Galactose-positive adjunct cultures prevent gas formation by *Paucilactobacillus wasatchensis* WDC04 in a model gas production test

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ABSTRACT

Gas production by obligatory heterofermentative lactic acid bacteria such as *Paucilactobacillus wasatchensis* is a sporadic problem in Cheddar cheese and results in undesired slits and cracks in the cheese. Growth of *Pa. wasatchensis* is not rapid, which makes investigations of gas production difficult to consistently execute. A primary objective of this study was to develop a model gas production test that could be used to investigate the effect of galactose and ribose utilization on gas production by *Pa. wasatchensis* and determine whether galactose-fermenting adjunct cultures could prevent gas formation. *Paucilactobacillus wasatchensis* WDC04 was inoculated at 10^1 to 10^6 cfu/mL into carbohydrate-restricted MRS broth containing different ribose and galactose levels and incubated for up to 21 d at 23°C. Gas production in the broth was detected using a Durham tube inverted on a 6-cm-long capillary tube; cells were enumerated at 4, 8, and 12 d; and residual galactose was also measured. Gas production was sporadic except for when 10^5 cfu/mL of *Pa. wasatchensis* WDC04 was inoculated into broth containing 0.3% ribose and 0.7% galactose. In those tubes, gas production was consistently observed after 8-d incubation, by which time galactose levels had decreased to 0.15%. Co-inoculation of *Pa. wasatchensis* WDC04 with as few as 10^3 cfu/mL of a lactose-negative galactose-positive adjunct culture (*Pediococcus acidilactici* 23F, *Lactocaseibacillus paracasei* UW4, or *Lactobacillus helveticus* 7995) resulted in galactose depletion by d 4 and no observable gas production by d 12. With less galactose available to the slower-growing *Pa. wasatchensis* WDC04, its growth was limited to 10^8 cfu/mL when any of the adjunct

cultures was co-inoculated, compared with 10^9 cfu/mL when grown on its own. We concluded that galactose-fermenting adjunct cultures have potential for preventing unwanted gas production in cheese by competition for resources and especially by removing the 6-carbon galactose before it can be utilized for energy by an obligatory heterofermentative lactobacilli such as *Pa. wasatchensis* and produce carbon dioxide.

Key words: cheese, nonstarter lactic acid bacteria, unwanted gas production, galactose

INTRODUCTION

In bacterial ripened cheeses such as Cheddar, the lactic acid bacteria (LAB) are separated into 2 distinct groups: starter LAB, which are bacteria purposely added by the producer during the cheesemaking process, and nonstarter LAB (NSLAB), which are not intentionally added and initially are present at low cell numbers ($<10^2$ cfu/g) compared with starter LAB, which are initially at 10^7 to 10^9 cfu/g (Broadbent et al., 2003). The NSLAB enter the cheese because they either survive pasteurization or are introduced post-pasteurization from the dairy plant environment and often become the predominant bacteria in Cheddar cheese by the time it is ready to be sold (Oberg et al., 2011; McMahon et al., 2014). Some NSLAB are viewed as positive or desired additions to the cheese microbiota, but others are associated with defects such as slits and cracks, off-flavors, and crystal formation in Cheddar cheese (Crow et al., 1995; Broadbent et al., 2003). The positive effects of some NSLAB on cheese quality has led to widespread use of adjunct cultures in starter LAB microbiology. These strains are used to improve cheese consistency, increase flavor intensity, provide unique flavors, inhibit other NSLAB, produce probiotic cheeses, accelerate ripening, improve aroma, and reduce bitterness (Wilkinson, 1993; Fox et al., 1998; Oberg et al., 2011). Such adjuncts are often isolated from the NSLAB populations of good-quality cheese.

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Paucilactobacillus wasatchensis, formerly designated *Lactobacillus wasatchensis*, is an obligatory heterofermentative NSLAB that causes late gas formation in aging Cheddar cheese (Ortakci et al., 2015b; Oberg et al., 2016). The ability of *Pa. wasatchensis* to utilize galactose by the heterofermentative phosphoketolase pathway results in CO₂ production when its preferential carbohydrate source, ribose, is not available (Ortakci et al., 2015a).

