A Novel Organism \textit{Lactobacillus wasatchensis}: Growth, Detection, Gassing Defects in Cheese, Control Strategy and Future Research Opportunities: A Review

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\textbf{ABSTRACT}

An obligate heterofermentative, \textit{Lactobacillus wasatchensis} has been recently isolated from an aged Cheddar cheese produced in Logan, Utah. The potential of this organism in causing gassing defects in aged cheese has raised concern among cheese manufacturers. The recent attention on this organism is attributed to its economic impact due to low-quality cheese. This comprehensive review provides the details about \textit{Lb. wasatchensis} characteristics, geographical distribution and effect of various physical and chemical factors such as heat treatment, carbohydrate utilization, pH, salt tolerance and growth temperature. \textit{Lb. wasatchensis} utilize ribose as a primary source for its growth, however, it can slowly utilize galactose resulting in gas generation. The details of testing methods along with suggestions for future research on improving these techniques using a phage as a selective medium are provided in this review. Recent research developments for controlling the growth of \textit{Lb. wasatchensis}, as well as potential research opportunities are summarized in this review.

\textbf{Key words:} Gas and texture defect in cheese, Galactose, \textit{Lactobacillus wasatchensis}, Nonstarter lactic acid bacteria, Ribose.

The use of lactic acid bacteria (LAB) as a starter culture (producing acid and flavor) and probiotic culture has been exploited for many years and it is continued to be utilized in the dairy and food industry. LAB either in the form of a defined or undefined starter culture is one of the key ingredients in cheese-making due to its rapid acid production capabilities and reducing the pH to the desired level, thus aiding in developing desired texture, mouthfeel and overall cheese quality throughout cheese making and ripening (Briggiler-Marco \textit{et al.} 2007). In the commercial cheese making, the milk is pasteurized (for food safety reason) to reduce the pathogens as well as competitive inherent and contaminant LAB flora (nonstarter lactic acid bacteria: NSLAB) to a safe level. Despite this pasteurization treatment, some of the NSLAB survive and are able to grow in the cheese during ripening process. This NSLAB on one side can contribute towards flavor intensity of the cheese during ripening (Tom \textit{et al.} 2001), whereas on the other side it is the main uncontrolled factor in today’s industrial cheese making and may be the cause of quality inconsistencies and defects in cheeses (Briggiler-Marco \textit{et al.} 2007). Facultative heterofermentative - NSLAB, salt tolerant and mesophilic lactobacilli can cause the development of undesirable flavor and body defects including the gas formation in Cheddar-type and brine salted cheeses (Lalvey \textit{et al.} 1987, Khalid and Marth 1990, Dacre 2009, Sheehan 2011). Gassing defects in cheddar cheese is a recurrent and widespread problem in the dairy industry that has a greater impact on most of the affected cheese plants (Mullan 2000). Studies on different microorganism responsible for the gas defect in cheese are abundant and numerous micro-organisms responsible for the gas defect in cheese have been identified so far (Lalvey \textit{et al.} 1987, Mullan 2000, Bassi \textit{et al.} 2015). Recently, Oberg \textit{et al.} (2016) isolated \textit{Lactobacillus wasatchensis} from aged Cheddar cheese produced in Logan, Utah. \textit{Lactobacillus wasatchensis} is an emerging microorganism of interest in the cheese industry for causing the unwanted gas production in aged during storage. \textit{Lactobacillus wasatchensis} is an obligate heterofermentative, gram-stain positive, catalase-negative, rod-shaped (non-motile rods occurring singly and in pairs; with 0.8-1.2 µm in width and 1.5-3.0 µm in length), non-spore-forming nonstarter lactic acid bacteria (NSLAB) (Oberg, \textit{et al.} 2016). This review is primarily targeted at cheese manufacturers as well as researchers. It is focused on comprehending all the research studies conducted related to this novel organism and aimed at identifying the future research needs to support the cheese manufacturer.
Geographical distribution

