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Growth and gas formation by *Lactobacillus wasatchensis,* a novel obligatory heterofermentative nonstarter lactic acid bacterium, in Cheddar-style cheese made using a *Streptococcus thermophilus* starter[1]

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ABSTRACT

A novel slow-growing, obligatory heterofermentative, nonstarter lactic acid bacterium (NSLAB), *Lactobacillus wasatchensis* WDC04, was studied for growth and gas production in Cheddar-style cheese made using *Streptococcus thermophilus* as the starter culture. Cheesemaking trials were conducted using *S. thermophilus* alone or in combination with *Lb. wasatchensis* deliberately added to cheese milk at a level of ~10^4 cfu/mL. Resulting cheeses were ripened at 6 or 12°C. At d 1, starter streptococcal numbers were similar in both cheeses (~10^9 cfu/g) and fast-growing NSLAB lactobacilli counts were below detectable levels (<10^2 cfu/g). As expected, *Lactobacillus wasatchensis* counts were 3 × 10^5 cfu/g in cheeses inoculated with this bacterium and below enumeration limits in the control cheese. Starter streptococci decreased over time at both storage temperatures but declined more rapidly at 12°C, especially in cheese also containing *Lb. wasatchensis*. Populations of fast-growing NSLAB and the slow-growing *Lb. wasatchensis* reached 5 × 10^7 and 2 × 10^8 cfu/g, respectively, after 16 wk of storage at 12°C. Growth of NSLAB coincided with a reduction in galactose concentration in the cheese from 0.6 to 0.1%. Levels of galactose at 6°C had similar decrease. Gas formation and textural defects were only observed in cheese with added *Lb. wasatchensis* ripened at 12°C. Use of *S. thermophilus* as starter culture resulted in galactose accumulation that *Lb. wasatchensis* can use to produce CO_2, which contributes to late gas blowing in Cheddar-style cheeses, especially when the cheese is ripened at elevated temperature.

Key words: cheese, late blowing, nonstarter lactic acid bacteria, *Streptococcus thermophilus*, galactose

INTRODUCTION

The manufacture of Cheddar cheese is characterized by use of mesophilic *Lactococcus lactis* starter strains and moderate cook temperatures (~39°C; Michel and Martley, 2001). However, the short method for Cheddar cheese manufacture (Bley et al., 1985) uses thermophilic *Streptococcus thermophilus* along with the regular mesophilic *Lc. lactis* ssp. *lactis* or *cremoris* starter culture so that rapid acid production continues even at cook temperatures of 42 to 43°C (Michel and Martley, 2001). Two advantages to using the short method include reduced manufacturing costs and a lower risk of bacteriophage infection (Cogan, 2011).

Unfortunately, this type of manufacturing process has also been linked to accumulation of up to 33 mmol/kg of galactose in cheese (~0.6% wt/wt) and to unwanted CO_2 production by nonstarter lactic acid bacteria (**NSLAB**). This can lead to development of slits and fractures in the aging cheese (Tinson et al., 1982b; Michel and Martley, 2001). Galactose accumulates because only the glucose moiety of lactose is used by *S. thermophilus*, thus galactose is excreted back into the milk or cheese as part of a Lac S-mediated antiport system for lactose uptake (Tinson et al., 1982a; Hutkins and Ponne, 1991; Vaillancourt et al., 2004). Heterofermentative NSLAB are known to use residual galactose to produce CO_2, leading to gassy defect in Cheddar cheese (Radford and Hull, 1982; Tinson et al., 1982b) that results in economic losses to the cheese manufacturer (Golnazarian, 2001).

We recently reported that *Lactobacillus wasatchensis* WDC04—a slow-growing, obligatory heterofermentative (**OHF**) NSLAB of cheese (Oberg et al., 2015)—can use galactose and produce gas in broth (Ortakci et al., 2015a) and in cheese (Ortakci et al., 2015b). When

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[1]In a previous paper (Ortakci et al., 2015) this bacteria was called *Lactobacillus wasatchii.* The accepted name is *Lactobacillus wasatchensis* (Oberg et al., 2015), in homage to the Wasatch mountain range running between Weber State University and Utah State University where this bacterium was first isolated and characterized.
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this NSLAB is present in high numbers in aging cheese it promotes gas production via fermentation of galactose (or other hexoses; Ortakci et al., 2015b). Based on these observations, we hypothesized that growth and gas production by *Lb. wasatchensis* would be promoted if *S. thermophilus* was included as part of a Cheddar cheese starter culture because the cheese would contain higher levels of residual galactose. We have also shown that *Lb. wasatchensis* readily grows on cell lysate material (presumably from the ribose that is released) so that starter culture lysis during cheese storage also promotes growth of *Lb. wasatchensis*. To confirm these findings, we inoculated milk with *Lb. wasatchensis* and then used *S. thermophilus* as a starter culture to make Cheddar-style cheese. Microbial populations, including starter culture, fast-growing NSLAB (which in our creamery is predominantly *Lactobacillus curvatus*), and the slow-growing NSLAB *Lb. wasatchensis* were then enumerated, along with measurements to determine the extent of gas formation, through 23 wk of cheese storage at 6 and 12°C.

