. monocytogenes* is just as pronounced at 7°C as it is at 30°C. While the preference in any species for glycine betaine in osmotic and chill adaptation has never been fully explained, in *L. monocytogenes* it may be due to the kinetics of transport, since the uptake rate for glycine betaine is severalfold higher than that for carnitine both at high osmolarity and at low temperatures (8, 19). Since glycine betaine could not inhibit carnitine uptake (19), competitive inhibition at the site of binding in the carnitine transport system is not a factor in the osmolyte preference exhibited by *L. monocytogenes*. These arguments do not address the issue of the preference of glycine betaine over endogenous osmolytes that are synthesized by the cell rather than transported into it. Presumably, glycine betaine affects the genetic control mechanisms of these systems.

L. monocytogenes adapted to stresses on meat surfaces in much the same way as it did in liquid broth. ^{13}C NMR spectroscopic studies of *L. monocytogenes* grown on bologna revealed that both carnitine and glycine betaine were accumulated and that no other identifiable osmolyte was accumulated to measurable levels. While a low level of glucose was observed, it probably did not result from the osmoregulatory pathway; glucose does not fit the paradigm of a compatible solute, since it is a reducing sugar that would react chemically with proteins. This sugar may instead accumulate intracellularly to low levels as part of the fermentation pathway of the organism. Another possibility is that the glucose observed in this extract arose as a contaminant from the meat surface, since the glucose concentration in bologna is quite high. Additional experiments must be carried out to determine which of these possibilities is correct. Of the other meat substrates tested, only those able to support the growth of *L. monocytogenes* yielded cells containing high levels of osmolytes. The amounts of osmolytes observed in these cells were too high to be accounted for by originating either from contamination with the meat itself or from the inoculum.

It is not surprising that *L. monocytogenes* responded in both meat and liquid broth environments by accumulating glycine betaine and carnitine, considering that the liquid broth used in this study is derived from beef extract. An unexpected result was that the carnitine level approached that of glycine betaine

in cultures 14 days and older whereas in liquid broth the carnitine level was typically 10-fold lower than that of glycine betaine. Hence, glycine betaine suppressed the accumulation of carnitine better in liquid broth than on processed meats. One plausible explanation for this difference is that while the overall concentration of osmolytes in the meats tested is high enough (Table 4) (1) to afford good growth, the osmolytes may not be readily accessible to the bacteria. Therefore, it is possible that the available glycine betaine was exhausted early during the course of the experiment, and so the cell then accumulated the less preferred osmolyte, carnitine, as well.

The capacity of *L. monocytogenes* to scavenge low levels of glycine betaine and carnitine from processed meats as a means of adaptation suggests that it may be possible to control the growth of this pathogen by creating a hostile environment devoid of osmolytes.

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