Culumber et al. (2017) in their study collected the different cheese samples (7 cheese samples manufactured in the USA with obvious gas production with puffy bags and 12 cheese samples with no apparent gassiness that had been manufactured in Australia, Ireland, New Zealand and the United States) and investigated the geographical distribution of *Lb. wasatchensis*. They reported positive detection of *Lb. wasatchensis* from the 6 of the 7 gassy cheese samples obtained from cheeses manufactured in western states and mid-western states in the USA. The 12 non-gassy cheese samples did not show the presence of *Lb. wasatchensis*. However, their argument was that this does not imply an absence of *Lb. wasatchensis* considering limitation of current detection method for isolating *Lb. wasatchensis* (detection limits requires *Lb. wasatchensis* to be a minimum of ~1.5 log cfu/g of the predominant faster-growing NSLAB population). They concluded that *Lb. wasatchensis* is not just a local phenomenon related to its first place of isolation but has a more general occurrence in cheese from several states across the United States. However, this study did not present any conclusive evidence to show its occurrence outside the USA. Further studies involving genomic analysis might be helpful in tracing the evolutionary spread of this organism between regions and manufacturing facilities, particularly outside the USA. This organism can cause a significant commercial impact on the cheese industry and understanding its biology and ecology will be of great importance to implement effective controls.

Detection and quantification methods

Oberg et al. (2016) isolated the *Lactobacillus wasatchensis* strain (WDC04T) from aged cheddar cheese following incubation for 35 days at 6°C on MRS agar (pH5.2). *Lb. wasatchensis* might be present as a part of NSLAB causing the gas defect in aged cheese, however, its presence might not be detected due to the following reasons. (i) current enumeration and identification method of NSLAB generally focused on lactic acid bacteria that can grow under the condition of 2 days incubation at 30 or 37°C, whereas *Lactobacillus wasatchensis* grows slower (5 days of incubation) than other NSLAB. (ii) the preferred temperature for the growth of *Lb. wasatchensis* is ~23°C and they grow marginally at the current method’s 37°C of incubation temperature (Culumber et al. 2017), (iii) the current method utilizes MRS or Rogosa agar with glucose as the sole carbohydrate source for enumeration and identification of NSLAB (Oberg et al., 2011), whereas *Lactobacillus wasatchensis* requires 5-carbon sugar such as ribose as a key nutrient for growth (Culumber et al. 2017). Hence, it appears that the current method (Glucose as growth substrate, 30 or 37°C incubation temperature for 2 days) for testing of NSLAB is not very well suited for the growth of *Lb. wasatchensis*.

Several researchers worked on developing the testing method suitable for the growth of *Lb. wasatchensis*. The plating method used by Ortakci et al. (2015) for enumeration and identification of *Lactobacillus wasatchensis* requires plating serially diluted cheese homogenate sample on MRS + vancomycin + Ribose agar and anaerobic incubation at 23°C for 48 h. After 48 h of incubation rapidly growing colonies are marked as NSALB followed by further incubation at 23°C for another 72 h. After 72 h, of incubation newly appearing small (0.5 to 1 mm in diameter) white circular smooth textured convex colonies can be counted as *Lb. wasatchensis*. The enumeration method has a detection limit of 1.5 log cfu/g for the *Lb. wasatchensis* within NSLAB population. This detection limit might not be a problem for cheese sample with gassing defect, as this sample will have significantly higher (>1.5 log) count of *Lb. wasatchensis* to produce enough gas to show visible gassing defect in cheese. However, this limitation of detection will pose challenges in testing raw milk, pasteurized milk, cream, ultrafiltered milk or environmental swab samples (an investigative study on identifying the source of *Lb. wasatchensis* where the population of *Lb. wasatchensis* might be lower than the detection limit of this method.

