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Changes in growth, rRNA content, and cell morphology of *Listeria monocytogenes* induced by CO_2 up- and downshift

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Abstract

Cell morphology, rRNA content, and growth were examined for *Listeria monocytogenes* LO28 and EGD, respectively, grown in brain–heart infusion (BHI) and on slices of sausage at 10 °C in 100% CO_2, 100% N_2, and air. In CO_2, filamentous cells were formed by both strains on sausage slices and by *L. monocytogenes* EGD in BHI. Filamentation was not induced by anaerobiosis only. Fluorescent in situ rRNA hybridization (FISH) of cells grown in BHI showed that the *L. monocytogenes* EGD filaments consisted of chains of individual slightly elongated cells. The rods formed by *L. monocytogenes* LO28 had the same size in air and CO_2. Septation and cell division were induced in the filaments after a CO_2 downshift (i.e., exposure to air). In BHI, the number of colony forming units increased rapidly when *L. monocytogenes* EGD grown in CO_2 was exposed to air whereas the number of *L. monocytogenes* LO28 remained almost unchanged. On sausage slices, the number of colony forming units also increased rapidly for both strains in response to CO_2 downshift. Large variations in rRNA content of individual cells were observed in the tested scenarios. The results demonstrate the risk of underestimating the number of infectious units under circumstances where filamentation may occur. Furthermore, the study illustrates the lack of residual inhibitory effect of CO_2 in this type of products after opening.

Keywords: *Listeria monocytogenes*; CO_2 downshift; Growth; Filamentous cells; Meat; Sausage; In situ rRNA

1. Introduction

Listeria monocytogenes is a food-borne, psychrotrophic pathogen which may cause the severe human infection listeriosis. Immuno-compromised individuals, i.e., elderly, newborns, pregnant, and permanently challenged patients are more susceptible to the

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infection than healthy individuals (Bell and Kyriakides, 1998). Listeriosis associated with consumption of contaminated meat products has been reported since the 1990s (Farber and Peterkin, 1999). The infection is reported worldwide with an annual frequency of typically two to five cases per million (Bell and Kyriakides, 1998). In Denmark, two to eight cases per million are reported per year. The mortality rate is approximately 30% for listeriosis patients in general (Bell and Kyriakides, 1998) and up to 75% in immunocompromised groups (Nørrung et al., 1999).

Modified atmosphere packaging (MAP) is used to control a range of food-borne bacteria including *L. monocytogenes* in foods. In MAP, the preservative effect is normally provided by a lowered oxygen (O_2) tension combined with the use of the antibacterial gas carbon dioxide (CO_2; Farber, 1991). Growth inhibition as a function of CO_2 concentration in the head space (Farber et al., 1996; Fernández et al., 1997) or dissolved CO_2 (Devlieghere et al., 2001) has been demonstrated for *L. monocytogenes*. The organism is fairly CO_2-tolerant and more than 70% CO_2 in anaerobic atmospheres is required to completely inhibit growth at chill temperatures (4 and 10 °C). If only 5% O_2 is present, growth may however occur even at 75% CO_2 (Wimpfheimer et al., 1990). A residual effect (i.e., continued inhibition of growth after CO_2 was replaced with air) has been reported. Inhibition of growth for more than 5 days was observed on beef steaks stored at 12.25 °C after prior storage for 5–8 weeks in a saturated CO_2 atmosphere at −1.5 °C and low pH (5.3–5.5). No residual effect was observed if inoculum was stored for <3 h in CO_2 (Avery et al., 1995).

Several hypotheses have been put forward regarding the inhibitive mechanism of CO_2 on bacteria. It has been suggested that changes in intracellular pH, enzyme activity (i.e., the actual activity level as well the expression of genes coding for the enzymes), membranes, or a combination of these may cause the growth inhibition as reviewed by Dixon and Kell (1989).

It has been observed that CO_2 may affect the morphology of the individual cell. Elongation was recently reported for *L. monocytogenes* (Nilsson et al., 2000; Li et al., 2003) and filamentation was reported for *Aeromonas hydrophila* (McMahon et al., 1998). Lack of septation may be part of a more general stress response because it has also been reported for osmotic stress in *L. monocytogenes* (Jørgensen et al., 1995) and later for *Salmonella* (Mattick et al., 2000). In these reports, morphology was studied using phase contrast microscopy. Other techniques such as fluorescent in situ rRNA hybridization (FISH) should allow study of filament morphology as well as growth activity because rRNA content has been reported to correlate with growth rate (Poulsen et al., 1993).

