

Effect of Temperature, Relative Humidity, and Suspending Menstrua on the Resistance of *Listeria monocytogenes* to Drying

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ABSTRACT

The ability of *Listeria monocytogenes* to survive dehydrated storage at different temperatures (5 vs. 25°C) and relative humidities (75, 59, 35, 14, and 1%) when suspended in different menstrua was studied. *L. monocytogenes* survived longer when held at 5°C compared to 25°C, and when suspended in beef extract, glycerol, Karo syrup, skim milk, and canned milk compared to distilled water. The contribution of relative humidity to survival was less clear, though survival tended to be longer at the lower relative humidities. At 5°C when suspended in beef extract and held at relative humidities of 59% and below, there was less than a 2 log₁₀ decline in the viable population (starting count ca. 10⁵/coverslip). *L. monocytogenes* was not injured during drying or storage at the various relative humidities. In addition, based on the *Listeria* selective media evaluated, these *Listeria* media permitted quantitative recovery of the organism dried on surfaces. These results suggested that once *L. monocytogenes* has contaminated a food processing plant, it can persist for long periods in the plant environment if the temperature is low and the organism is protected by various food components.

Listeria monocytogenes has been known as a pathogen of humans since 1929 when Nyfeldt (12) described the first cases of human listeriosis. It is only in recent years that food has become recognized as a vehicle for the transmission of the organism to humans (10,13,14). This recognition was brought about by three documented major outbreaks in which milk (11), shredded cabbage (21), and cheese (15) were the incriminated foods. It was only recently that foods, particularly processed foods, were surveyed for their content of *L. monocytogenes*.

Raw foods might be expected to contain low numbers of *L. monocytogenes* since the organism occurs widely in the environment such as water, sewage, and soil as well as the gastrointestinal tract of food animals (1,12). The presence of *L. monocytogenes* in heat processed foods such as pasteurized milk, ice cream products, or frankfurters could be explained in two ways: 1) the organism can survive the heating step used in the preparation of these foods, or 2) the heating step destroys any *L. monocytogenes* present in the raw food, but the food becomes recontaminated during further handling of the food by organisms present in a

contaminated plant environment. For example, recent work from our laboratory has indicated that *L. monocytogenes* is destroyed by the heating cycle of the standard frankfurter process (Zaika et al. 1989, J. Food Protection in press). However, frankfurters have been linked both epidemiologically (22) and definitively (3) with cases of human listeriosis. Thus, contact of *Listeria*-free frankfurters with a contaminated plant environment undoubtedly contributed to the presence of *L. monocytogenes* in the finished product.

The ability of various bacteria to survive under different environmental conditions has been studied (5,6,9,17,18,19,20). In general, Gram positive bacteria survive better than Gram negative bacteria, and most bacteria survive better at a low temperatures (4-5°C) compared to room temperature (15-25°C). Specific instances of survival of *L. monocytogenes* are available. Dickgiesser (5) observed that *L. monocytogenes* survived for 20 to 30 d (temperature and relative humidity unspecified) on hospital tile. Welshimer (24) observed the survival of *L. monocytogenes* in fertile soil held at 24 to 26°C in sealed tubes for periods up to 295 d; when held in clay, the number declined. When held in cotton plugged tubes at 24 to 26°C, the numbers declined in both clay and fertile soil. Doyle et al. (8) reported on the storage of *L. monocytogenes* in nonfat dry milk powder (3.6 to 6.4% moisture — organism dried in the milk powder) at 25°C; the numbers decreased more than 4 log₁₀ CFU/g during 16 weeks of storage.

The purpose of this study was to investigate the ability of *L. monocytogenes* to survive under various simulated food plant environments and in the presence of various model foods. Specifically, we determined the influence of temperature (5 vs. 25°C), relative humidity (75 to 1%), and suspending menstrua (sterile "model" fluid foods) on the ability of *L. monocytogenes* to survive drying on glass microscope coverslips.

MATERIALS AND METHODS

Organisms

Seven strains of *L. monocytogenes* (V7, RMII, RMI, Murray B, Scott A, V97, and ATCC 7644) were used in this study. Each was grown overnight in individual tubes of Tryptose broth (Difco) at

37°C. After growth, 1 ml from each culture was added to a sterile centrifuge tube, and the mixture centrifuged (10,000 × g, 5 min 5°C). The pellet was then resuspended by adding 7 ml of the different suspending menstrua and mixing on a Vortex mixer until a uniform suspension was obtained. Thus, the cells placed on the coverslip represent a cocktail — a mixture of the seven strains of *L. monocytogenes*.