Another method for identification of *Lb. wasatchensis* is based on extracting the *Lb. wasatchensis* DNA from a cheese sample in conjunction with an amplification step using polymerase chain reaction (PCR) with *Lb. wasatchensis* specific primers followed by agarose gel electrophoresis (Culumber et al. 2017). Oberg et al. (2017) developed new primers, LW86Fa and LW258Ra, which will be useful in traditional and real-time PCR for the rapid detection of *Lb. wasatchensis* contamination in gassy cheeses. The PCR / qPCR method, is time consuming and the detection limit is high, at least ~10⁴ log cfu/g (Bowen 2018), which required high DNA extraction yield as well (Tyler 2018). Bowen (2018) studied the DNA extraction of *Lb. wasatchensis* from cheese sample using phenol-chloroform extraction method and efficacy of qPCR at amplifying *Lb. wasatchensis* DNA extracted from cheese samples. This method produced a relatively pure DNA product. However, the amount of DNA harvested was not proportional to the amount of *Lb. wasatchensis* in the cheese and too much variation in the concentration of DNA extracted from each sample was observed. The threshold for detection using qPCR was ~8 x 10⁷ cfu/g of cheese. Overall, the work involving the qPCR method was not successful and it was recommended to conduct further study for improving the consistency in extracting the DNA amount and detection threshold for this method to be effective at measuring low levels of *Lb. wasatchensis* concentrations in cheese. Tyler (2018) investigated the effectiveness of a direct DNA extraction method and an indirect DNA extraction method on broth media, milk and cheese sample to purify and concentrate the DNA for downstream PCR-based analysis and visualization using gel electrophoresis. In direct method, the bacterial cells were lysed within the cheese sample followed by separation of the released DNA from the sample. In an indirect method, the cells were first separated from...
the cheese sample by mechanical or, chemical means, or by a combination of both, followed by lysis of the cell. Generally, the direct DNA extraction method yields a higher volume of DNA whereas the indirect extraction method yields higher purity of DNA. The significantly higher yield of DNA extracts among all three samples was reported with the direct DNA extraction method. Significantly higher purity of DNA extracts was reported from milk and cheese samples using the indirect method, whereas the direct method yielded purer extracts from the broth sample. Subsequent analysis of DNA extracts using Gel electrophoresis method revealed that the direct method had a lower presumptive detection threshold for Lb. wasatchensis in milk and cheese samples (<10 cells/ml or g) but it was considered as an erroneous result because of the sample contamination by extracellular DNA extract. Based on the indirect method the detection threshold for Lb. wasatchensis was 10^6 cells/ml for DNA extracts from milk or broth. Also, the very faint bands were observed at 10^6 cells/ml level, However, the bands could only be considered as presumptive positive. In case of cheese sample, the detection threshold was 10^4 cfu/g which is about the limit for the detection of Lb. wasatchensis in aged cheese in which the other NSLAB lactobacilli may be at levels of 10^4 cfu/g or higher.

Some limitations of these two methods are: (i) With a plating method of Lb. wasatchensis, it is very slow growing and takes 5 days to appear on the agar plates. (ii) Lb. wasatchensis needs to be higher than ~1.5 log cfu/g of the other faster growing NSLAB or it won’t be detected (Culumber et al. 2017). Moreover, typical day 1 levels of NSLAB including Lb. wasatchensis in young cheese is <10^2 cfu/g thus placing further constraints on isolating slow-growing NSLAB, such as Lb. wasatchensis, in younger cheeses (Culumber et al. 2017). (iii) With plating method, the organism other than Lb. wasatchensis able to grow in the test condition would also be counted as Lb. wasatchensis colonies resulting in false higher counts. (iv) With PCR based methods higher detection threshold continues to be challenged.

Tyler (2018) suggested assessing the suitability of indirect DNA extracted via the spin-column method from cheese for qPCR for future work. It may also be of some value to investigate ways to block the extraction of extracellular DNA to improve the direct phenol-chloroform method.