Neither the effect of CO_2 downshift nor the strain variation has previously been described for *L. monocytogenes*. The aim of our investigations was to examine and quantify changes in cell morphology and growth characteristics of a CO_2-tolerant and a CO_2-sensitive *L. monocytogenes* as a function of CO_2 up- and downshift in a laboratory model as well as a real food model.

2. Materials and methods

2.1. Bacterial strains

L. monocytogenes LO28 (clinical strain received from P. Cossart, Institute Pasteur, Paris, France via J.E. Olsen, The Royal Veterinary and Agricultural University, Denmark) and *L. monocytogenes* EGD (originally obtained from G.B. Mackaness, Saranac Lake, NY; Kaufmann, 1984) were selected for the work as our preliminary experiments showed LO28 to be relatively CO_2-tolerant and EGD relatively CO_2-sensitive.

2.2. Preparation and analysis of sausages

2.2.1. Preparation

One batch of sausages with pH 6.4 was produced at The Danish Meat Research Institute (Roskilde, Denmark) following a standard recipe and procedure (Budde et al., 2003) to which 0.04% ascorbic acid was added. In a parallel production, a batch of the sausages with pH 5.9 was produced by addition of 0.5% glucono-δ-lactone to the recipe.

2.2.2. Chemical analyses

Chemical analyses were conducted on the sausage prior to inoculation to determine the content of fat [Nordic Committee on Food Analysis (NMKL, 1991) No. 131], water [(NMKL, 1974) No. 23], salt [Association of Analytical Chemists (AOAC, 1995)

39.1.10], water activity (rotronic hygroscope), L-lactate (Boehringer Mannheim Cat. No. 0139084), D-glucose (UV determination method, Boehringer Mannheim Cat. No. 716251), nitrite (Lachat Ionanalysator Quick Chem® FIA+8000 series), as well as pH (combined pH electrodes, Radiometer, Copenhagen, Denmark). Furthermore, pH was measured on the meat surface during storage using strips (pH 5.3–7.2, Merck, Darmstadt, Germany). The amount of dissolved CO_2 was determined in 2 g of sausage 24 h after packaging and at weekly intervals using a titrimetric method (Gill, 1988). Preliminary experiments had shown equilibrium to be reached within this time period.

2.3. Examination of individual cell morphology and activity

2.3.1. Microscopic analysis

Cell morphology was studied in an Olympus BH-2 microscope (Olympus, Japan) with 100× magnification and pictures were taken using a microscope-adaptable camera (Kodak Microscopy Documentation System MDS 290).

2.3.2. Quantification of cell morphologic changes and growth activity

2.3.2.1. Oligonucleotide probe. The oligonucleotide probe EUB338 (5′-GCTGCCTCCCGTAGGAGT-3′; Amann et al., 1990) specific for most species in the bacterial domain (Daims et al., 1999) was used for all 16S rRNA hybridizations. The CY3-labelled probe was purchased from Hobolth DNA Syntese A/S (Hillerød, Denmark).

2.3.2.2. Cell fixation and whole cell hybridization. Cells were fixed at the time of harvest using the following procedure: 2 ml culture was harvested by centrifugation at 8000×*g* for 5 min. Pellet was washed in physiological saline and resuspended in 1 ml saline and 1 ml 96% ethanol. The cells were stored overnight at 5 °C. The suspension was centrifuged (8000×*g*, 5 min), all supernatants were removed, and 100 μl 96% ethanol and 100 μl TE buffer (TRIS EDTA buffer solution pH 7.4, Fluka) were added. The samples were stored at −20 °C until staining. The hybridization procedure was performed according to the procedure of Poulsen et al. (1993). The cells were studied in a Carl Zeiss Axioplan epifluorescence microscope using a XF40 filter set (Omega Optical, Brattlebror, VT). Digital images were captured with a slow-scan charge-coupled device camera (CH250, Photometrics, Tucson, AZ). Using the Cellstat software (Department of Microbiology and Institute of Mathematical Modelling, Technical University, Denmark), the area as well as the intensity (i.e., amount of rRNA) of the cells were compared. Changes in cell area were considered to reflect changes in length because the width of the cells was constant.