Traditionally, Cheddar cheese is made using *Lactococcus lactis* as the starter culture, with a cook temperature of about 39°C (Michel and Martley, 2001). Alternatively, a “short method” for producing Cheddar cheese can be used, in which the rate of lactic acid production is increased by including a thermophilic LAB, *Streptococcus thermophilus*, along with the *L. lactis* culture (Bley et al., 1985; Michel and Martley, 2001). Addition of *S. thermophilus* maintains faster acid production during and after cooking rather than the slowdown that occurs with *L. lactis*. Using *S. thermophilus* also provides the benefit of not being vulnerable to *L. lactis* bacteriophages (Michel and Martley, 2001). However, the majority of *S. thermophilus* strains do not utilize the galactose moiety of lactose but, rather, export it using an antiporter that brings fresh lactose into the cell. This results in galactose accumulating in the cheese (Hutkins and Ponne, 1991).

Tinson et al. (1982) observed that Cheddar cheese made with *L. lactis* ssp. *cremoris* starter and *S. thermophilus* adjunct cultures resulted in higher levels of residual galactose (0.56% wt/wt) and CO₂ production. Ortakci et al. (2015c) added *Pa. wasatchensis* WDC04 to Cheddar cheese made with *S. thermophilus* as a starter culture and observed gas formation when the cheese was ripened at elevated temperature (12°C).

A challenge in studying gas production by *Pa. wasatchensis* is that it requires both growth to high numbers and utilization of galactose for energy production by the cell. When grown with galactose as the only carbohydrate, *Pa. wasatchensis* has very limited growth (Ortakci et al., 2015a); whatever CO₂ is formed is insufficient to exceed its solubility in water in a test tube, and no bubbles are observed in Durham tubes. In contrast, with ribose as the carbohydrate, *Pa. wasatchensis* grows to high numbers (~10⁹ cfu/mL) but, because it is already a 5-carbon sugar, no CO₂ is produced. According to Ortakci et al. (2015a), when both ribose and galactose are present, they are co-utilized, with ribose being used for energy and galactose for other cellular needs, which also results in a lack of CO₂ production. An objective of this research was to develop a gas production test (GPT) that could be used to study the conditions under which *Pa. wasatchensis* produces CO₂. A further objective was to then use the GPT to deter-

mine whether an additional culture that would ferment galactose (Gal⁺) but not lactose (Lac⁻) would prevent gas formation by *Pa. wasatchensis*.

MATERIALS AND METHODS

Cultures

***Paucilactobacillus wasatchensis*.** *Paucilactobacillus wasatchensis* WDC04 was obtained from the culture collection at Weber State University (Ogden, UT), grown in lactobacilli de Man, Rogosa, and Sharpe broth (MRS; Hardy Diagnostics) supplemented with 1% ribose (wt/vol; MRS+R; Sigma Aldrich Inc.), and incubated at 23°C for 48 to 72 h. These stock cultures were frozen and stored at -80°C until needed. Working cultures were prepared by inoculating thawed stock solution into MRS+R broth, incubating at 23°C for 72 h, and then diluting to an optical density at 600 nm (OD₆₀₀) of 1.3, which had been determined to be the OD₆₀₀ indicating a cell concentration of 10⁹ cfu/mL (Supplemental Figure S1, <https://doi.org/10.6084/m9.figshare.14488896.v1>). Enumeration of *Pa. wasatchensis* WDC04 was performed on MRS+R agar plates incubated at 23°C for 48 h in a Gaspak EZ system (Becton Dickinson).

Protective Cultures. Three Gal⁺ Lac⁻ protective cultures, *Lactocaseibacillus* (*La.*) *paracasei* UW4, *Lactobacillus* (*Lb.*) *helveticus* 7995, and *Pediococcus* (*Pd.*) *acidilactici* 23F, were selected from the culture collections at Utah State University (Logan, UT) or Weber State University based on carbohydrate fermentation patterns from API50 CH panels (bioMérieux Inc.). Carbohydrate fermentation profiles for the protective cultures confirmed they all fermented glucose and galactose but not lactose. *Lactocaseibacillus paracasei* UW4 and *Pd. acidilactici* 23F were also able to utilize ribose and *N*-acetylglucosamine. Stock cultures were prepared by propagation in MRS broth at 30°C for 48 h, frozen, and stored at -80°C until needed. Working cultures were prepared from thawed stock culture and inoculated into MRS broth supplemented with 1% galactose (MRS+G; Sigma Aldrich Inc.), and incubated at 30°C for 10 h (previously determined to be the time when cell concentration reached 10⁹ cfu/mL). Protective cultures were enumerated by plating on MRS+G agar and incubating anaerobically at 30°C for 48 h.