Survival and growth conditions for Lb. wasatchensis in cheese

Pasteurization and source of contamination

Bowen (2018), in their study reported about 4.5 log reduction of Lb. wasatchensis cells when bacterial cells were exposed to HTST pasteurization in a test tube at 72°C in lab conditions. In another study, Ortakci et al. (2015) investigated the thermo-tolerance (HTST at 72°C for 15 s and LTLT at 63°C for 30 min) of Lb. wasatchensis using UHT milk as a base medium for growth and reported ~4.5-log reduction (from 6 × 10^6 cfu/ml to 9.2 × 10^1 cfu/ml) with HTST and no detectable colonies (i.e., <10^1 cfu/ml) were observed with LTLT. Similarly, Oberg et al. (2016) reported about 4 log reduction of Lb. wasatchensis cells as a result of pasteurization (HTST-72°C for 15 s). However, Bowen (2018) in their study observed a greater than 7 log reduction with no survival of this bacterial cells, when samples run through an industrial heat exchanger with an increased heat exposure due to the regeneration sections had an even at sub-pasteurization temperatures of 69.4°C. Based on their finding it was suggested that post-pasteurization contamination cross-contamination could be the major cause for the presence of Lb. wasatchensis in cheese. However, this study was done using pasteurized milk as a Lb. wasatchensis inoculum base which does not represent background micro present in raw milk and effect of other liquid ingredients (cream, ultra-filtered milk) used for raw milk standardization in a commercial cheese plant. Hence, this observation does not stand true for all the conditions, including raw milk and other liquid ingredients with diverse microflora. Also, there is always a possibility of having a significantly higher log of initial NSLAB (8 to 9 log) in raw milk which results in a considerable amount of (2-3 log) of NSLAB in pasteurized milk.

The finding that Lb. wasatchensis (~ >10^6 cfu/ml in milk) can survive HTST conditions indicates that the survived organism could have the potential to form biofilms and grow in the cheese-processing environment that can subsequently get released from the loose biofilm and gain entry into cheese (Ortakci et al. 2015). In our opinion, considering higher standards of environmental control (Equipment cleaning, air quality, floor cleaning, good manufacturing practices etc.,) practiced in commercial cheese manufacturing, the likely hood of Lb. wasatchensis introduction in cheese through environmental contamination (which are under manufacturing plant control) is lower compared to raw milk being a source (not strictly under the control of manufacturing plant). It will also be interesting to study the role of rework (cheese fines/cheese rework) in introducing the contaminant Lb. wasatchensis back to the cheese during manufacturing. Generally this rework tends to be subjected to some degree of temperature abuse (for example, cheese sitting in the plant at ambient temperature before being transfer to the cold store for further use in future and cheese fines stored at higher temperature in whey before being separated and added back in the cheese), which can provide growth opportunity to the small number of pasteurization survived Lb. wasatchensis. Understanding the source as well as the factors supporting the growth of Lb. wasatchensis is important in finding the possible tools to prevent and control the growth of this unwanted organism during cheese ageing.

Carbohydrate utilization for growth

Lb. wasatchensis could primarily utilize one of the 50 carbohydrate substrates (API 50 CH carbohydrate fermentation panels) tested, ribose. The gas production tests
indicated that, the *Lb. wasatchensis* is also capable of slowly utilizing galactose and N-acetylglucosamine as evidenced by both the gas production in the Durham tubes and observable turbidity in the MRS broth (Oberg *et al.* 2016). Ortakci *et al.* (2015) investigated the growth and the gas production of a *Lb. wasatchensis* in CR-MRS base media (carbohydrate restricted - de Man, Rogosa and Sharpe) with ribose and galactose either alone or in combination. They also discussed the different possible metabolism pathways and their end products under different growth conditions and scenarios. The gas (CO$_2$) production was reported only when *Lb. wasatchensis* was grown in CR-MRS plus galactose or CR-MRS plus ribose and galactose and no gas production when ribose was used as a sole sugar source. Based on their observation they proposed that when *Lb. wasatchensis* is provided with both ribose and galactose in the growth medium, the ribose is primarily utilized for ATP production, whereas the galactose is utilized for the synthesis of peptidoglycans and other cellular macromolecules. Once ribose is depleted, the galactose gets utilized to provide energy to the cell. Ortakci *et al.* (2015) investigated the effect of accelerated cheese ripening along with ribose and galactose supplementation on growth of *Lb. wasatchensis*. It was reported that the *Lb. wasatchensis* were slow in utilizing galactose in the absence of ribose. The addition of ribose in cheese curd stimulated the growth of *Lb. wasatchensis* where most of the growth occurring during the first 8 wk. of ripening over total 23 wk. ripening time. The combination of ribose and galactose in the cheese also showed an increase in the gas production. Co-utilization of galactose with ribose by *Lb. wasatchensis* displayed an increase in the extent and occurrence of late gas formation in cheese. They proposed that *Lb. wasatchensis* initially consumed ribose to promote the growth with co-utilization of galactose and once ribose is depleted, the remaining galactose is used in producing energy, with CO$_2$ being generated. They also reported about 2-log growth of *Lb. wasatchensis* in cheese even without ribose supplementation, indicating that starter cell autolysis provided adequate pentoses (ribose and other sugars) to support growth.

