Adaptive Growth Responses of *Listeria monocytogenes* to Acid and Osmotic Shifts above and across the Growth Boundaries

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ABSTRACT

The effect of acid and osmotic shifts on the growth of *Listeria monocytogenes* was evaluated at 10°C. Two types of shifts were tested: (i) within the range of pH and water activity (a_w) levels that allow growth of *L. monocytogenes* and (ii) after habituation at no-growth conditions back to growth-permitting conditions. A *L. monocytogenes* cheese isolate, with high survival capacity during cheesemaking, was inoculated (10^2 CFU/ml) in tryptic soy broth supplemented with 0.6% yeast extract at six pH levels (5.1 to 7.2; adjusted with lactic acid) and 0.5% NaCl (a_w 0.995), or four a_w levels (0.995 to 0.93, adjusted with 0.5 to 10.5% NaCl) at pH 7.2 and grown to early stationary phase. *L. monocytogenes* was then shifted (at 10^2 CFU/ml) to each of the aforementioned growth-permitting pH and a_w levels and incubated at 10°C. Shifts from no-growth to growth-permitting conditions were carried out by transferring *L. monocytogenes* habituated at pH 4.9 or a_w 0.90 (12.5% NaCl) for 1, 5, and 10 days to all pH and a_w levels permitting growth. Reducing a_w or pH at different levels in the range of 0.995 to 0.93 and 7.2 to 5.1, respectively, decreased the maximum specific growth rate of *L. monocytogenes*. The lag time of the organism increased with all osmotic downshifts, as well as by the reduction of pH to 5.1. Conversely, any type of shift within pH 5.5 to 7.2 did not markedly affect the lag times of *L. monocytogenes*. The longer the cells were incubated at no-growth a_w (0.90), the faster they initiated growth subsequently, suggesting adaptation to osmotic stress. Conversely, extended habituation at pH 4.9 had the opposite effect on subsequent growth of *L. monocytogenes*, possibly due to cell injury. These results suggest that there is an adaptation or injury rate induced at conditions inhibiting the growth of the pathogen. Thus, quantifying adaptation phenomena under growth-limiting environments, such as in fermented dairy and meat products or products preserved in brine, is essential for reliable growth simulations of *L. monocytogenes* during transportation and storage of foods.

In dynamic growth modeling, it is assumed that the growth rate of cells is instantaneously adapted to momentary temperature changes, whereas there is contrasting evidence for induction of short lag times before growth is resumed (1, 3, 20, 21). Studies also suggest that precultivation of *Listeria monocytogenes* at 4°C results in a faster growth rate at 7°C than preculture at 7°C (19). Therefore, there seems to be marked uncertainty about the role of previous environmental conditions or the effect of inoculum history on subsequent microbial growth.

The lag phase of a microorganism in a new environment is affected by the intrinsic (e.g., pH and water activity [a_w]) and/or extrinsic (e.g., temperature) properties of the environment and the physiological state of cells, as determined by the previous environment. Subsequent changes of environmental factors may induce an “intermediate” lag phase (27), the duration of which depends on the magnitude and direction of the environmental shift. Quantification of the effect of temperature shifts on the lag time of bacteria has received more attention (2, 18, 27, 28, 30, 31) than the corresponding effect of osmotic shifts (15–17), whereas relevant information for shifts in pH is currently lacking.

The most widely used approach for quantifying the effect of sudden changes in the environment on bacterial lag time is based on the amount of work that cells need to undertake and the rate at which this work is accomplished (work to be done, h_0) (15, 23). According to the latter approach, if changes in the environment do not pose additional adaptation work, then the inverse of lag time (the so-called lag rate) (18) and growth rate should follow the same trend as a function of the shift and they should be linearly correlated. This may be true for shifts of low magnitude (e.g., 3 to 5°C) that are far from the growth boundaries. However, linearity is lost when the intensity of the stress imposed is so high that the bacteria cannot adapt to the new conditions with only the available homeostatic mechanisms and require additional effort to overcome the imposed stress. This is the case with osmotic or acid shifts, which are considered more severe stresses than temperature (23, 24), or with shifts close to the growth boundaries. Regarding the latter, even though the lag time of *Lactobacillus plantarum* was consistently well predicted.
after temperature upshifts, marked variations in lag time were evident in temperature downshifts close to the growth boundaries, which rendered the prediction of growth difficult (31).

