Short Communication: Growth Characteristics of *Streptococcus uberis* in UHT-Treated Milk

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ABSTRACT

Streptococcus uberis is an important environmental pathogen associated with bovine mastitis as well as with high total bacterial numbers in bulk tank milk. This study was conducted to determine whether *S. uberis* reproduction is likely to contribute to high bacterial numbers in bulk tank milk. Four *S. uberis* raw milk isolates were individually inoculated into UHT-treated milk and incubated at 4.4 or 7°C for up to 5 d to simulate appropriate cooling; at 10°C for 5 d to simulate marginally inadequate cooling; at 21 or 25°C for 7 h to simulate ambient temperatures; or at 32°C for 7 h to simulate elevated temperature conditions. None of the *S. uberis* isolates grew at either 4.4 or 7°C. *Streptococcus uberis* growth at 10°C appeared to be ribotype-specific. Although ribotype 116-520-S-1 isolates did not grow at 10°C, ribotype 116-520-S-2 isolate numbers increased up to 3.5 log₁₀ cfu/mL within 5 d. Generation times were calculated as 2.7 ± 0.1 h, 2.1 ± 0.1 h, and 1.0 ± 0.1 h for 116-520-S-1 isolates and 1.8 ± 0.4 h, 1.3 ± 0.3 h, and 0.8 ± 0.1 h for 116-520-S-2 isolates at 21, 25, and 32°C, respectively. Our results suggest that high numbers of *S. uberis* in bulk tank milk are more likely to reflect high numbers of *S. uberis* shed by mastitic cows, rather than multiplication of these organisms under cooling conditions required for production of Grade A milk.

(Key words: *Streptococcus uberis*, growth, bulk tank milk, total bacterial counts)

Abbreviation key: BHI = brain heart infusion, PMO = Pasteurized Milk Ordinance, TBC = total bacterial counts.

Streptococcus uberis is an important environmental pathogen associated with bovine mastitis (Hogan and Smith, 1997; Leigh, 1999). Cows with clinical mastitis caused by this organism have been shown to shed up to 10⁷ cfu/mL of *S. uberis* in their milk (Leigh, 1999). This organism is also commonly isolated from bovine

body sites, including the skin surface, genital tract, and tonsils, as well as from manure, soil, and bedding (Cullen and Little, 1969; Sharma and Packer, 1970; Razavi-Rohani and Bramley, 1981; Bramley, 1982). *Streptococcus uberis* has been demonstrated to contribute to elevated bacterial counts in farm bulk tank milk (Auldish and Hubble, 1998; Hayes et al., 2001). To illustrate, *S. uberis* was identified as the predominant organism in over 50% of brief, sporadic increases (so-called “spikes”) in bulk tank milk total bacteria counts observed during daily monitoring of bulk tank microflora on multiple farms (Hayes et al., 2001).

Total bacterial count (TBC) of bulk milk is an important measure of milk quality and can assist in evaluating mastitis outbreaks, farm sanitation efficacy and proper milk handling practices (Farnsworth, 1993). According to Pasteurized Milk Ordinance (PMO) standards, TBC of Grade A milk for an individual producer should not exceed 100,000 cfu/mL (FDA, 2001). Elevated numbers of bacteria in milk generally arise from at least one of 4 common sources: dirty teats, soiled equipment, mastitis infections, and poor refrigeration (Blowey et al., 1999; Murphy and Boor, 2000).

Growth characteristics for several bacteria in milk have been reported previously (Lawton and Nelson, 1954; Andrey and Frazier, 1959; Fang et al., 1993). No similar studies have been reported for *S. uberis*. A better understanding of the growth characteristics of *S. uberis* will provide insight into the causes of TBC increases associated with this organism. The objective of this study was to determine experimental time/temperature conditions that allow *S. uberis* growth.

The *S. uberis* isolates shown in Table 1 were selected from a strain collection previously described by Hayes et al. (2001). Briefly, isolates were obtained by spreading bulk tank milk samples (0.1 mL) on modified Edwards medium (Oxoid, Hampshire, England). Plates were incubated at 32°C. Selected isolates were identified with the API 20 Strep test according to the manufacturer's instructions (BioMerieux Vitek, Hazelwood, MO). All *S. uberis* isolates in the strain collection grouped into one of 2 EcoRI ribotypes: 116-520-S-1 or 116-520-S-2 (<http://www.pathogen tracker.net>, accessed August 30, 2003). Ribotyping, based on scoring

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Table 1. Growth of *Streptococcus uberis* at 4, 7, 10, 21, 25, and 32°C in UHT milk.