**Growth under different temperature conditions**

Oberg *et al.* (2016) studied the effect of temperature (6, 12, 20, 25, 30 and 37 °C) on the growth of *Lb. wasatchensis* using MRS-R broth and incubation for 8 days. They reported that, the *Lb. wasatchensis* can grow in the temperature range of 6 to 30°C with an optimum temperature range of 23 to 25°C. The growth at 6°C was very slow and growth at 37°C was limited with a very long lag phase. Ortakci *et al.* (2015) reported 23°C as an optimum growth temperature among 3 different growth temperature (12, 23 and 37°C) they studied using CR-MRS as a growth medium with ribose and galactose either alone or in combination as an energy source. Ortakci *et al.* (2015) reported an increased likelihood of the gas formation in cheese at accelerated ripening temperature (12°C). Ortakci *et al.* 2015 investigated the growth of *Lb. wasatchensis* along with *S. thermophilus* as a starter culture and the gas production at accelerated ripening (12°C). The cheese was made using *S. thermophilus* as a starter culture alone (Control) or in combination with *Lb. wasatchensis* deliberately added to cheese milk at a level of ~ 4 log. On day one of the cheese made both control and test cheese sample contained similar ~ 9 log of *S. thermophilus*. Whereas *Lb. wasatchensis* were below enumeration limits in the control cheese and ~ 5 log in test cheese sample. Both control and cheese samples subsequently ripened at 6°C (regular ripening temperature for most of the cheeses) and 12°C (accelerated ripening temperature to attain flavor in short ripening time) for up to 23 weeks. The gas formation and textural defects were only observed in test cheese (inoculated with *Lb. wasatchensis*) ripened at 12°C (accelerated ripening), also the highest reduction in *S. thermophilus* from 9 log on day 1 to ~ 5.5 log after 23 weeks and the highest growth of *Lb. wasatchensis* (log ~ 8.5) after 23 weeks. This study was very interesting to us considering the answer we were searching that, how *Lb. wasatchensis* can produce gas in cheese considering that milk contains lactose as major carbohydrate while *Lb. wasatchensis* are efficient at utilizing ribose and galactose and not lactose. The possible answer can be found in above results and arguments made by researchers in this study. The results showed a significant amount of galactose (0.6 to 0.7%) in all cheese samples and the source of this galactose in cheese is *S. thermophilus* known to produce galactose from lactose present in milk (Michel and Martley, 2001). They made an argument that the higher cell reduction of *S. thermophilus* in test cheese ripened at 12°C indicating higher cell death and release of cellular ribose via its subsequent lysis. Hence, the presence of ribose (key growth nutrient) and galactose (gas production via galactose metabolism) accompanied by a higher count of *Lb. wasatchensis* under accelerated ripening provides a suitable environment for the growth and gas formation. To achieve efficiency in cheese making, the commercial cheese manufacturers have indulged in various fast cheese making process and fast ripening process. The use of *St. thermophilus* has increased significantly during the past few decades in manufacture of cheddar cheese including some swiss and italian cheese varieties due to their rapid acid production capabilities and hence providing short cheese making time (Michel and Martley, 2001, Callanan and Ross, 2004). On the other hand, in downstream of cheese making manufacturers are also trying accelerated ripening practices to develop desired flavor intensity in a short time. Such practices provide benefit in increasing cheese production efficiency, however, it may come with side effects such as quality defects (texture, color and gassing) especially in longer aged cheese such as cheddar and parmesan. Looking at beneficial sides, the cheese manufacturers are less likely to back down on these practices and hence there is a strong need to find the solutions to these side effects and one of them is gassing defects caused by *Lb.*
wasatchensis specially when the combination of St. thermophilus as starter culture and accelerated cheese ripening is used. Further research in developing galactose negative strains of St. thermophilus and also looking at converting ribose and galactose in some type of non-utilizable carbohydrate form for Lb. wasatchensis will be interesting.

