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Growth and gas production of a novel obligatory heterofermentative Cheddar cheese nonstarter lactobacilli species on ribose and galactose

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ABSTRACT

An obligatory heterofermentative lactic acid bacterium, *Lactobacillus wasatchii* sp. nov., isolated from gassy Cheddar cheese was studied for growth, gas formation, salt tolerance, and survival against pasteurization treatments at 63°C and 72°C. Initially, *Lb. wasatchii* was thought to use only ribose as a sugar source and we were interested in whether it could also utilize galactose. We conducted experiments to determine the rate and extent of growth and gas production in carbohydrate-restricted (CR) de Man, Rogosa, and Sharpe (MRS) medium under anaerobic conditions with various combinations of ribose and galactose at 12, 23, and 37°C, with 23°C being the optimum growth temperature of *Lb. wasatchii* among the three temperatures studied. When *Lb. wasatchii* was grown on ribose (0.1, 0.5, and 1%), maximum specific growth rates (μ_{max}) within each temperature were similar. When galactose was the only sugar, compared with ribose, μ_{max} was 2 to 4 times lower. At all temperatures, the highest final cell densities (optical density at 640 nm) of *Lb. wasatchii* were achieved in CR-MRS plus 1% ribose, 0.5% ribose and 0.5% galactose, or 1% ribose and 1% galactose. Similar μ_{max} values and final cell densities were achieved when 50% of the ribose in CR-MRS was substituted with galactose. Such enhanced utilization of galactose in the presence of ribose to support bacterial growth has not previously been reported. It appears that *Lb. wasatchii* co-metabolizes ribose and galactose, utilizing ribose for energy and galactose for other functions such as cell wall biosynthesis. Co-utilization of both sugars could be an adaptation mechanism of *Lb. wasatchii* to the cheese environment to efficiently ferment available sugars for maximizing metabolism and growth. As expected, gas formation by the heterofermenter was observed only when galactose was present in the medium. Growth experiments with MRS plus 1.5% ribose at pH 5.2 or 6.5 with 0, 1, 2, 3, 4, or 5% NaCl revealed that *Lb. wasatchii* is able to grow under salt and pH conditions typical of Cheddar cheese (4 to 5% salt-in-moisture, pH ~5.2). Finally, we found that *Lb. wasatchii* cannot survive low-temperature, long-time pasteurization but survives high-temperature, short-time (HTST) laboratory pasteurization, under which a 4.5 log reduction occurred. The ability of *Lb. wasatchii* to survive HTST pasteurization and grow under cheese ripening conditions implies that the presence of this nonstarter lactic acid bacterium can be a serious contributor to gas formation and textural defects in Cheddar cheese.
Key words: nonstarter lactic acid bacteria, late blowing, ribose, cofermentation

INTRODUCTION

Lactic acid bacteria (**LAB**) present in ripening cheese include deliberately added starter LAB and a variety of adventitious LAB referred to as nonstarter LAB (**NSLAB**). The NSLAB gain access to cheese through the milk or processing environment (Naylor and Sharpe, 1958; Peterson and Marshall, 1990; Martley and Crow, 1993; Somers et al., 2001).

The predominant NSLAB in Cheddar cheese are facultative heterofermentative (**FHF**) lactobacilli and, less frequently, pediococci or obligatory heterofermentative (**OHF**) lactobacilli (Jordan and Cogan, 1993; Crow et al., 2001; Banks and Williams, 2004). Presence of OHF lactobacilli are a particular concern because these microbes may promote the development of undesirable flavor and body defects including gas formation in Cheddar cheese (Dacre, 1953; Laleye et al., 1987; Khalid and Marth, 1990). Unwanted gas formation in Cheddar cheese is a recurrent and widespread problem in the dairy industry that has probably affected most cheese plants (Mullan, 2000). Our group recently isolated a new *Lactobacillus* species from a "gassy" Cheddar cheese after incubation on de Man, Rogosa, and Sharpe (**MRS**) agar for 35 d at 6°C. This bacterium was designated *Lactobacillus wasatchii* sp. nov. (our unpublished

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data; GenBank accession number: AWTT00000000 as *Lactobacillus* spp. WDC04).

Lactobacillus wasatchii is an OHF species and therefore uses the pentose phosphate pathway (**PP**) to generate energy from pentose and hexose sugars. Its preferred sugar is ribose, although hexoses such as galactose are also a potential energy source in cheese. More importantly, hexose sugars can be fermented by OHF to lactate, acetate or ethanol plus CO_2, making *Lb. wasatchii* a potential contributor to gassy defect in Cheddar cheese.