2.4. CO_2 downshift of L. monocytogenes grown in BHI

Preinoculum was made by inoculating a loop-full cells of *L. monocytogenes* EGD and LO28, respectively, from a frozen stock (−80 °C) into 10 ml brain–heart infusion (BHI, Merck) at 10 °C for 7 days. The preinoculum was diluted and transferred to fresh BHI (pH 7.2) to give an initial cell density of 10^5 cfu/ml and incubated in 100% CO_2 or air at 10 °C. Cultures grown in CO_2 were placed in anaerobic jars (Oxoid, Basingstoke, UK) which were evacuated and subsequently filled with gas three times. The gas/medium ratio was above 50:1 to ensure that the medium was saturated with CO_2. Cultures grown in air with agitation were placed in an incubator. Cultures grown in CO_2 were downshifted (i.e., exposed to air) in the exponential and stationary phases, respectively, by transferring the broth from the anaerobic jar to the incubator. Cell morphology and rRNA content in individual cells were studied at the time of downshift and at fixed intervals during the following days in the microscope. Cell density was monitored spectrophotometrically (OD_{600}) and by enumeration of colony-forming units on TSA plates (Tryptone Soya Agar, Oxoid). Using strips (Merck), pH was measured in the cultures at the time of downshift.

2.5. CO_2 downshift of L. monocytogenes grown on sausage slices

Cells from a frozen stock (−80 °C) were inoculated in 10 ml BHI and incubated at 30 °C overnight, and 0.1 ml was then transferred to 10 ml BHI with 2.0% NaCl and incubated for 13 days at 5 °C in order to preadapt the cells. For both strains, preinoculum was diluted and spread on 1.0-cm thick sausage slices resulting in

an initial concentration of approximately $5 \cdot 10^3$ cfu/g. The slices were placed in plastic trays in plastic pouches (15.5×22.5 cm, NEN 40 HOB/LLDPE 75, Danisco Flexible, Denmark) with very low O_2 and CO_2 transmission rates (OTR=0.45 $cm^3/m^2/24$ h at 23°C, 5/95% RH, 1 atm and CO_2TR=1.8 $cm^3/m^2/24$ h at 23°C, 5/95% RH, 1 atm). The packages were filled with air, 100% N_2, or 100% CO_2 and stored at 10 °C for up to 36 days. The gas/product ratio was approximately 4:1. Growth was followed by enumeration on BHI agar as well as on *Listeria* selective base (Oxford Formula, Oxoid) for tentative verification of *Listeria*. Cell morphology was examined during the experiment. The inoculated slices were downshifted (i.e., exposed to air by opening). Five packages from each atmosphere/sausage combination were opened per strain and these were replaced in the 10 °C incubator in a large permeable plastic bag to avoid to dehydration. Growth and cell morphology were followed in the packages from the time of downshift and days on. pH was measured using strips placed directly on the sausage slices. Immediately after opening of packages, samples were collected for analysis of dissolved CO_2. In the sausage samples, FISH could not be performed satisfactorily on the cells due to auto-fluorescence from the matrix.

2.6. Data analysis

For cultures growing on sausage slices, the growth rate was determined for each growth curve using the slope function in the Microsoft Excel software. Linear model analyses were conducted to determine significant effects using SAS (SAS Institute, Cary, NC).

3. Results

3.1. pH and chemical analysis

3.1.1. pH changes

In the broth, pH decreased for both strains from the original 7.2 to 5.5–5.7 during the exponential phase and further to 5.3 in the stationary phase.

In all packaging atmospheres, pH of the sausage slices decreased 0.2–0.5 units in the sausage with pH 6.4 and 0.1–0.2 units in the sausage with pH 5.9 during storage.

3.1.2. Sausage composition

The composition of the sausage with pH 6.4 was: 0.13% D-glucose, 11.1% fat, 0.3% L-lactate, 2.00% NaCl, 2.94% salt in water, 36 ppm Na-nitrite, 68.1% water, and a_W=0.98. For the sausage with pH 5.9, the same figures were: 0.09%, 10.9%, 0.3%, 2.08%, 3.06%, 24 ppm, 68.0%, and 0.98, respectively.

3.1.3. Dissolved CO_2

The amount of initially dissolved CO_2 was higher ($P<0.001$) in the sausages with pH 6.4 than in the sausages with pH 5.9 when packaged in 100% CO_2 (Table 1). The initial level did not change markedly during storage (results not shown). The amount of initially dissolved CO_2 was independent of pH ($P>0.05$) in air and 100% N_2, respectively. In these two atmospheres, the amount increased slightly after 2 weeks of storage corresponding to the growth of the cells on the slices (results not shown).