The response of bacteria to dynamic environmental conditions varies with the Gram type, genus, etc. For instance, the lag time of gram-negative Escherichia coli and Salmonella increased nonlinearly as osmotic conditions became less favorable for growth, suggesting the need for additional adaptation work after the shift. Conversely, it has been suggested that gram-positive bacteria have a higher ability to withstand osmotic stress than gram-negative bacteria, due to inherent constitutive turgor pressure mechanisms available to the former (15). Nonetheless, the findings of the aforementioned studies are restricted to observations following shifts within the growth domain of microorganisms. Another key hypothesis in growth modeling addresses whether shifts in the environment across the growth boundaries induce additional adaptation work to microorganisms compared with shifts in the growth region, or allow cells to adapt to adverse conditions and, hence, enhance their subsequent growth. Mitchell et al. (21) stressed that extended exposure to temperatures that do not support growth of Salmonella may affect subsequent growth of the bacterium at growth-permitting temperatures. However, although aw shifts to harsher conditions are known to induce adaptation work (15–18), growth under dynamic pH or for osmotic and pH shifts across the growth boundaries is not clearly understood. Such knowledge is crucial for development or optimization of dynamic growth models for L. monocytogenes, because it is ubiquitous in nature and may be sequentially exposed to growth-limiting or growth-permitting conditions in various products of low pH and/or low aw, as well as during fermentation and ripening of meat and dairy foods.

Based on the above, the objectives of this work were to evaluate the growth responses of L. monocytogenes under (i) environmental shifts within the range of pH and aw levels that allow growth of the bacterium and (ii) shifts from optimum aw (0.995) or pH (7.2) conditions to no-growth conditions, habituation for 1, 5, or 10 days, and then back to growth-permitting conditions.

**MATERIALS AND METHODS**

**Bacterial strain and culture preparation.** L. monocytogenes C3 (serotype 4b), shown previously to survive the stressful environment of cheesemaking better than other strains, was used in this study. The strain was selected among other isolates from dairy products, factory equipment, and human outbreaks (including Scott A), after a preliminary experiment where all the strains were grown at the pH and NaCl levels tested in the present study. The particular strain demonstrated the highest growth rate and the shortest lag time among all the tested strains (data not shown). The strain was maintained at −22°C in tryptic soy broth supplemented with 0.6% yeast extract (TSBYE; Biolife Italiana Srl, Milan, Italy) and 20% glycerol (Glycerine 4810, Oleo, Ertvelde, Belgium). Cultures were activated by transferring 0.1 ml of stock culture to 10 ml of TSBYE followed by incubation at 30°C for 24 h. Then working cultures were obtained by subculturing 0.1 ml of the previous culture to 10 ml of TSBYE followed by incubation at 30°C for 16 h, in order to obtain late-exponential-phase to early-stationary-phase cells. The culture was harvested by centrifugation (3,600 × g for 15 min at 4°C; Megafuge 1.0R, Heraeus Instruments, Hanau, Germany). The cell pellet was washed twice and resuspended in 10 ml of maximum recovery diluent (MRD; Biolife). The bacterial population of the latter suspension, which was constantly at 10^6 CFU/ml (confirmed by four independent cultures, duplicate plates), was serially diluted in MRD to the level of 10^4 CFU/ml in order to be used as the initial inoculum for the experiments.