MATERIALS AND METHODS

Bacterium and Growth

Working cultures of *Lb. wasatchensis* WDC04 were prepared from frozen stocks stored at −80°C by sequential transfer twice into de Man, Rogosa and Sharpe (**MRS**; Becton Dickinson Inc., Sparks, MD) broth containing 1.5% (wt/vol) ribose (donated by Bioenergy Life Science Inc., Ham Lake, MN; **MRS+R**). Cultures were incubated anaerobically using GasPak EZ (Becton Dickinson Inc.) at 23°C for 40 h. Cells for the cheesemaking experiments were propagated in 400 mL of MRS+R for 40 h at 23°C. Cells were harvested by centrifugation at 7,500 × *g* for 10 min at 4°C, washed twice with sterile 0.1% (wt/vol) peptone water, and harvested after each wash. The cell suspensions were used in the cheesemaking trials. Cell suspension concentrations were determined by spread plate counts on MRS+R agar incubated anaerobically for 5 d at 23°C.

Cheesemaking

Fresh bovine milk was obtained from the George B. Caine Dairy Research and Teaching Center (Wellsville, UT) and transported to the Aggie Creamery at Utah State University (Logan, UT). The milk was standardized to a protein-to-fat ratio of 0.84, pasteurized at 73°C for 15 s, and 136 kg was added into an open stainless steel vat (each vat had previously been cleaned then heat sanitized for 30 min). All vats of milk were warmed to 31°C, and then 0.25 g/kg of frozen pellets containing *S. thermophilus* M6 starter culture (Chr. Hansen Inc., Milwaukee, WI) were added. For the experimental vats, ~10^4 cfu/mL of *Lb. wasatchensis* was also added and the milk allowed to ripen for 10 min. Then, 0.12 mL/kg of a 32% (wt/wt) $CaCl_2$ solution (Nelson-Jameson Inc., Marshfield, WI), 0.13 mL/kg of annatto, and 0.16 mL/kg of double-strength (~650 international milk clotting units/mL) chymosin rennet (Maxiren; DSM Food Specialties USA Inc., Eagleville, PA) were added and the milk allowed to set undisturbed for 20 min. After cutting and healing, the curd and whey mixtures were stirred for 10 min, heated to 39°C over 35 min, and then stirred for another 10 min. Curd was stirred until a curd pH of 6.3 was reached with partial whey drainage. Remaining whey was then drained and curd was allowed to mat together, cut into slabs, and cheddared until the curd pH reached 5.25. Curd was milled and salted (30 g/kg of curd) in 3 applications with 5 min between each application. Salted curd from each vat was separated into two 7-kg portions and placed into open plastic containers. Curd was packed into plastic hoops and pressed overnight (140 kPa, ~18 h, ~20°C). The cheese was then dehooped and each block cut into 10 pieces of ~600 g; each piece was vacuum packaged. Five pieces were stored at 6°C and 5 at 12°C. Control cheeses were made using the same procedure except that no *Lb. wasatchensis* was added. Cheesemaking was conducted in triplicate.

Microbial Enumerations

At 0, 8, 16, and 23 wk, cheese samples (11 g) were collected from the interior of each cheese and homogenized in 99 mL of sterilized 2% (wt/vol) sodium citrate (previously warmed to 45°C) using a Stomacher 400 Circulatory laboratory blender (Seward Laboratory Systems Inc., Bohemia, NY) set for 3 min at 230 rpm (Broadbent et al., 2013). Serial dilutions were prepared in 0.1% sterile peptone water. In this experiment, the lowest dilution used for bacterial enumeration was 10^{-2}. For calculating mean numbers and when making plots of microbial numbers, a value of 5×10^1 cfu/g was used for samples with counts $<10^2$ cfu/g.

Streptococcus thermophilus. *Streptococcus thermophilus* was enumerated as described by Tabasco et al. (2007) using M17 agar (Becton Dickinson Inc.) containing 1% (wt/vol) lactose (Sigma-Aldrich Inc., St. Louis, MO) incubated aerobically at 45°C for 24 h.

Fast-Growing NSLAB. The method of Oberg et al. (2011) for enumerating NSLAB on MRS agar supplemented with 2 μg/mL of vancomycin incubated anaerobically at 37°C for 48 h was used to enumerate