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A Research Note

Survival of *Listeria monocytogenes* During the Manufacture and Storage of Nonfat Dry Milk

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ABSTRACT

The ability of *Listeria monocytogenes* to survive in skim milk during spray drying and to persist in nonfat dry milk during storage was examined. Concentrated (30% solids) and unconcentrated skim milks were inoculated with ca. 10^5 to 10^6 *L. monocytogenes*/ml and spray dried (inlet temperature, 165 ± 2°C; outlet temperature 67 ± 2°C) to a moisture content of 3.6 to 6.4%. The nonfat dry milk was packaged in moisture-resistant film and stored at 25°C for up to 16 wk. A reduction of ca. 1 to 1.5 $\log_{10}$ *L. monocytogenes*/g occurred during the spray drying process, irrespective of whether the milk was concentrated or not before spray drying. The organism progressively died during storage at 25°C, with a >4-$\log_{10}$ CFU/g decrease occurring within 16 wk of storage.

An outbreak of listeriosis which occurred in 1983 was linked to drinking a specific brand of pasteurized milk *(4)*, and has prompted concern about the survival and growth characteristics of *Listeria monocytogenes* in dairy products. Nonfat dry milk can serve as a vehicle for infectious disease agents, e.g., several outbreaks of salmonellosis have been traced to this product *(3,5,6)*. Dried milk may become contaminated with pathogens in many ways, both before and after drying. Hence, it is useful to know how well such organisms survive the drying process and during storage of the dried product. This is particularly important for *L. monocytogenes*, which reportedly has unusual thermal resistance *(2)*. The purpose of this study was to determine the survival characteristics of *L. monocytogenes* in skim milk during spray drying and in nonfat dry milk during storage.

MATERIALS AND METHODS

Preparation of cultures

Two strains of *L. monocytogenes*, Scott A (human isolate, serotype 4b) and V7 (milk isolate, serotype 1) provided by R. M. Twedt, Food and Drug Administration, Cincinnati, OH, were tested individually. Cultures were maintained at 4°C on tryptose agar (Difco Laboratories) slants or held frozen in skim milk at -20°C. Cells used to inoculate skim milk were grown in 100 ml of tryptose broth (Difco Laboratories) in a 500-ml side-arm Erlenmeyer flask which was evacuated three times to 20 in. Hg and refilled with a microaerobic atmosphere (5% O_2, 10% CO_2, 85% N_2). Cultures were grown with (100 gyrations/min) or without agitation at 35 to 37°C for 48 h. Cells were then washed with 0.1% peptone and recovered by centrifugation (8000 × *g*, 15 min). This was done three times after which the cells were diluted to the appropriate concentration.

Inoculation and spray drying of milk

Skim milk [concentrated (30% solids) and unconcentrated (10% solids)] was obtained from the University of Wisconsin Dairy Plant on the morning of each trial. One ml of a suspension of *L. monocytogenes* cells (ca. 1×10^8 to 1×10^9 CFU/ml) was inoculated per liter of milk, and one trial of each type of milk (concentrated or unconcentrated) inoculated with the same strain of *L. monocytogenes* was done during a single day. Each strain of *L. monocytogenes* and each type of milk was spray dried and evaluated in duplicate.

Milk was dried in a gas-fired Nerco-Niro portable spray drying unit, with an inlet air temperature of 165 ± 2°C and an outlet air temperature of 67 ± 2°C. The nonfat dry milk was collected in a cyclone collector, and packaged in oxygen impermeable film (Saran-coated Mylar, oxygen transfer rate less than 0.5 cm^2/1 atm/100 cm^2; Curwood, Inc., New London, WI). The packages of nonfat dry milk (25 g/package) were heat-sealed to prevent moisture uptake during storage, and held at 25°C. Duplicate samples of each trial of each type of milk were assayed for *L. monocytogenes* immediately after spray drying and at 1, 2, 4, 6, 8, 10, 12 and 16 wk of storage. Additionally, before spray drying, duplicate 25-ml samples of each milk before and after inoculation with *Listeria* were assayed for *L. monocytogenes*.

Determination of moisture content

Moisture content of nonfat dry milk was determined by drying samples in a vacuum oven at 100°C until constant weights were obtained *(1)*. Duplicate samples of each trial of each type of milk were tested.

Enumeration of L. monocytogenes

Each sample (25 g) was added to 225 ml of tryptose broth in a Stomacher bag (Tekmar, Cincinnati, OH; 500 ml capacity) and blended in a Stomacher 400 for 30 to 45 s. One ml of blended sample was then serially diluted (1:10) in tryptose broth, and 0.1-ml portions of each dilution were surface plated in duplicate onto McBride's Listeria agar (MLA). Additionally, duplicate 0.1-ml portions of the initial 25 g/250 g-dilution were plated on MLA. When *L. monocytogenes* counts were $<10^3$ CFU/g, 10 plates were each inoculated with 0.1 ml of the initial 25 g/250 g-dilution to provide a minimum level of sensitivity of 10 *L. monocytogenes*/g. The remaining sample (25 g/250 g-dilution) was incubated in the Stomacher bag at 4°C for cold enrichment.