Model Gas Production System

Carbohydrate-restricted MRS (CR-MRS) broth was prepared as described in McMahon et al. (2020). Stock 10% (wt/vol) solutions of galactose and ribose were filter sterilized and stored at 22°C. Test solutions

containing 1% (wt/vol) sugars were prepared by adding ribose and galactose to CR-MRS broth at levels of 0.5% ribose + 0.5% galactose, 0.4% ribose + 0.6% galactose, 0.3% ribose + 0.7% galactose, 0.2% ribose + 0.8% galactose, and 0.1% ribose + 0.9% galactose. Half of the test tubes contained a Durham tube (Fisher Scientific) inverted onto a 6-cm-long capillary tube (Drummond Scientific Company; Figure 1) that was added before autoclaving. Test solutions (with and without Durham tubes) were inoculated with *Pa. wasatchensis* WDC04 at 10^1 , 10^2 , 10^3 , 10^4 , 10^5 , 10^6 , or 10^7 cfu/mL on 3 different occasions. All of the test samples were incubated at 23°C. Test tubes with Durham tubes were observed for gas production over a 21-d period, and OD₆₀₀ was measured in test tubes without a Durham tube. After

this initial trial, the most consistent treatment combinations for producing a gas bubble were repeated an additional 5 times (on separate days). The combination of ribose + galactose concentration and *Pa. wasatchensis* WDC04 inoculum that produced a gas bubble 8 out of 8 times was selected as the GPT.

To explore the effects of protective cultures on gas production, each protective culture was added to the GPT at 10^3 , 10^4 , 10^5 , 10^6 , or 10^7 cfu/mL. This was performed as biological replicates on 3 different days. Test tubes containing CR-MRS with 0.3% ribose and 0.7% galactose with and without Durham tubes were inoculated with *Pa. wasatchensis* WDC04 and then with the protective culture; then growth (OD₆₀₀) and gas production were monitored daily. Plate counts were performed on d 4, 8, and 12 by serial dilution and plating on MRS+R and MRS+G agars. Control GPT tubes were inoculated with *Pa. wasatchensis* WDC04 only.

Galactose Analysis

Galactose levels in the GPT tubes were measured enzymatically (LACGAR; Megazyme Ltd.) according to kit instructions. Samples were collected at 1, 4, 8, and 12 d and diluted 20-fold before measurement.

Statistical Analysis

Differences in growth curves as a result of adding a protective culture were analyzed using a random-effects model. The remaining experiments were conducted using a nested factorial design. Statistical analysis was performed using Tukey HSD in JMP (version 14; SAS Institute Inc.).

RESULTS AND DISCUSSION

Gas Production Test

When gas bubbles in the Durham tubes did form, they were generally observed between d 8 and d 16. In the initial trial, many of the treatments (with various levels of ribose, galactose, and *Pa. wasatchensis* WDC04 inoculation) had gas formation observed in 1 or 2 of the broth samples but not in all 3 (Table 1). Gas production was more frequently observed when higher (i.e., $\geq 10^5$ cfu/mL) numbers of *Pa. wasatchensis* WDC04 were added, except at the lowest ribose level (0.1% ribose, 0.9% galactose), in which no gas was observed at any inoculation level (Table 1). This was attributed to only minimal growth by *Pa. wasatchensis* WDC04 with galactose, as previously shown (Ortakci et al., 2015; McMahon et al., 2020). If ribose concentration

Figure 1. Test tube containing an inverted Durham tube resting on top of a 60-mm-long capillary tube so as to increase volume beneath the Durham tube from which gas could be collected.

Table 1. Number of times gas production was observed in 3 independent replicate tubes of CR-MRS¹ broth containing a total of 1% ribose and galactose at different ratios and with different inoculum levels of *Paucilactobacillus wasatchensis* WDC04²

Sugar ratio		Inoculation level (cfu/mL)						
Ribose	Galactose	10 ¹	10 ²	10 ³	10 ⁴	10 ⁵	10 ⁶	10 ⁷
50	50	0	1	2	0	2	2	2
40	60	2	2	2	2	2	3	2
30	70	0	2	2	2	3	3	2
20	80	0	0	1	1	3	2	2
10	90	0	0	0	0	0	0	0

¹Carbohydrate-restricted lactobacilli de Man, Rogosa, and Sharpe broth, with ribose and galactose as the only added sugars.