Isolate	Ribotype	Growth at					
		4°C	7°C	10°C	21°C	25°C	32°C
FSL A3-068	116-520-S-1	—	—	—	+	+	+
FSL A3-092	116-520-S-1	—	—	—	+	+	+
FSL A3-079	116-520-S-2	—	—	+	+	+	+
FSL C1-308	116-520-S-2	—	—	+	+	+	+

rRNA gene restriction fragment polymorphisms, can type bacterial isolates to the genus, species and strain levels (Bruce et al., 1995; Bruce, 1996). To represent different genetic groups among *S. uberis* isolates, we selected 2 isolates from each *EcoRI* ribotype for this study. Isolates were grown overnight in 5 mL of commercial UHT-treated milk incubated at 37°C. Overnight cultures were diluted serially in PBS, and 0.1 mL of the appropriate dilutions were inoculated into 5 mL of UHT milk to give approximately 10^3 to 10^4 cfu/mL of *S. uberis* in each of 6 pairs of tubes. For each isolate, one pair of tubes was incubated for 5 d at each 4.4°C or at 7°C to represent appropriate refrigeration conditions as described in the PMO (FDA, 2001). A third pair of tubes was incubated at 10°C for 5 d to simulate marginally inadequate cooling. Fourth and fifth pairs were incubated at 21 or 25°C for 7 h to simulate ambient temperatures. The last pair was incubated at 32°C for 7 h to simulate elevated temperatures. Daily, 0.1-mL volumes of the cultures from the samples incubated at 4.4, 7, or 10°C were serially diluted in PBS and surface plated in duplicate (0.1 mL) onto brain heart infusion (BHI; Becton Dickinson, Sparks, MD) agar. Samples incubated at 21, 25, or 32°C were plated in duplicate onto BHI at 0 and 7 h postinoculation. Brain heart infusion plates were incubated at 37°C for 24 h prior to enumeration of bacterial colonies. All experiments were repeated twice. Generation times were calculated by the following formula:

$$g = (T \log 2) / (\log b - \log a),$$

where g is generation time, T is the time interval for the growth experiment, a is the initial bacterial number, and b is the bacterial number at the end of the incubation period (e.g., 7 h at 21, 25, or 32°C).

Table 1 summarizes the abilities of the *S. uberis* isolates to grow in UHT milk at different temperatures. None of the 4 isolates grew at 4.4°C or 7°C. Ribotype 116-520-S-2 isolate numbers increased by either 1.5 or 3.5 $\log_{10}$ cfu/mL following 5 d at 10°C (Figure 1). Ribotype 116-520-S-1 isolates did not grow at 10°C. Generation times at 21, 25, and 32°C were determined for all isolates. Ribotype 116-520-S-2 isolate generation times

were shorter than those for ribotype 116-520-S-1 isolates. Generation times were 2.7 ± 0.1 h, 2.10 ± 0.1 h, 1 ± 0.1 h for 116-520-S-1 isolates and 1.8 ± 0.4 h, 1.3 ± 0.3 h, and 0.8 ± 0.1 h for 116-520-S-2 isolates at 21, 25, and 32°C, respectively.

Temperature and duration of milk storage on the farm affect microbial numbers in raw milk (Cousins and Bramley, 1981). According to PMO standards, raw milk for pasteurization must be cooled to 10°C or less within 4 h or less after initiation of the first milking, and to 7°C or less within 2 h after the completion of milking. Blend temperatures of multiple milkings should not exceed 10°C (FDA, 2001). Although *S. uberis* can reproduce in <3 h at temperatures $\geq 21^\circ\text{C}$, we have shown that this organism should not reproduce during storage if PMO standards for bulk tank milk temperature control are followed. If milk were to be produced and stored at the high temperatures used in this study (e.g., 21, 25, or 32°C), *S. uberis* would not necessarily

Figure 1. Growth of *Streptococcus uberis* at 10°C in UHT milk. Mean $\log_{10}$ values (cfu/mL) obtained from duplicate tubes in one experiment are shown. Although absolute bacterial numbers at each time point differed between experiments, in both trials, postinoculation bacterial growth initiated at the same times, yielding similar relative increases in bacterial numbers.

predominate the total microflora, especially if it had been initially present at low numbers in the raw milk, as many different types of organisms also would be capable of reproducing under these conditions. This work has demonstrated that *S. uberis* does not grow at refrigeration temperatures required by the PMO (FDA, 2001). These findings suggest that high levels of *S. uberis* in properly cooled bulk tank milk are indicators of mastitis infection and are unlikely to reflect *S. uberis* growth in the bulk tank.

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