Suspending menstrua

The following substances were used to simulate various food/food components (all were sterile): distilled water, tryptone broth (Difco), 10% nonfat dry milk, canned milk, 10% glycerol, 10% light Karo syrup, 5% beef extract (Difco); 0.5 ml of the cells suspended in the different food components was placed on sterilized glass coverslips (11 × 22 mm) and the coverslips rotated to give a uniform layer over the entire surface of the coverslip. The inoculated coverslips were then air-dried under a biological hood and placed in their respective desiccators as described below.

Survival system

The microscope coverslip system described by Doyle and Roman was used (7). As described by Doyle and Roman (7), saturated salt solutions were prepared in desiccators to give relative humidities of 75, 59, 35, 14, and 1% (25); before use, one set was equilibrated for several days at 5°C and a second set at 25°C.

Recovery of cells from coverslips

At appropriate intervals, inoculated coverslips were removed from the desiccators, placed in 9.9 ml peptone dilution tubes, and mixed for 15 s on a Vortex mixer; the cells washed off and counted at the beginning became the numbers of cells added, and thus, the zero time value. After standing for 5 min, the tubes were again mixed for 15 s. It was determined visually that this procedure removed the suspended bacteria from the coverslip. Surviving *L. monocytogenes* were determined by surface plating of the dilution tube contents onto duplicate Tryptose agar (TA, Difco) plates using a Spiral plater (Model D, Spiral Systems, Bethesda, MD). Colonies were counted after 3 d at 37°C. The lower limit of detection of this plating system was 20 cells; any counts less than 20 cells are represented on the figures as zero.

Injury and *Listeria* selective media

In certain experiments, survivors were also surface plated using a Spiral plater on MBA (McBrides agar, without blood; Difco), modified Vogel Johnson agar (MVJ; 2), cyclohexanedione-nalidixic acid-phenylethanol agar (CNP; 16), TPBAP (Tryptose phosphate broth + 2% agar [TPBA] + 1% sodium pyruvate), and TPBAS (TPBA containing 5% added NaCl) (23). The difference between the count on TPBAP and TPBAS represents the number of cells injured by exposure to the drying and storage conditions of the various experiments; the counts on the *Listeria* selective media represent the ability of these media to recover cells exposed to various drying and storage conditions.

RESULTS AND DISCUSSION

Definition of the system

The glass coverslip system developed by Doyle and Roman (7) for use with *C. jejuni* appeared also to be a satisfactory one for use with *L. monocytogenes* and seemed adequate to investigate the influence of % RH, temperature, and suspending menstrua on the survival of the organism. Though all variables were studied at least twice and similar responses were observed, data presented are from individual experiments. Data from a typical experiment with 5% beef extract and distilled water as suspending menstrua are presented in Figs. 1 and 2, respectively. Survival was better at 5 than at 25°C for both suspending menstrua. In addition, survival was better in beef extract compared with distilled water. Further, within the individual temperatures with beef extract, survival was favored by conditions of lower % RH. However, for beef extract, at 14 and 1% RH, survival was similarly good at both 25 and 5°C.

Distilled water (Fig. 2) was quite detrimental to *L. monocytogenes*, especially at 25°C where there was a rapid decline in the number of viable cells. Further evidence for this is presented in Fig. 3 where the influence of drying menstrua and plating media can be seen. On the left (w/o drying) are the viable counts of *L. monocytogenes* placed on the coverslip before drying. In the middle are the number of viable *L. monocytogenes* (just after drying and prior to storage) recovered when the cells were suspended in 10% Karo syrup. There was at least 3 log cycle (99.9%) decline in the number of viable cells during the drying step itself. On the right are shown the number of viable *L. monocytogenes*