²Incubation was performed at 23°C.

was at least 0.2% (with 0.8% galactose), gas production was observed for inoculum levels of $\geq 10^3$ cfu/mL. The inconsistency of observable gas production in the test medium is reflective of the sporadic nature of unwanted gas production in actual cheese during ripening. The most consistent gas production (i.e., gas present in all 3 replicates) occurred at inoculum levels of 10^5 or 10^6 cfu/mL and ribose levels of 0.2 to 0.4% (along with 0.8 to 0.6% galactose, respectively; Table 1).

In the follow-up trial, the most rapid growth of *Pa. wasatchensis* WDC04 occurred with an inoculum level of 10^6 cfu/mL in CR-MRS containing 0.4% ribose and 0.6% galactose (reaching OD₆₀₀ of 1.3 in 3 d; Figure 2),

but gas bubbles were not always produced (Table 2). The combinations using inoculum levels of 10^5 or 10^6 cfu/mL in CR-MRS containing 0.3% ribose and 0.7% galactose both reached OD₆₀₀ of 1.3 in 5 d. Growth was slowest (requiring 10 d to reach OD₆₀₀ of 1.3) with the combination of a 10^5 cfu/mL inoculum and sugar levels of 0.2% ribose and 0.8% galactose. The only combination that always (8 out of 8 times) showed gas production was an inoculum level of 10^5 cfu/mL and sugar content of 0.3% ribose and 0.7% galactose. This CR-MRS broth formulation was designated as the gas production broth and, in combination with an inoculation of 10^5 cfu/mL *Pa. wasatchensis* WDC04, was selected as GPT.

Figure 2. Growth at 23°C of *Paucilactobacillus wasatchensis* WDC04 inoculated at varying levels into carbohydrate-restricted de Man, Rogosa, and Sharpe broth containing 1% sugar consisting of varying ratios of ribose:galactose, as follows: (■) 20:80 ratio with 10^5 cfu/mL, (▲) 30:70 ratio with 10^5 cfu/mL, (●) 30:70 ratio with 10^6 cfu/mL, and (◆) 40:60 ratio with 10^6 cfu/mL based on optical density at 600 nm (OD₆₀₀). Data reported as the mean $\pm$ SE of 5 independent replicates.

Table 2. Time (d) during incubation at 23°C before gas production by *Paucilactobacillus wasatchensis* WDC04 was observed in carbohydrate-restricted de Man, Rogosa, and Sharpe broth formulations (5 independent replicates) that showed the most consistent results in preliminary trials (Table 1)

Replicate	Ribose:galactose (inoculum level, cfu/mL)			
	20:80 (10^5)	30:70 (10^5)	30:70 (10^6)	40:60 (10^6)
1	— ¹	7	9	6
2	16	6	6	8
3	6	6	6	6
4	7	6	7	9
5	—	7	—	—

¹—: no gas formation observed over 21 d of incubation.

Growth and Interaction of WDC04 and Protective Cultures in GPT

When *Pa. wasatchensis* WDC04 was grown using the GPT, exponential growth was observed through 4-d incubation at 23°C and then slower growth over the next 4 d to an OD $\sim$ 1.5, followed by a slight decrease (Figure 3). Based on prior correlations of OD₆₀₀, the cell numbers would be predicted to be 10^9 cfu/mL (see Supplemental Figure S1). Gas bubbles were observed in the Durham tube starting on d 6 (Table 2) and at least by d 8, when growth appeared to reach a plateau. This growth is fueled by ribose along with co-utilization of galactose (Ortakci et al., 2015a), and growth slows down as ribose is exhausted and the cell needs to use galactose for energy production (McMahon et al., 2020). To be used for energy production, galactose is converted into 6-P-gluconate, and then a molecule of CO₂ is released to produce ribulose-5-P, which can then be metabolized through the pentose phosphate pathway.

When grown in the gas production broth (CR-MRS + 0.3% ribose + 0.7% galactose), the protective cultures all grew faster than *Pa. wasatchensis* WDC04 and reached maximum OD₆₀₀ ($\sim$ 2.5) within 2 to 4 d, depending on the initial inoculation level (Figure 4). Plate counting was performed every 4 d, and by d 4 all the protective cultures had reached 10^9 cfu/mL. This level was maintained for *La. paracasei* UW4, but for *Pd. acidilactici* 23F and *Lb. helveticus* 7995 the cell numbers declined to $\sim$ 10^8 cfu/mL by d 12 (Figure 5).