**Media preparation.** A total of seven pH values (7.2, 6.0, 5.8, 5.5, 5.3, 5.1, and 4.9) and five aw levels (0.995, 0.97, 0.95, 0.93, and 0.90) by adding NaCl were evaluated in TSBYE. Of these conditions, one pH and one aw level, namely pH 4.9 and aw 0.90, respectively, did not allow growth of L. monocytogenes at 10^5°C; these were selected for the experiments involving habituation of the bacterium at no-growth conditions, as will be further described. We did not use TSBYE without glucose, because none of the experimental cases was expected to cause inactivation. Thus, potential acid adaptation due to glucose levels as low as 0.25%, contained in commercially available TSBYE formulation, was assumed to have no influence on the growth of L. monocytogenes. In order to achieve the target pH values of the tested media by using the smallest possible volume of lactic acid, a concentrated lactic acid solution of 85% (wt/wt) was used (Sigma-Aldrich Ltd., Taufkirchen, Germany), along with 10 N NaOH (when necessary; sodium hydroxide pellets, Panreac, Castellar del Valles, Spain). To achieve the lowest tested pH (4.9) in 100 ml of commercial TSBYE (initial pH 7.3) prepared according to manufacturer’s recommendations in 250-ml Duran bottles (Duran Group GmbH, Mainz, Germany), the maximum volume required was 0.6 ml of the concentrated lactic acid solution (85% [wt/wt]). Therefore, the total volume of the lactic acid added was not considered to change the total volume of the broth. The target aw levels were achieved by adding 0, 4.5, 7.5, 10, and 12 g of NaCl in 100 ml of TSBYE, also in 250-ml Duran bottles. Given that the commercial composition of TSB contains 0.5% NaCl, the final NaCl concentrations of the tested media were 0.5, 5, 8, 10.5, and 12.5%. The pH and aw of all media were measured after autoclaving with a pH electrode (pH 691, Metrohm, Zofingen, Switzerland) and aw meter (HygroLab, Rotronic, Bassersdorf, Switzerland), respectively. Two pairs of growth media adjusted at each of the aforementioned pH and aw levels were prepared for two independent experiments.

**Experimental procedure.** To evaluate the growth of L. monocytogenes in response to environmental shifts within the range of pH or aw levels permitting growth, the following procedure was applied: pairs of sterile broths (100 ml) adjusted to all the growth-permitting pH values (7.2, 6.0, 5.8, 5.5, 5.3, and 5.1) or aw levels (0.995, 0.97, 0.95, and 0.93) were inoculated with 1 ml of the L. monocytogenes C3 suspension in MRD (10^5 CFU/ml) in order to obtain an initial level of 100 CFU/ml. Inoculated media were placed in high-precision (±0.5°C) incubation chambers (MIR-153, Sanyo Electric Co., Osaka, Japan) and stored at 10°C. Changes in viable counts were monitored at regular intervals, ranging from 6 h to 3 days, depending on the growth rate and lag time of the pathogen at each tested condition, until late exponential to early stationary phase. Enumeration of viable cells was performed by serial dilutions in MRD, surface plating using a spiral plater (Autoplate 400, Automated Spiral Plater, Spiral Biotech, Inc., Norwood, MA) onto duplicate tryptic soy agar supplemented with 0.6% yeast extract (Biolife) and incubation at 30°C for 24 to 48 h. After the L. monocytogenes culture had reached the late exponential to early stationary phase (ca. 8 to 9 log
CFU/ml), aliquots (1 ml) were serially diluted and then shifted to duplicate media adjusted at all the growth-permitting levels of the same factor, as illustrated in Figure 1, at an initial population of 100 CFU/ml. In particular, six pH shifts were performed from each of the six initial pH levels, resulting in a total of 36 shifts and four transfers from each of the four initial a_w levels, resulting in 16 total NaCl shifts. We performed both downshifts (toward lower pH or a_w levels) and upshifts (toward optimal pH and a_w levels); we also tested transfers of L. monocytogenes to noninoculated medium of the same pH or a_w level as those applied before shift (Fig. 1).