3.2. Cell morphology

3.2.1. Broth experiment

Microscopic examinations of cell morphology showed that *L. monocytogenes* EGD formed long filaments (10–50× normal length) when grown in 100% CO_2 and short rods when grown in air (Fig. 1). This was more pronounced during exponential growth than in the stationary phase. When *L. monocytogenes* LO28 was grown in 100% CO_2, the culture consisted almost solely of short rods (Fig. 1). Filaments did occur but were estimated to be less than 1% of the cells. Under standard aerobic conditions, *L. monocytogenes* EGD had a visually determined width/length ratio of 1:5 while *L. monocytogenes* LO28 had a ratio of 1:3–1:4. Filamentation was not induced by

Table 1
The amount of CO_2 dissolved in sausages with different initial pH and packaged in three different atmospheres

	Dissolved CO_2[a] (ml/kg meat)	
	pH 6.4	pH 5.9
Air	46.0±12.0	50.2±13.9
100% N_2	58.2±39.7	46.2±87.5
100% CO_2	843.0±136.5	652.8±243.2

[a] Each concentration represents the average of four determinations measured 24 h after packaging.

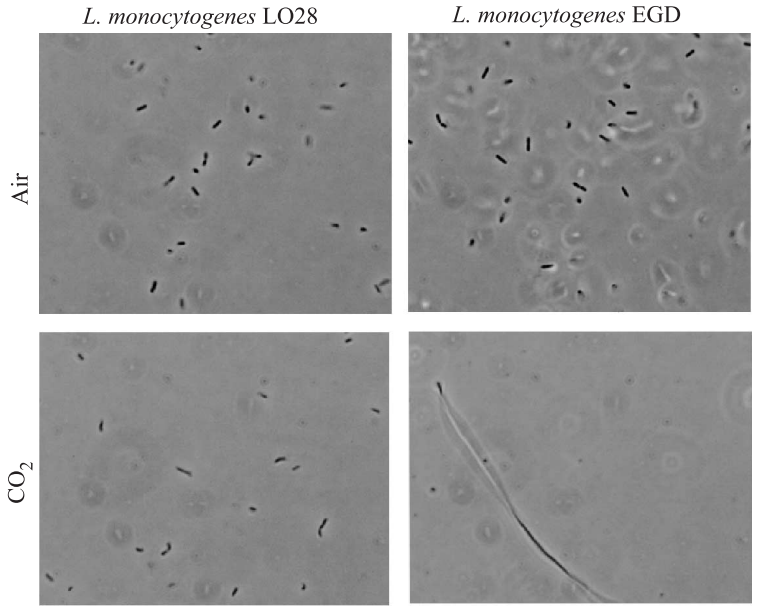

Fig. 1. Phase contrast microscopic examination of the cell morphology of *Listeria monocytogenes* grown to the exponential phase in two atmospheres in BHI at 10 °C.

anaerobiosis (100% N_2) alone in any of the strains. In CO_2, motility was reduced for *L. monocytogenes* LO28 and completely lacking in the filaments of *L. monocytogenes* EGD.

The in situ hybridizations showed that each filament in CO_2 consisted of a chain of cells with no visible septa rather than one long cell (Fig. 2). The cells in the chains formed by *L. monocytogenes* EGD were elongated and approximately 1.2 times longer ($P<0.001$) than cells grown aerobically, i.e., the cell area increased, and with a constant cell width, this reflects an increased cell length (Fig. 3). The rod size

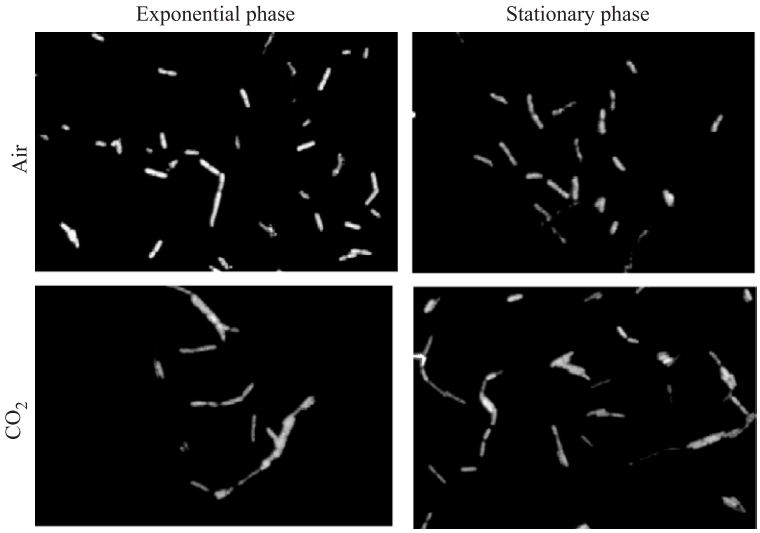

Fig. 2. Epifluorescent staining of the ribosomal RNA in *Listeria monocytogenes* EGD grown to the exponential and stationary phases in air and 100% CO_2 in BHI at 10 °C.