This study examined growth characteristics of *Lb. wasatchii* with respect to ribose and galactose utilization, gas formation, tolerance to the salt and pH values found in Cheddar cheese, and its ability to survive pasteurization treatments. To our knowledge, this is the first report on growth and gas formation of a slow-growing OHF lactobacillus species isolated as an NSLAB from a "gassy" Cheddar cheese.

MATERIALS AND METHODS

Materials

Lactobacilli MRS broth, proteose peptone, polypeptone, beef extract, yeast extract, GasPak EZ gas-generating pouches, and agar were purchased from Becton Dickinson and Co. (Sparks, MD); ribose was donated by Bioenergy Life Science Inc. (Ham Lake, MN), UHT milk was from Gossner Foods Inc. (Logan, UT), Tween-80 and bromocresol purple were from Sigma-Aldrich Inc. (St. Louis, MO), dipotassium phosphate was from Fisher Scientific Inc. (Fair Lawn, NJ), sodium acetate trihydrate and diammonium citrate were from Mallinckrodt Baker Inc. (Paris, KY), galactose, and triammonium citrate were from Alfa Aesar Inc. (Ward Hill, MA), and magnesium sulfate was from Alfa Aesar Inc. (Heysham, UK).

A carbohydrate-restricted version of MRS (**CR-MRS**) was prepared by omitting glucose from the MRS broth formula. To 2 L of deionized water was added 20.0 g of proteose peptone No. 3, 20.0 g of beef extract, 10.0 g of yeast extract, 2.0 g of Tween-80, 4.0 g of ammonium citrate, 10.0 g of sodium acetate, 0.2 g of magnesium sulfate, 0.1 g of manganese sulfate, and 4.0 g of dipotassium phosphate. The CR-MRS was supplemented with different levels of ribose and galactose to study the growth properties of *Lb. wasatchii*.

Bacterium and Growth

Stock cultures of *Lb. wasatchii* were maintained at −80°C in MRS broth supplemented with 1.5% ribose (**MRS+R**) and 10% glycerol. Working cultures was prepared by 2 successive transfers into 10 mL of MRS+R broth, with anaerobic incubation using GasPak EZ at 23°C for 40 h after each transfer. Growth of *Lb. wasatchii* was evaluated by inoculation of the working culture into 10 mL of CR-MRS broth acidified to pH 5.20 with HCl and supplemented with 0.1% galactose or ribose, 0.5% galactose or ribose, 1.0% galactose or ribose, or a 0.50:0.50 combination or 2.0% sugar (1% ribose plus 1% galactose). Optical density of the cell suspensions were followed at 640 nm ($\mathbf{OD_{640}}$) after inoculation and every 12 h thereafter at 12, 23, or 37°C and during anaerobic incubation in jars containing GasPak EZ. Maximum specific growth rate ($\boldsymbol{\mu_{max}}$) was calculated as the slope of the steepest linear portion of the growth rate curves. Broth samples containing Durham tubes were similarly prepared, inoculated, and incubated to test for gas production. Working cultures were prepared in duplicate to conduct growth curves and gas formation experiments.

To test NaCl tolerance of *Lb. wasatchii* at pH 5.2 or 6.5, *Lb. wasatchii* working cultures were prepared in triplicate and inoculated into MRS+R broth containing 0, 1, 2, 3, 4, or 5% (wt/wt) NaCl. Growth at 23°C under anaerobic conditions was followed by spectrophotometrical (OD_{600}) measurements every 8 h until the stationary phase was reached.

Thermotolerance

The ability of *Lb. wasatchii* to withstand pasteurization treatment was assayed by heating 9.9 mL of UHT milk to 63°C and 72°C in sterile polypropylene tubes. Once the desired temperature was reached, each tube was inoculated with 0.1 mL of *Lb. wasatchii* working culture (prepared in triplicate) containing ~6×10^8 cfu/mL and the samples held at 63°C and 72°C for 30 min or 15 s, respectively. Samples were then placed in a 31°C water bath (the set temperature commonly used for making Cheddar cheese) for 2 h. These treatments were designed to mimic the HTST continuous pasteurization used in large-scale cheese operations and the low-temperature, long-time (**LTLT**) batch pasteurization often used by small-scale artisan cheese makers. Samples were then plated on MRS+R agar in duplicate and incubated at 23°C anaerobically for 5 d.

Statistical Analysis

Statistical analysis of the effect of different temperature, sugar, pH, and NaCl treatments on μ_{max} and final cell density of *Lb. wasatchii* were performed using PROC GLM in SAS (version 9.1, SAS Institute, Cary, NC), and differences between means were determined