The application of shifts from optimum a_w (0.995) or pH (7.2) conditions to no-growth conditions for 1, 5, or 10 days and then back to growth-permitting pH and a_w levels, as shown in the lower graph.

The number of days of habituation prior to the shift to growth-permitting conditions was chosen to examine the effect of the length of incubation at no-growth conditions on the ability of L. monocytogenes to adapt to subsequent environments. These shifts were also considered as a simulation of cross-contamination between those products with physicochemical properties that do not allow proliferation of the pathogen and those products with optimal or suboptimal properties. Incubation of tested media at growth-permitting or growth-inhibiting pH and a_w levels was carried out at 10°C in high-precision (±0.5°C) incubation chambers, in four replicates (two replicates per condition in two separate experiments). The incubation temperature (10°C) was chosen to ensure a broader range of pH and a_w conditions that permit growth of L. monocytogenes compared with conditions at a refrigeration temperature of 4°C, thus yielding more data about the effect of acid and osmotic shifts on the growth of the bacterium. Also, 10°C is a common temperature at dairy processing sites or in cheese ripening chambers.

Fitting of growth curves. Changes in viable counts during incubation at 10°C were transformed to log values. Log-transformed data were fitted to the Baranyi model (3) in order to determine the maximum specific growth rate \( \mu_{max} \) (h\(^{-1} \)), the lag time (h), and the dimensionless parameter \( h_0 \) representing the “work-to-be-done” before entering the exponential phase of growth (23), also analogous to the “relative lag time” (15, 18). The estimated \( \mu_{max} \), lag times, and \( h_0 \) values were subjected to one-way analysis of variance, and
RESULTS AND DISCUSSION

Effect of shifts between growth-permitting levels of pH and $a_w$. The $\mu_{\text{max}}$ of L. monocytogenes was influenced by the direction, but not by the magnitude, of $a_w$ shifts ($\Delta a_w$), as shown by the almost parallel position of $\mu_{\text{max}}$ curves in response to shifts for different starting $a_w$ values (i.e., conditions before the shift; Fig. 2a). More specifically, upshifts in $a_w$ increased $\mu_{\text{max}}$, and the opposite occurred when $a_w$ decreased (Fig. 2a). The change of $\mu_{\text{max}}$ in response to pH shifts indicated a similar trend to that caused by $a_w$ changes, independently of the pH before shift (Fig. 2b). Nevertheless, as expected, $\mu_{\text{max}}$ was influenced by the pH and $a_w$ levels after each shift ($P < 0.05$; Fig. 2). Therefore, our results suggest that in growth simulations under dynamic acidic or osmotic conditions, $\mu_{\text{max}}$ is instantaneously adapted to the new environment, similarly to the bacterial growth responses to fluctuating temperatures.

Lag times were influenced by both the direction and the magnitude of $a_w$ shifts (Fig. 3a). Specifically, lag times of L. monocytogenes sharply increased ($P < 0.05$) as the conditions of $a_w$ became harsher (i.e., in the case of negative shifts; Fig. 3a). This is in accordance with Mellefont et al. (15), who observed increased relative lag times at low $a_w$s for Salmonella, although this effect was less evident for L. monocytogenes. Lebert et al. (13) also demonstrated an exponential increase in the lag time of Listeria innocua grown under optimum $a_w$ conditions (0.995) and subsequently exposed to various $a_w$ levels in broth and gelatine, from 0.995 down to the growth-inhibiting $a_w$ level of 0.91. However, in our study we also showed that lag times are markedly reduced ($P < 0.05$) when shifts occurred toward more favorable conditions than the initial $a_w$ (Fig. 3a). This suggests that any possible injury due to low $a_w$ is quickly repaired when the conditions become more favorable for growth. Similar observations regarding the effect of environmental shifts on the intermediate lag time of microorganisms have been reported for temperature downshifts in studies predicting growth of Salmonella and Brochothrix thermosphacta (4, 20).
and for temperature upshifts in studies involving *E. coli* growth (27, 28). Therefore, changes in the $a_w$ of the food have a marked influence on the lag time of *L. monocytogenes*, similar to the effect posed by temperature shifts on the growth of the bacterium.