of *L. monocytogenes* LO28 was similar in CO_2 and air. The size of the cells in the filaments was similar for exponential and stationary phase cultures grown in CO_2 (Fig. 3). The filaments could consist of up to 40–50 cells. The average cell area was larger for *L. monocytogenes* EGD than for *L. monocytogenes* LO28 in the exponential phase ($P<0.001$) while similar in the stationary phase (Fig. 3).

In the microscope, septum formation and cell division were observed within a few days for the filamentous cells after exposure to air and more rapid in exponential than in stationary cultures. For *L. monocytogenes* EGD grown in 100% CO_2 to the exponential phase, the downshift resulted in a significant decrease ($P<0.001$) in the area of the single cells within 2 days (Fig. 3) while it took between 2–4 days in the stationary culture. Because the cell area of *L. monocytogenes* LO28 was unaffected by the presence of 100% CO_2, it remained unchanged after the CO_2 downshift ($P>0.05$; Fig. 3).

Due to large cell to cell variations, the average rRNA content was not significantly different ($P>0.05$) in air and CO_2 (results not shown). Likewise, no significant differences were observed in the average rRNA content of the cells in the CO_2 atmosphere before and after downshift.

3.2.2. Sausage experiment

Both *L. monocytogenes* EGD (Fig. 4) and LO28 formed long filamentous cells consisting of 10–50 single cells on sausage slices packaged in 100% CO_2 and normal rods in air and N_2. However, filaments were also occasionally observed in air and N_2 for cells in the late stationary phase. After downshift, septum formation was observed for both *L. monocytogenes* EGD (Fig. 4) and LO28. Within 4 days after the downshift, the majority of the filaments had divided.

3.3. Changes in cell density

3.3.1. Broth experiment

In the case of *L. monocytogenes* EGD, both OD and the number of colony forming units increased after the CO_2 downshift and the increase was considerably larger and more rapid for exponentially growing cultures (Fig. 5). In the case of *L. monocytogenes* LO28, only small increases were observed.

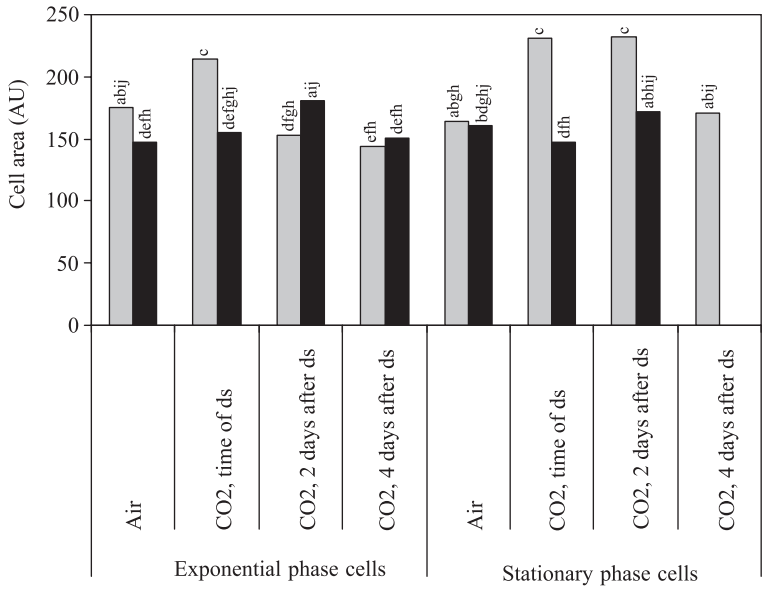

Fig. 3. In situ rRNA in *Listeria monocytogenes* EGD (light bars) and LO28 (dark bars) grown in BHI at 10 °C in air or 100% CO_2. Cultures grown in CO_2 were downshifted (i.e., exposed to air) in the exponential and the stationary phases, respectively. Bars with the same letter are not significantly ($P>0.001$) different. The calculations are based on a total of 16,372 cells.

