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Late blowing of Cheddar cheese induced by accelerated ripening and ribose and galactose supplementation in presence of a novel obligatory heterofermentative nonstarter *Lactobacillus wasatchensis*[1]

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ABSTRACT

Lactobacillus wasatchensis sp. nov. has been studied for growth and gas formation in a control Cheddar cheese and in cheese supplemented with 0.5% ribose, 0.5% galactose, or 0.25% ribose plus 0.25% galactose using regular and accelerated cheese ripening temperatures of 6 and 12°C, respectively. Milk was inoculated with (1) *Lactococcus lactis* starter culture, or (2) *Lc. lactis* starter culture plus *Lb. wasatchensis* (10^4 cfu/mL). In the control cheese with no added *Lb. wasatchensis*, starter numbers decreased from 10^7 initially to ~10^4 cfu/g over 23 wk of ripening at 6°C. When the cheese was ripened at 12°C, or if *Lb. wasatchensis* was added, the final starter counts were 1 log lower. In contrast, nonstarter lactic acid bacteria in the cheese increased from $<10^2$ cfu/g at press to 10^6 to 10^7 cfu/g after 23 wk, with higher numbers being observed with ripening at 12°C. In cheese with no added *Lb. wasatchensis*, levels of *Lb. wasatchensis* were initially below the enumeration threshold but counts of up to 10^3 cfu/g were detected after 23 wk. When the cheese was inoculated with *Lb. wasatchensis*, it could be enumerated throughout ripening, with final levels at 23 wk being dependent on whether ribose had been added to the cheese curd. With added ribose (with or without added galactose), *Lb. wasatchensis* grew to 10^7 to 10^8 cfu/g after 23 wk, whereas without added ribose it was 1 log lower. In all cheeses with added *Lb. wasatchensis*, greater gas formation was observed at 12°C, with most gas production occurring after ~16 wk. Very little gas production was detected in cheese without added *Lb. wasatchensis* ripened at 12°C or in cheese with added *Lb. wasatchensis* ripened at 6°C. Adding a combination of ribose and galactose caused more gas formation, putatively because of the ability of *Lb. wasatchensis* to co-utilize both sugars and grow to high numbers, and then produce gas from galactose as ribose levels were depleted. Even without sugar supplementation, gas was observed in cheese with added *Lb. wasatchensis* after 16 wk. We also observed that *Lb. wasatchensis* could grow to high cell densities when grown in carbohydrate-restricted broth containing lactococcal cell lysate. This suggests that during cheese ripening, lysis of starter bacteria provides sufficient substrates (such as ribose) to allow growth of *Lb. wasatchensis* and, if fermentable hexose is available, the cheese will become gassy. We conclude that *Lb. wasatchensis* is a previously undetected contributor to late gas formation in Cheddar cheese and the defect is more pronounced when elevated ripening temperatures are used.

Key words: nonstarter, lactic acid bacteria, gas, cheese, starter autolysis

INTRODUCTION

Ripening cheese at elevated temperatures is technically the simplest method to accelerate maturation, and the lower refrigeration costs may provide overall savings to the producer (Wilkinson, 1993; Folkertsma et al., 1996). However, late gas formation ("gassy defect") in Cheddar cheese has been a recurring problem for over 100 yr and this defect continues to be one of the main concerns during accelerated ripening (Van Slyke and Hart, 1903; Mullan, 2000). This defect is manifest as openness of cheese texture (slits or cracks) or "blown wrappers" without any texture change in cheeses aged longer than 3 mo (Laleye et al., 1987). Gassy defect tends to be sporadic and recurrent and has probably been experienced at most cheese-making plants (Mullan, 2000). The slits, cracks, or voids caused by gassy defect in cheese are not usually evident until the cheese

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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 bacteria was first isolated and characterized.
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is graded unless the cheese pack is loose. Even though this textural defect may not create a specific sensory defect, it may result in the inability of a cheese converter to cut the blocks with uniformity and also affects the slicing ability of the cheese. If the defect is severe, the block will crumble upon cutting, which can increase cutting losses from 10% (nondefective) up to 50% (Elliott et al., 1981; Donnely et al., 2014). In addition, the time and money spent on the aging process is lost, as such cheese is downgraded and sold at a lower price (Martley and Crow, 1996; Golnazarian, 2001).