When a protective culture was grown along with *Pa. wasatchensis* WDC04 as part of the GPT, less growth of both bacteria occurred. Each protective culture was at consistently lower numbers at d 4 when grown with *Pa. wasatchensis* WDC04, regardless of the inoculum level used, and cell numbers reached only $\sim$ 10^8 cfu/mL (Figure 5). Having to compete for resources with the protective cultures also reduced the growth of *Pa. wasatchensis* WDC04 ($P < 0.05$), which reached only $\sim$ 5×10^7 cfu/mL, compared with 1×10^9 cfu/mL when grown on its own (Table 3).

Figure 3. Growth at 23°C of *Paucilactobacillus wasatchensis* WDC04 when inoculated at 10^5 cfu/mL into gas production broth (carbohydrate-restricted de Man, Rogosa, and Sharpe broth containing 0.3% ribose and 0.7% galactose) monitored by optical density at 600 nm (OD₆₀₀), showing first observation of gas bubble (arrow). Data reported as the mean $\pm$ SE of 3 independent replicates.

Figure 4. Growth measured daily using optical density at 600 nm (OD₆₀₀) of protective cultures (A) *Pediococcus acidilactici* 23F, (B) *Lactobacillus helveticus* 7995, and (C) *Lactocaseibacillus paracasei* UW4 in gas production broth (carbohydrate-restricted de Man, Rogosa, and Sharpe broth containing 0.3% ribose and 0.7% galactose) at 23°C and inoculated at levels of 10⁵ cfu/mL on its own (●) or inoculated at 10³ (■), 10⁴ (▲), 10⁵ (◆), 10⁶ (★), or 10⁷ (▼) cfu/mL along with 10⁵ cfu/mL of *Paucilactobacillus wasatchensis* WDC04. Data reported as the mean ± SE of 3 independent replicates.

Figure 5. Growth measured every 4 d using plate counting of protective cultures (A) *Pediococcus acidilactici* 23F, (B) *Lactobacillus helveticus* 7995, and (C) *Lactocaseibacillus paracasei* UW4 in gas production broth (carbohydrate-restricted de Man, Rogosa, and Sharpe broth containing 0.3% ribose and 0.7% galactose) at 23°C and inoculated at levels of 10^5 cfu/mL on its own (●) or inoculated at 10^3 (■), 10^4 (▲), 10^5 (◆), 10^6 (★), or 10^7 (▼) cfu/mL along with 10^5 cfu/mL of *Paucilactobacillus wasatchensis* WDC04. Data reported as the mean $\pm$ SE of 3 independent replicates.

Table 3. Mean numbers (cfu/mL) of *Paucilactobacillus wasatchensis* WDC04 grown in carbohydrate-restricted de Man, Rogosa, and Sharpe broth containing 0.3% ribose and 0.7% lactose in the absence or presence of a protective culture during incubation over 12 d at 23°C from 3 independent replicates

Protective culture	Incubation time			
	0 d	4 d	8 d	12 d
Control ¹	1.5×10^5 ^a	1.9×10^9 ^a	3.2×10^9 ^a	1.2×10^9 ^a
23F ²	1.8×10^5 ^a	1.5×10^8 ^c	6.8×10^7 ^b	4.4×10^7 ^c
UW4 ³	2.3×10^5 ^a	2.8×10^8 ^{bc}	6.0×10^7 ^b	5.2×10^7 ^{bc}
7995 ⁴	1.8×10^5 ^a	3.2×10^8 ^b	2.5×10^7 ^b	4.6×10^7 ^c

^{a-c}Means in the same column with different letters were significantly different; $\alpha = 0.05$.

¹No protective culture added.

²*Pediococcus acidilactici* 23F.

³*Lactocaseibacillus paracasei* UW4.

⁴*Lactobacillus helveticus* 7995.

Influence of Protective Cultures on Gas Production by *Pa. wasatchensis* WDC04

When any of the protective cultures were added to the GPT, no gas production was observed, even at the lowest inoculum level (10^3 cfu/mL). Utilization of galactose by the protective cultures can reduce the growth rate of *Pa. wasatchensis* so that less galactose is available to participate in exponential growth via co-utilization with ribose, and it removes the availability of any hexoses that can result in CO₂ production.