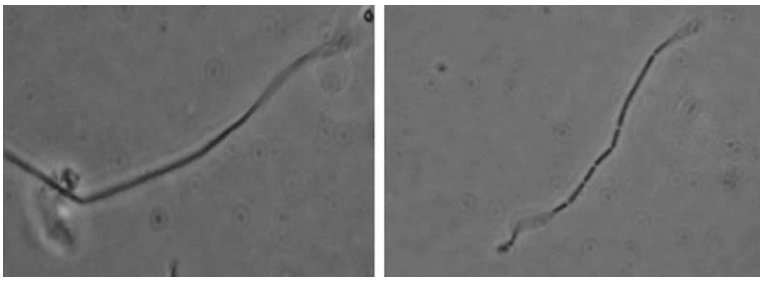

Fig. 4. Growth of *Listeria monocytogenes* EGD on slices of a meat model sausage (pH 5.9) to the stationary phase in 100% CO_2 and subsequently downshifted (i.e., exposed to air). Photos were taken at time of downshift (left) and 2 days after the downshift (right).

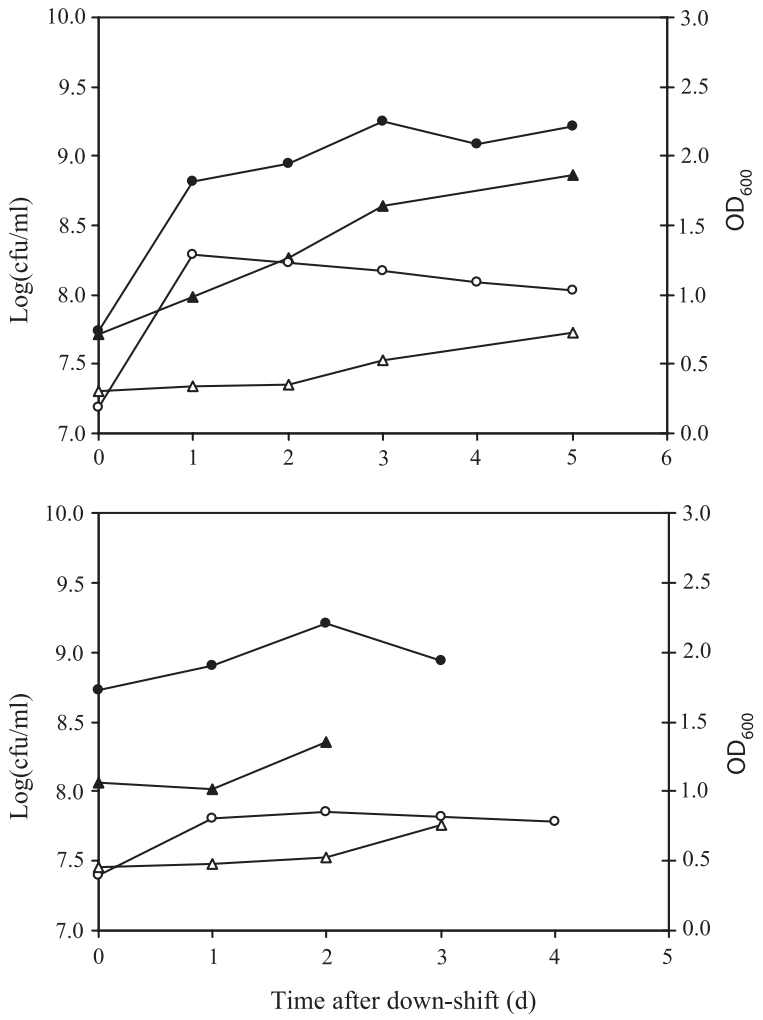

Fig. 5. Log (cfu/ml; closed symbols) and OD_{600} (open symbols) as functions of time after downshift (i.e., transfer from 100% CO_2 to air) for *Listeria monocytogenes* EGD (top) and LO28 (bottom) initially grown in BHI at 10 °C in 100% CO_2 to the exponential growth phase (circles) as well as to the stationary phase (triangles).

3.3.2. Sausage experiment

For all packaging atmospheres, the growth of exponentially growing cultures was more rapid after opening (Fig. 6) whereas changes in the stationary phase were moderate. The increase of growth rates (Table 2) was higher in CO_2 packages (2.9-fold to 7.8-fold, respectively) than in N_2 (1.5-fold to-2.7-fold) or air packages (1.1-fold to 2.1-fold). In the case of *L. monocytogenes* LO28 grown on sausages with pH 6.4, the increase of the colony forming units was slightly higher in opened CO_2 packages than in opened air packages. For the other scenarios, the growth rates in CO_2 packages after opening were similar to those in air packages after opening. For both strains, growth proceeded faster on slices of sausages with pH 6.4 than on slices of sausages with pH 5.9. The relative inhibition (RI) of growth in CO_2 compared with anaerobic growth was lower at pH 6.4 than at pH 5.9 for *L. monocytogenes* LO28 while it was similar for *L. monocytogenes* EGD (Table 2). If comparing the rates with those predicted with Growth Predictor (http://www.ifr.ac.uk/safety/GrowthPredictor), a publicly available modelling tool, using 2% NaCl, initial count $5 \cdot 10^3$ cfu g^{-1}, and appropriate pH in the model, the growth rates in the sealed packages were all between one third and half of the predicted values while the rates post-opening although lower