MLA plates were incubated at 37°C for 48 h in a reduced oxygen atmosphere (5% O_2, 10% CO_2, 85% N_2). Plates were counted for typical (bluish gray, translucent, slightly raised, 0.5 to 1.5 mm in diameter and weakly β-hemolytic) colonies of *L. monocytogenes*. *Listeria*-like colonies developing on plates with the most dilute sample were selected randomly and confirmed as *L. monocytogenes*. Confirmatory tests included: characteristic tumbling motility (when grown at 21°C) determined microscopically, β-hemolysis, catalase reaction (positive), Gram stain reaction (positive) and serology. Serological slide agglutination tests were done on isolates thought to be *L. monocytogenes* using commercially prepared antiserum (Difco) to confirm that the isolates were serotypes 1 and 4.

If no *L. monocytogenes* grew on plates that were inoculated directly with the sample, the corresponding sample held at 4°C was assayed for *L. monocytogenes* by plating 0.1-ml portions on duplicate MLA plates at 2, 4, 6 and 8 wk of incubation. *Listeria*-like colonies that grew on MLA plates were confirmed by the procedures described above. If *L. monocytogenes* was enumerated on selective agar by direct plating, samples held at 4°C were not assayed.

RESULTS AND DISCUSSION

The moisture content of the nonfat dry milk was 3.6 to 4.5% when a concentrate (30% solids) was dried, and 4.9 to 6.5% when unconcentrated skim milk was dried (Table 1).

In all instances, a large population of *L. monocytogenes* survived the spray drying process; however, some death occurred. Considering that there was approximately a three- [beginning with concentrated (30% solids) milk] to nine- [beginning with unconcentrated (10% solids) skim milk] fold concentration of the milk by spray drying, there was, in general, about a 1- to 1.5-$\log_{10}$ *L. monocytogenes* (per gram of fluid milk) decrease during the spray drying process, irrespective of whether the milk was concentrated or not before drying (Table 1). Similarly, Miller et al. *(7)* showed that both salmonellae and *Escherichia coli* survived in milk during spray drying; however, a larger portion of their initial population (generally about a 3-$\log_{10}$ CFU/g reduction) than that of *L. monocytogenes* was killed during drying.

Although exact comparisons regarding survival of the two *L. monocytogenes* strains cannot be made because of differences in the number of viable cells present before and after spray drying, strain Scott A was generally more sensitive than strain V7 to both the spray drying process

TABLE 1. *Fate of* L. monocytogenes *in skim milk during spray drying and in nonfat dry milk during storage*[a].

Strain	Type of milk	Trial No.	Moisture content[b] (%, wt/wt)	$\log_{10}$ *L. monocytogenes* (CFU/g)[b]: Fluid milk (Day 0)	After spray drying (Day 0)	1 wk	2 wk	4 wk	6 wk	8 wk	10 wk	12 wk	16 wk
Scott A	Concentrated	1	3.6	5.08	4.26	3.48	3.53	3.65	<1.00(–)[c]	<1.00(+)[d]	<1.00(–)	<1.00(–)	<1.00(–)
		2	3.9	4.76	4.00	3.72	3.66	3.74	<1.00(–)	<1.00(–)	<1.00(–)	<1.00(–)	<1.00(–)
	Unconcentrated	1	4.9	5.20	4.59	4.92	4.36	3.66	<1.00(–)	<1.00(–)	<1.00(–)	<1.00(–)	<1.00(–)
		2	5.0	5.00	4.74	4.36	3.86	3.83	<1.00(–)	<1.00(–)	<1.00(–)	<1.00(–)	<1.00(–)
V7	Concentrated	1	3.7	5.40	5.20	4.79	4.36	2.93	3.18	2.93	<1.00(+)	2.53	<1.00(–)
		2	4.5	5.53	5.28	4.23	4.15	2.34	2.40[e]	2.79	2.57	2.38	<1.00(–)
									<1.00(+)[e]				
	Unconcentrated	1	5.7	5.54	5.70	4.89	5.15	3.18	3.15	2.67	2.59	<1.00(–)	<1.00(–)
		2	6.4	6.34	5.76	4.30	3.04	1.95	<1.00(–)	<1.00(–)	<1.00(–)	<1.00(–)	<1.00(–)

[a]Following spray drying, milk powder was held in sealed, water-impermeable Saran-coated Mylar bags at 25°C.
[b]Average of duplicate determinations.
[c](–), negative by cold enrichment.
[d](+), positive by cold enrichment.
[e]Individual counts reported, not averaged.

and to long-term storage in dried milk. When present in nonfat dry milk at 25°C, Scott A cells decreased by more than 3 $\log_{10}$ CFU/g by 6 wk of storage (regardless of whether the milk powder was prepared from concentrated or unconcentrated milk), whereas the same >3-$\log_{10}$ CFU/g reduction in number of V7 cells occurred between 12 and 16 wk of storage in nonfat dry milk made from concentrated skim milk. Strain V7 died more rapidly in nonfat dry milk that was not concentrated before spray drying, with a >3-$\log_{10}$ CFU/g reduction occurring by 4 wk in one trial and by 10 to 12 wk in another trial. Perhaps the higher moisture content (i.e., 5.7 and 6.4%) of these two lots of nonfat dry milk had an influence on the organism's ability to survive.

Results indicate that *L. monocytogenes* can survive the process of spray drying and may persist for at least 12 wk in nonfat dry milk held at room temperature (25°C) if sufficient numbers (ca. 10^5 CFU/g) are present initially. However, the organism does die off in nonfat dry milk during storage, with >4-$\log_{10}$ CFU/g reduction occurring by 16 wk. Hence, holding *L. monocytogenes*-contaminated nonfat dry milk at room temperature for several months may be a suitable means of decontaminating suspect product.

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