Adding any of the protective cultures significantly ($P < 0.05$) reduced the growth of *Pa. wasatchensis* WDC04 by d 4 and through 12 d of incubation (Table 3). Protective culture *Pd. acidilactici* 23F had the largest effect, with *Pa. wasatchensis* WDC04 counts only reaching 1.5×10^8 cfu/mL by d 4, compared with 1.9×10^9 cfu/mL when grown its own. Both *Pd. acidilactici*

23F and *La. paracasei* UW4 have the ability to ferment ribose (as shown by API50 CH panels), and this could also contribute to lower growth of *Pa. wasatchensis* WDC04 as well as depletion of galactose. In contrast, *Lb. helveticus* 7995 cannot ferment ribose, and it had the least effect on growth of *Pa. wasatchensis* WDC04 (Table 3).

In the GPT when *Pa. wasatchensis* WDC04 is the only bacteria (inoculated at 10^5 cfu/mL), the galactose level stayed at its initial mean ($\pm$ SE) level of 0.66% ($\pm 0.18\%$) for 1 d, then steadily decreased to 0.15% by d 8 before tapering more slowly to 0.01% at d 12 (Figure 6). This period from d 1 to d 8 corresponds with the time of exponential growth for *Pa. wasatchensis* WDC04 (Figure 3) and, when no gas production was observed, supports the finding of Ortakci et al. (2015a) that galactose is co-metabolized with ribose when they are present together. Ribose is primarily utilized for ATP production via the pentose phosphate pathway, whereas galactose is directed toward biosynthesis of peptidoglycans and other cellular macromolecules (Ortakci et al., 2015a).

Once ribose is depleted, *Pa. wasatchensis* must shuttle some galactose through the heterofermentative phosphoketolase pathway (which results in CO₂ production) to produce ribulose-5-P that can then be used to generate energy. Galactose consumption was essentially linear between d 2 to 8, by which time *Pa. wasatchensis* WDC04 achieved maximum biomass, and ribose would have been depleted sometime before d 8 (actual ribose levels were not measured). More gradual galactose utilization after d 8 likely reflects the need for maintaining energy in the cells. When any of the protective cultures were included in the GPT, galactose was depleted and completely consumed by d 4 (Figure 7), even with the lowest inoculum level of 10^3 cfu/mL. At the highest inoculum level (10^7 cfu/mL), galactose depletion was rapid, with about half consumed by d 1.

Figure 6. Galactose concentration in gas production broth (carbohydrate-restricted de Man, Rogosa, and Sharpe broth containing 0.3% ribose and 0.7% galactose) inoculated with 10^5 cfu/mL *Paucilactobacillus wasatchensis* WDC04 and incubated at 23°C. Data reported as the mean $\pm$ SE of 3 independent replicates.

Figure 7. Galactose concentration in gas production broth (carbohydrate-restricted de Man, Rogosa, and Sharpe broth containing 0.3% ribose and 0.7% galactose) inoculated with adjunct cultures (A) *Pediococcus acidilactici* 23F, (B) *Lactobacillus helveticus* 7995 and (C) *Lactocaseibacillus paracasei* UW4 inoculated at 10^5 cfu/mL (●) or inoculated with 10^5 cfu/mL *Paucilactobacillus wasatchensis* WDC04 and 10^3 (■), 10^4 (▲), 10^5 (◆), 10^6 (★), or 10^7 (▼) cfu/mL of the adjunct culture and incubated at 23°C. Data reported as the mean $\pm$ SE of 3 independent replicates.

CONCLUSIONS

Gas production by *Pa. wasatchensis* WDC04 was consistently observed in Durham tubes in 6 to 8 d when using an inoculation level of 10^5 cfu/mL in CR-MRS broth containing 0.3% ribose and 0.7% galactose as the only sugars and incubating at 23°C. This combination of nutrients provided for growth of *Pa. wasatchensis* WDC04 to 10^9 cfu/mL based on co-utilization of ribose and galactose, with sufficient galactose remaining by d 8 to generate enough CO₂ to create a gas bubble. When a faster-growing Gal⁺ Lac⁻ adjunct culture such as *La. paracasei* UW4, *Lb. helveticus* 7995, or *Pd. acidilactici* 23F was included, growth of *Pa. wasatchensis* WDC04 was impeded, and all the galactose was utilized while *Pa. wasatchensis* WDC04 was still in exponential growth. With no residual galactose that could be utilized after the ribose had been depleted, no CO₂ was produced. This shows the potential for using a Gal⁺ Lac⁻ adjunct culture in Cheddar cheese manufacture to prevent unwanted gas production and formation of cracks and slits.

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