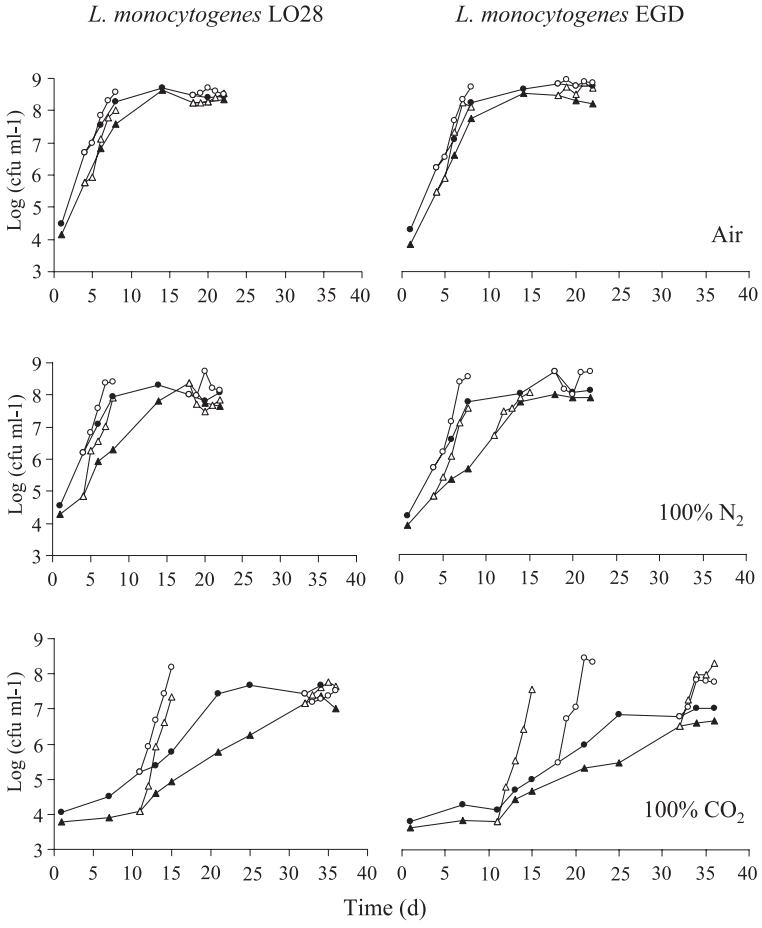

Fig. 6. Growth of *Listeria monocytogenes* LO28 (A) and EGD (B) grown on slices of a meat model sausage with pH 6.4 (●) or pH 5.9 (▲) packaged in air (top), 100% N_2 (middle), or 100% CO_2 (bottom) in sealed packages (closed symbols) as well as in packages opened in the exponential or the stationary phase (open symbols), respectively.

Table 2
Growth rates[a] calculated[b] for *Listeria monocytogenes* LO28 and EGD grown on sausage slices with different pH in three atmospheres at 10 °C in sealed and opened packages

		Sealed				Opened[c]		
		Air	N_2	CO_2	RI[d]	Air	N_2	CO_2
pH 6.4	LO28	0.62	0.51	0.26	49%	0.66	0.75	0.75
	EGD	0.47	0.48	0.17	64%	0.84	1.01	0.78[e]
pH 5.9	LO28	0.54	0.36	0.14	61%	0.96	0.60	0.90
	EGD	0.53	0.29	0.11	62%	1.10	0.78	0.86

[a] log(cfu)/day.
[b] Δlog(cfu/g)/Δday.
[c] Packages were opened in the exponential growth phase: day 4 for air and N_2 atmospheres, day 12 for CO_2 atmospheres.
[d] Relative inhibition of growth in the CO_2 versus N_2 atmospheres was calculated as $RI=((\mu_{N2}-\mu_{CO2})/\mu_{N2})\times 100$.
[e] Opened on day 18 instead of day 12.

were more comparable to the predicted values (data not shown).