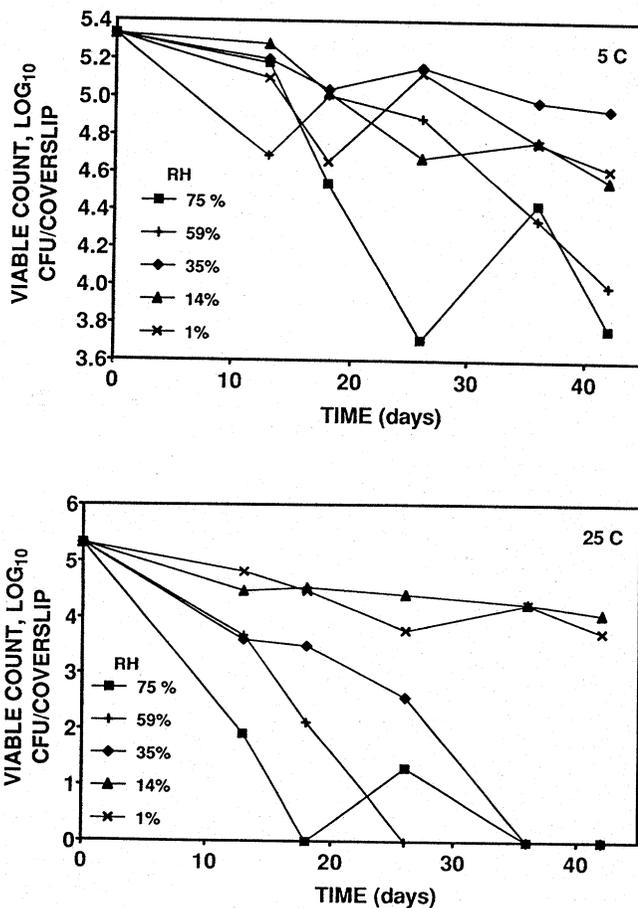


Figure 1. Influence of % relative humidity and temperature on the survival of *L. monocytogenes* suspended in 5% of beef extract.

- 5°C
- 25°C

recovered immediately after drying cells suspended in distilled water. There is further decline of about 2 log cycles in the number of viable cells. Thus, there are decreases in viable cells both from the drying step itself and drying in distilled water compared to Karo syrup.

We also studied the ability of *L. monocytogenes* to survive for extended periods at 5°C when suspended in beef extract. These data are presented in Fig. 4 and suggested that

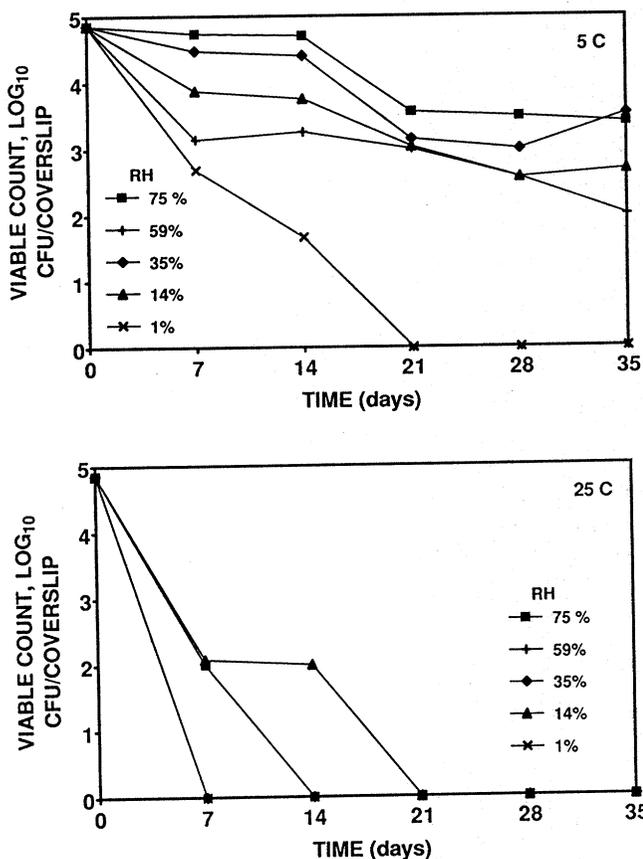


Figure 2. Influence of % relative humidity and temperature on the survival of *L. monocytogenes* suspended in distilled water.
a) 5°C
b) 25°C

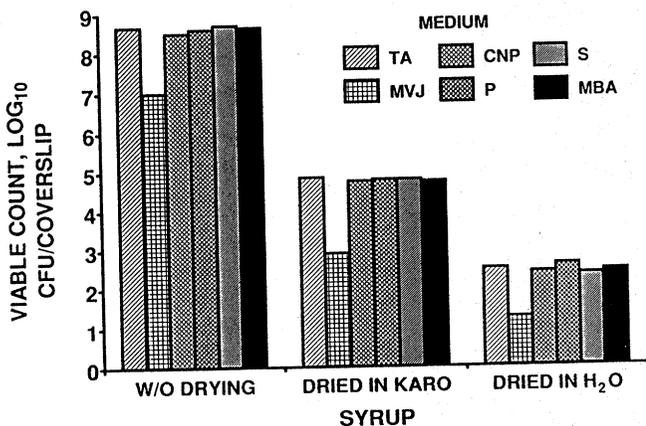


Figure 3. Influence of suspending menstrua and plating media on the number of surviving *L. monocytogenes*.