In contrast with the observations of $a_w$ shift effects, lag times were not influenced by pH shifts within the range of 5.3 to 7.2 ($P \geq 0.05$; Fig. 3b), and the magnitude of $h_0$ was relatively lower (0 to 0.4; data not shown) compared with that caused by $a_w$ shifts ($\Delta a_w$ in the range 0 to 1.5). However, remarkable increases in lag times were caused by shifts close to the growth-limiting pH value of 5.1 ($P < 0.05$; Fig. 4b), in agreement with Robinson et al. (23). It is suggested that cells are capable of restoring their homeostasis rapidly when mild pH stress is imposed, whereas the combination of low temperature (10°C) with growth-limiting pH values may have posed a marked energetic burden that necessitated the expenditure of additional energy by the cells in order to initiate growth. Therefore, these data indicate that the intermediate lag times induced by pH shifts should be considered only when they occur near the growth boundaries (i.e., pH 4.9 to 5.2). This is notable in regard to the risk of *L. monocytogenes* growth during the shelf life of some dairy products (commonly semihard or hard cheeses) that may marginally support growth of the bacterium due to their low pH values (5.0 to 5.5).

Notably, the hypothesis that the adaptation work of the bacterium to a new environment is independent of the environmental factor controlling its growth is not valid for $a_w$. To reach this conclusion, first, we calculated $h_0$ as a product of lag time and $\mu_{\text{max}}$ for each shift, and no meaningful trend of $h_0$ with $a_w$ was evident. Then we applied the following transformation:

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h_0 = \mu_{\text{max}} \times (\text{lag}_{\text{new}} - \text{lag}_{\text{same}})
\]

where $\text{lag}_{\text{new}}$ is the lag time of *L. monocytogenes* after shift to a different $a_w$ from that in which the organism was precultured and $\text{lag}_{\text{same}}$ is the lag after shifting to fresh medium with the same $a_w$ as in preculture. These calculations revealed a trend: habituation at low $a_w$ enhanced subsequent adaptation to $a_w$ in the range of 0.93 to 0.995 (Fig. 4). No such trend was obtained under the pH conditions tested in the present study (results not shown). Consequently, models for the effects of osmotic shifts on the intermediate lag time of *L. monocytogenes* should consider not only the $a_w$ after the shift but also the direction and the magnitude of shift. Cheroutre-Violette and Lebert (5) presented a dynamic growth model for *L. monocytogenes* in response to shifts in NaCl and pH based on recurrent neural networks. Even though good agreement was obtained between predictions and data, the model was complex; its learning base was oversimplified and perhaps inadequate to cover all potential shifts from different initial NaCl and pH levels. Our findings will assist in the development of simpler growth models based on biologically meaningful assumptions related to potential adaptation phenomena of *L. monocytogenes*. Such phenomena may occur during transfer of *L. monocytogenes* from environments of low $a_w$ (e.g., brines) or pH (fermented product) to environments of higher $a_w$ or pH (e.g., milk or other raw materials) and vice versa.