Gassy defect in cheese has been associated with poor sanitation (usually observed as early gas formation) and germination of *Clostridium* spores (late blowing; Fox et al., 1990; McSweeney and Fox, 2004). In properly manufactured Cheddar cheese with a pH <5.4 and salt-in-moisture (**S/M**) content >4.0% (Lawrence et al., 2004), gassiness has been attributed to obligate heterofermentative (**OHF**) nonstarter lactobacilli species such as *Lactobacillus brevis* and *Lactobacillus fermentum* (Sherwood, 1939; Laleye et al., 1987, 1990). It has often been difficult to identify the cause of gassiness, however, and Elliott et al. (1981) linked gassy defect in Cheddar cheese to an unidentified and slow-growing bacterium.

Our group has isolated a novel slow-growing OHF species, *Lactobacillus wasatchensis*, from a "gassy" Cheddar cheese manufactured at Utah State University (Oberg et al., 2015). We reported that *Lb. wasatchensis* produces gas from hexose sugars and can co-utilize ribose and galactose (Ortakci et al., 2015). These are potential substrates for nonstarter lactic acid bacteria (**NSLAB**) in Cheddar cheese (Tinson et al., 1982; Thomas, 1987; Rapposch et al., 1999; Michel and Martley, 2001). We also showed that *Lb. wasatchensis* is able to grow under salt and pH conditions typical of Cheddar cheese (4 to 5% S/M, ~pH 5.2), and that there is potential for some survival of *Lb. wasatchensis* during HTST pasteurization (Ortakci et al., 2015).

The ability of *Lb. wasatchensis* to readily utilize both ribose and galactose, to grow at cheese ripening temperatures, and to survive in the relatively harsh environment of cheese led us to hypothesize that *Lb. wasatchensis* is a contributor to late gas blowing and textural defects in Cheddar cheese (Ortakci et al., 2015). The aims of this work were to confirm that gas formation occurs in Cheddar cheese when *Lb. wasatchensis* is present, and to examine the effect of temperature (6 or 12°C) and substrate availability (ribose and galactose) on this phenomenon. Using these experimental parameters, we monitored the microbiota of the cheese for *Lb. wasatchensis*, starter lactococci, and NSLAB, as well as gas formation throughout 23 wk of ripening.

MATERIALS AND METHODS

Bacteria and Growth

Working cultures of *Lb. wasatchensis* WDC-04 (Oberg et al., 2015) 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 (**R**; donated by Bioenergy Life Science Inc., Ham Lake, MN), in which the cultures were incubated anaerobically using GasPak EZ (Becton Dickinson Inc.) at 23°C for 40 h. Cells for the cheese-making experiments were propagated in 400 mL of MRS+R for 40 h at 23°C. Cells were harvested by centrifugation at 6,000 × *g* for 10 min at 4°C, washed twice with sterile 0.1% (wt/vol) peptone water, and then collected again by centrifugation. Concentration of cell suspensions was determined by anaerobic spread plate counts on MRS+R agar after 5 d at 23°C. The cell suspensions were subsequently used in cheese-making experiments after proper dilutions were made to reach desired numbers in cheese milk.

Frozen pellets of *Lactococcus lactis* ssp. *lactis/cremoris* (DVS850, Chr. Hansen Inc., Milwaukee, WI) suspended in peptone water (optical density at 600 nm, $\mathbf{OD_{600}}$, of ~0.9) was inoculated into 750 mL of M17 broth (Becton Dickinson Inc.) supplemented with 1% lactose (**M17-L**; Sigma-Aldrich Inc., St. Louis, MO) and incubated at 30°C for 24 h under aerobic conditions. The cells were then harvested by centrifugation (7,500 × *g*; 10 min; 4°C), washed twice in 50 mL of sterile phosphate buffer and then suspended in 10 mL of sterile phosphate buffer. A 0.1-mL aliquot was diluted and plated on M17-L agar to enumerate starter numbers (Branen and Keenan, 1969; Thomas, 1987; Rapposch et al., 1999). The cell suspensions were stored at −80°C until used in cell-free extract experiments.

Cheese Making

Fresh bovine milk was obtained from the George B. Caine Dairy Research and Teaching Center (Wellsville, UT) and transported to the Gary Haight Richardson Dairy Products Laboratory at Utah State University (Logan). The milk was standardized to a protein-to-fat ratio of 0.84 and pasteurized at 73°C for 15 s; then, 273 kg was transferred into each of 2 open stainless steel vats (which had previously been cleaned and heat sanitized for 30 min). Both batches of milk were warmed to 31°C, and then 0.2 g/kg of frozen pellets containing *Lc. lactis* ssp. *lactis/cremoris* starter culture DVS850 were added. To one of the vats, 10^4 cfu/mL of *Lb.*