the organism can easily persist on surfaces in a contaminated food plant environment except at the higher relative humidities, and thus, readily contaminated any food which comes in contact with the surface. The extended survival observed in this study (see Fig. 4) of 136 d and beyond, confirms by the observations of Welshimer (24) for *L. monocytogenes* in soil at room temperature and McEldowney and Fletcher (20) for *Pseudomonas* sp. at 4°C and 75% relative humidity.

Effect of suspending "food" menstrua

The data presented in Figs. 1 and 2 indicate that the suspending menstrua can contribute significantly to the ability of *L. monocytogenes* to survive drying. Data on the effect of suspending menstrua as well as % relative humidity and temperature on survivors are given in Table 1, and indicate that these menstrua also contribute to survival,

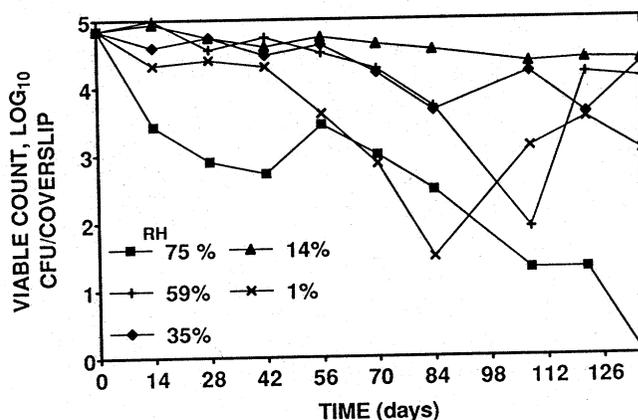


Figure 4. Influence of % relative humidity on the survival of *L. monocytogenes* suspended in beef extract and held at 5°C.

especially at 5°C. Further, at 5°C, survival tended to be higher at the higher relative humidities, though temperature seems to be a more important factor influencing survival. Our observations on the protective effect of suspending menstrua (compared to distilled water) agree with the observations on survival of dried *S. aureus*: survival was enhanced when the cells were suspended in broth, serum, or saliva compared to distilled water (18).

Injury and *Listeria* selective media

One factor which microbiologists must take into account in the attempt at quantitative recovery of any organism (spoilage or pathogen) from a food is the presence of injured cells. Injured cells often won't grow on the selective media typically used for their recovery from foods. Smith and Archer (23) have studied injury to *L. monocytogenes* by heat and developed the TPBAP/TPBAS plating system to detect and quantitate heat injury in *L. monocytogenes*.

We plated some of our samples for survivors on TPBAP and TPBAS. Data for one of these platings are presented in Fig. 5, and indicate that *L. monocytogenes* is not injured during the drying process itself and is not injured during continued storage at different relative humidities at 5°C

TABLE 1. Effect of food menstruum, temperature, and % relative humidity on the percent^a *L. monocytogenes* surviving storage.

	Percent Survivors ^a									
	% Relative Humidity at 5°C					% Relative Humidity at 25°C				
Suspending menstruum	75	59	35	14	1	75	59	35	14	1
10% Glycerol ^b	35 ^a	50	12	1.8	4.4	0.05	0.08	0.014	0.08	0.028
10% Karo syrup ^c	15	11	43	100	46	0 ^d	0	0.056	46	32
Canned milk ^e	2.4	20	9.1	9.1	5.4	0	0	0	0.35	0
10% Skim milk ^f	13	16	0.9	2.6	0.05	0	0	0.01	0.28	0.01
5% Beef extract ^g	2.8	10	41	28	22	0	0	0	15	13
Distilled water ^c	5.1	0.41	8	1.3	0	0	0	0	0	0

^aCount at the end of storage divided by the count at zero time x 100 yields.

^bAfter 36 d of storage.

^cAfter 35 d of storage.

^d0% Survival indicates less than 20 viable cells at the end of storage.

^eAfter 42 d of storage.

^fAfter 30 d of storage

^gAfter 36 d of storage.