**Effect of shifts from growth-inhibiting to growth-permitting levels of pH and $a_w$.** The duration of habituation at no-growth conditions did not influence the $\mu_{\text{max}}$ of *L. monocytogenes* at subsequent shifts to growth-permitting levels (data not shown). However, an apparent effect on lag time and, hence, on $h_0$ estimates was evident (Fig. 5). Specifically, for the same level of $a_w$, the $h_0$ tended to decrease ($P < 0.05$) proportionally to the time the cells were held at 0.90 (Fig. 5a). Furthermore, lag times and $h_0$ estimates at $a_w$ 0.93 and 0.95 after 10 days at 0.90 were lower ($P < 0.05$) than the corresponding values after growth at 0.995 (Fig. 6); however, no differences ($P \geq 0.05$) in lag times were observed at $a_w > 0.95$ in cultures previously maintained at $a_w$ 0.90 compared with those grown at 0.995 (Fig. 6). These results suggest that the cells are able to carry out some of the “work to be done” required for growth in $a_w$ 0.93 to 0.995, during habituation at 0.90. Nonetheless, the lag times and $h_0$ values of *L. monocytogenes* after preadaptation to $a_w$ 0.90 decreased with increasing $a_w$ (Fig. 5a). This in turn means that the adaptation work represented by the parameter $h_0$ decreased as the magnitude of the shift from no-growth to growth conditions increased. The observation that lag time is proportional to the magnitude of the shift toward the growth limits (i.e., in negative shifts) is established for bacterial growth under fluctuating temperatures and suggests a positive temperature history effect on lag time on bacteria (8, 30). More specifically, although lag was induced upon shifts from high temperature (25 to 37°C) to 3 to 4°C, preincubation at 4°C markedly reduced such a lag time (7). The putative mechanisms associated with resistance of *L. monocytogenes* to osmotic stress involve the uptake of compatible solutes such as glycine, betaine, and carnitine (11). Our results suggest that during incubation at $a_w$ 0.90, the metabolic activity of cells continues and probably assists in accumu-
found that *E. coli* and *L. monocytogenes* at growth-permitting *a_w* (0.90 or pH 4.9) before the shock. Conversely, preadaptation at pH 7.0 enhanced subsequent survival at pH 3.0, as opposed to cells initially grown at pH 7.0. It may thus be postulated that the conditions that increase acid resistance (e.g., gradual drop of pH during fermentation) or even reduce the minimum pH allowing growth may not impact the adaptation work required for entrance into the exponential phase of growth under favorable conditions. Additional research involving the transcriptional or proteomic changes mediated by habituation at growth-inhibiting pH would offer a better insight to the above-specified mechanisms. A potential area of application of such findings is the quantitation of the necessary resources that will enable cells to grow once they encounter favorable conditions. Furthermore, although the growth rate of exponentially growing cells is thought to adapt instantaneously to changes in *a_w*, our results suggest that the bacterial lag time depends on the duration of preadaptation at no-growth conditions. Not taking this adaptation into account may lead to an underestimation of the actual growth of *L. monocytogenes*, thereby compromising the safety of foods of low *a_w* (e.g., 0.90 to 0.94), especially close to the growth boundaries of this bacterium. These findings are valuable in quantitative microbial risk assessment; they provide quantitative data to confirm implications on the adaptive responses of *L. monocytogenes*, an important area of uncertainty that is not sufficiently addressed by the existing predictive models. However, based on these implications, further research is required to quantify the rate at which cells adapt while they are in no-growth status, especially various *L. monocytogenes* strains that are involved in outbreaks or that persist in processing and retail environments.