4. Discussion

Both in BHI and on sausages, cell numbers increased after CO_2 downshift without any sign of residual CO_2 effect. This is in contrast to previously reported observations (Avery et al., 1995) and indicates that major residual CO_2 bacteriostatic effects cannot be relied on for safety at the pH and temperature values employed here. On the contrary, filament formation due to lack of septation was observed, and while the filaments may form one colony, they have the potential of forming up to 50 single cells each indicating a risk of underestimating the number of *Listeria* in CO_2-packaged meat products.

The filamentous cells populations were shown to have a growth potential after the opening of the packages. In BHI, only *L. monocytogenes* EGD displayed massive filamentation in CO_2 and this strain had a larger and more rapid increase in cell number and density after CO_2 downshift than the nonfilamentous *L. monocytogenes* LO28. On sausage slices in CO_2, both strains formed filaments and rapid growth was detected for both after the downshift.

Theoretically, one 50-cell filament could multiply to 100 cells within one to two generations whereas one cell would require seven generations. Given the same generation time, the filamentous strain should therefore display a markedly more rapid growth rate under conditions where septation takes place. The single cell in situ rRNA content displayed large variations in part due to the morphological changes, and although it was useful to examine the cell morphology changes and septation, it could not as intended be used to show differences in growth activities in this scenario. However, based on cfu, the application of estimated rates of increase (Table 2) showed that the time needed for a 2 log increase (100 cfu g^{-1} is the limit set by Danish legislation for similar products) was within the same order for the three atmospheres post-opening. The time to reach 100 cfu g^{-1} from an initial 1 cfu g^{-1} after opening was within in 2.2–2.7 days in CO_2 packages (both strains and pH values), 1.9–3.3 days in N_2 packages, and 1.8–3.1 days in air packages. Because the time needed to reach 100 cfu/g was not markedly shorter in CO_2 packages post-opening than in air or N_2 packages, it indicates that septation proceeds relatively slowly, which corresponds to the microscopical findings, and no sudden increase in cell numbers are expected at this temperature where the packages were opened and continuously stored at 10 °C. However, the scenario in consumers' homes includes opening and exposure of the product to ambient temperatures for a few minutes and up to several hours. Such a temperature increase would undoubtedly speed up the cell division as also reported for salt downshifted *L. monocytogenes* where reversion to normal cells was completed within 8 h at 30 °C (Jørgensen et al., 1995).

The effect of CO_2 downshift on cell morphology has not been reported for *L. monocytogenes*, but formation of filamentous cells in *L. monocytogenes* Scott A subjected to osmotic stress (9% NaCl) and septum formation followed by cell division in response to osmotic downshift has been reported previously (Jørgensen et al., 1995). Later work has also observed filamentation during osmotic and acidic stress (Bereksi et al., 2002), and elongation of individual cells of *L. monocytogenes* Scott A grown in buffered BHI in 100% CO_2 at 4 °C has been reported (Nilsson et al., 2000; Li et al., 2003). There therefore seems to be strong indications that filament formation is a common response used by several bacteria under certain stress, but the mechanisms involved are not yet understood.

Comparing relative inhibition (RI) of the two strains using the anaerobic atmosphere (100% N_2) as reference showed that the acid-tolerant *L. monocytogenes* LO28 was less affected by the atmosphere than the acid-sensitive *L. monocytogenes* EGD at pH 6.4 whereas the inhibition was similar at pH 5.9. These differences could be due to differences in H^+/HCO_3^- sensitivity rather than in CO_2 sensitivity, because at pH 5.9, almost all dissolved gas would be on the CO_2 form while at pH 6.4 up to 10% appears as H^+/HCO_3^-. If so, *L. monocytogenes* LO28 is less inhibited by H^+/HCO_3^- than *L. monocytogenes* EGD, but the two strains are equally inhibited by CO_2. This corresponds to *L. monocytogenes* LO28 having an approximately 100-fold higher natural level of glutamate decarboxylase than *L. monocytogenes* EGD (Cotter et al., 2001). The glutamate dehydrogenase complex allows *L. monocytogenes* to grow in the presence of acid as it converts extracellular glutamate to extracellular γ-amino-butyric-acid (GABA) using an intracellular proton.

In conclusion, we report a strain-dependent morphologic CO_2 response in *L. monocytogenes* including extensive filamentation which increases the risk of underestimating the number of cells in the packages. No residual CO_2 inhibition was observed after opening of packages.

Acknowledgments

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