(compare data in Fig. 5 with the data in Figs. 1a and 2a); counts on TPBAP and PBAS are essentially the same as counts on TA for the samples stored at 5°C. Similar responses were observed for samples stored at 25°C (data not shown). To determine if suspending menstruum could influence cell injury in *L. monocytogenes* stored at different temperatures and relative humidities, cells were also suspended in 10% glycerol and 10% Karo syrup, and samples for viable count were plated on TPBAP and TPBAS. For both of these suspending menstrua, the response observed was similar to that for beef extract shown in Fig. 5 [the counts on TPBAP and TPBAS were similar to TA for samples stored at both 5 and 25°C (data not shown)].

The data shown in Fig. 3 indicate that, although many cells of *L. monocytogenes* died during the process of air drying, the samples suspended in Karo syrup or distilled water on the coverslips, there was no cell injury evident in water (the count on TPBAS = count on TPBAP or TA). Further, counts on MBA and CNP media were similar to the TA or TPBAP, indicating that these two selective media were capable of quantitatively detecting dried *L. monocytogenes* cells. Recovery on MVJ was lower than on TA or the other *Listeria* selective agars; this lower recovery is typical of the MVJ medium (2).

In conclusion, the data presented here indicate that *L. monocytogenes* would survive for extended periods under conditions found in many food processing plants, and especially in refrigerated areas and when suspended (entrapped) in food components. These "zones of surviving cells" can contaminate *Listeria*-free foods and thus become foci for reinfection of properly processed foods. Further, it is possible to conceive of these surviving cells as existing in a biofilm (4) which protects these cells from sanitizers, detergents, and other procedures for removing them. Besides being protected, cells in a biofilm on a surface may be capable of growth even though the surface is located in an environment of low moisture availability. McEldowney and Fletcher (20) have presented data for a strain of *Pseudomonas* which suggest that the number of microcolonies and number of cells within microcolonies increased on a dry

surface held under an atmosphere of relative humidity as low as 0% or as high as 75%. Other data also suggest that metabolism and growth take place within the microenvironment of the biofilm (20). Therefore, the production of biofilms may be an explanation for fairly good survival of *L. monocytogenes* in the presence of food components since

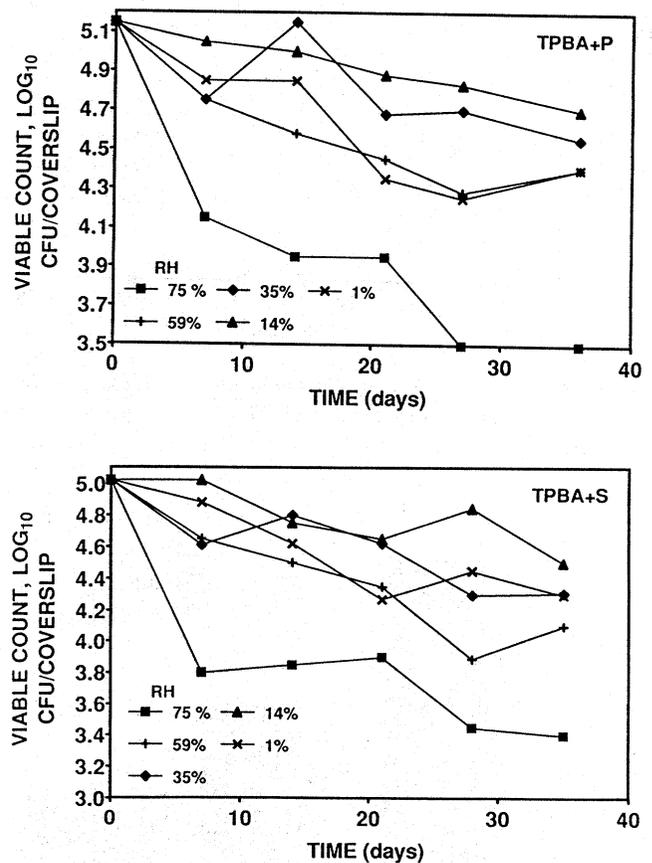


Figure 5. Effect of plating media, TPBAP, and TPBAS on the number of surviving *L. monocytogenes* suspended in 5% beef extract and stored at 5°C.

a) TPBAP

b) TPBAS

these foods also provide nutrients which help cells maintain their viability.

Though only a few media selective for *L. monocytogenes* were evaluated, it would appear that present *Listeria* media are capable of recovering any *L. monocytogenes* present on food contact surfaces. Further, it does not appear that *L. monocytogenes* is injured by being dried on a surface and that the number recovered should represent the number present.

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