In contrast with the observations with *a_w*, the longer the cells were held at no-growth pH (4.9), the greater was the work needed for growth initiation in a more favorable environment (Fig. 5b). Specifically, the value of *h*₀ at pH levels decreasing from 7.2 to 5.1, increased from 0 (no lag) to 0.7, 0.6 to 2.5, and 1.1 to 4.2, after 1, 5, and 10 days of incubation at pH 4.9, respectively (Fig. 5b). Furthermore, lag times and *h*₀ estimates at pH 5.1 (near-growth and no-growth interface), after 10 days at pH 4.9 were higher (*P* < 0.05) than the corresponding values at pH 7.2 and 5.5, following the same habituation scenario (Fig. 5b). This may be explained on the basis of the higher energetic burden posed by pH on cells compared with *a_w* under no-growth conditions (24, 29). The present experimental approach consisted of three consecutive steps: (i) growth at optimum pH; (ii) shift to no-growth pH for 1, 5, and 10 days; and (iii) regrowth at different pH above the growth boundaries. According to Goodson and Rowbury (9), prior growth of *E. coli* at pH 7.0 reduced the ability of cells to grow at the same pH after short exposure (26 min) to pH 3.0 (adjusted with HCl). It is likely that habituation at pH 4.9, in the present study, induced physiological changes to cells which rendered them more resistant to subsequent lethal acid challenges (6, 26) or reduced their minimum pH for growth initiation. For instance, Skandamis et al. (25) found that habituation of *E. coli* O157:H7 in acidic meat decontamination run-off fluids (pH 4.9) enabled growth of cells at lower pH than nonhabituated cells. Note, however, that the pH-dependent acid resistance mechanisms of *E. coli* O157:H7 are different from those of *L. monocytogenes* (10, 14). Moreover, such mechanisms presumably differ from those needed to resume growth under subsequent favorable conditions. Kroll and Patchett (12) found that growth of *L. monocytogenes* at pH 5.0 before acid shock at pH 3.5 delayed regrowth at pH 7.0, in comparison with growth at pH 7.0 before the shock. Conversely, preadaptation at pH 5.0 enhanced subsequent survival at pH 3.0, as opposed to cells initially grown at pH 7.0. It may thus be postulated that the conditions that increase acid resistance (e.g., gradual drop of pH during fermentation) or even reduce the minimum pH allowing growth may not impact the adaptation work required for entrance into the exponential phase of growth under favorable conditions. Additional research involving the transcriptional or proteomic changes mediated by habituation at growth-inhibiting pH would offer a better insight to the above-specified mechanisms. A potential area of application of such findings is the quantitation of the necessary resources that will enable cells to grow once they encounter favorable conditions.
could also be associated with the passage of *L. monocytogenes* from the stomach (low pH) to the intestine (high pH).

In the present study, shifts were applied on bacterial cells in late exponential to early stationary phase. Therefore, our results may not be directly comparable to those of relevant studies, in which cells in the midexponential or late stationary phase were used. According to previous reports (16, 17, 22), cells in the exponential phase are more sensitive to osmotic shifts than cells in stationary phase. Our findings represent the response of cells in a state between exponential and stationary phase. Furthermore, the present results may provide inferences for the effects of $a_w$ and pH shifts on the growth of *L. monocytogenes* and, hence, reduce this area of uncertainty in dynamic growth modeling and quantitative microbial risk assessment. In particular, we indicated that habitation of *L. monocytogenes* under growth-inhibiting conditions, (e.g., in a product with pH <4.4 or $a_w$ <0.92 or a combination of pH 5.0 and $a_w$ 0.94) is not only a period when the pathogen simply does not grow. Instead, these situations may trigger adaptation phenomena, which could enhance growth of the bacterium upon subsequent transfer to a growth-supporting environment (e.g., due to cross-contamination). Therefore, the predictions of models for microbial responses near the growth boundaries of pathogens should be viewed with caution, and perhaps proper adjustments of existing models might be necessary in order to take into account the above adaptation phenomena. However, the effects of $a_w$ and pH shifts were studied independently, and further work is needed to study the effect of simultaneous shifts on the intermediate lag time. In addition, the effects of such shifts (pH and $a_w$) on the growth of *L. monocytogenes* need to be studied not only on liquid cultures but also on cultures growing on solid surfaces or immobilized in emulsified (oil-in-water or water-in-oil) foods. Even though the present findings refer to a single strain, they could be considered representative of the potential behavior of the bacterium in dairy processing environments, since the selected strain was a persistent dairy isolate. However, our findings should be evaluated for multiple strains in order to account for potential strain variability in growth and adaptive responses (24).

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