Salt tolerance and growth under different pH conditions
Oberg et al. (2016) studied the effect of pH (4, 5, 6 and 6.5) on growth of Lb. wasatchensis using MRS-R broth and incubation for 3 days. They reported that Lb. wasatchensis was able to grow at pH 4 and robustly at the pH range of 5 to 6.5. Sodium chloride (NaCl at 0, 1, 2, 3, 4, 5 or 6.5% w/w) tolerance was examined by Oberg et al. (2016) in MRS broth + 1.5% ribose and incubation for 3 days. Lb. wasatchensis grew well up to a NaCl concentration of 5% (w/w). Oratcki et al. (2015) also have shown that Lb. wasatchensis is quite tolerant of the salt (0 to 5%) and pH (6.5 to 5.2) conditions that usually exist in aged cheese such as cheddar and parmesan. It appears that the cheese environment (pH and high salt tolerance) is very well suited for the growth of Lb. wasatchensis and they should be able to grow if nutrients and accelerated temperature requirements are available. Considering this as a slow growing organism, it has a more potential of affecting medium to long aged (6 month and above) cheeses over short aged cheeses.

Developments to tackle Lb. wasatchensis induced gassing issue in cheese
Oberg et al. (2017) investigated the effectiveness of common NSLAB lactobacilli and potential bio-protective lactic acid bacteria (BP-LAB) strains in inhibiting Lb. wasatchensis using the spot test along with the agar flip method. The BP-LAB strains did not show any signs of inhibition on Lb. wasatchensis up to 48 h of incubation as compared to competitive inhibition caused by the NSLAB cultures Lactobacillus brevis or Lactobacillus fermentum LF7469. When incubation time was extended to 72 h, the largest Lb. wasatchensis inhibition zone was displayed by BP-LAB P200 (commercial culture). The next inhibitory BP-LAB was LB-3 (commercial culture) with the NSLAB, Lb. fermentum LF7469, also produced a large inhibition zone. A paper disc assay test using cell free extracts confirmed several BP-LAB strains produced a bacteriocin, showing a very small zone of inhibition for Lb. wasatchensis around the paper disc. The researcher suggested the potential of some selected NSLAB strains and BP-LAB in inhibiting this problematic bacterium during cheese ripening. Ireland et al. (2018) investigated the effectiveness of selected organic acids (lactic, formic, propionic, citric and acetic) in inhibiting Lb. wasatchensis at their minimum, median and maximum concentrations range of what is naturally found in aged Cheddar cheese. In their testing method, the MRS broth with 1% ribose (MRS + R) inoculated with Lb. wasatchensis were tested against these organics acids and its growth rates was determined at two different pH (7 and 5) on a Tecan Infinite 200 PRO spectrophotometer over 40 h. The organic acids exhibited some inhibition at pH 7. Significant inhibition of Lb. wasatchensis was reported by both formic and citric acid at pH 5.0. Formic acid at the maximum concentration (100 mM) displayed the most inhibitory effect among all 5 acids. Citric acid at minimum (12 mM) and median (13.5 mM) concentrations also displayed some inhibition. They suggested the use of formic and citric acids at concentrations normally found in Cheddar cheese as a potential antimicrobial agent to prevent or reduce late gas defects in ageing cheese. The results showing inhibition of Lb. wasatchensis by Formic acid and Citric acid at pH 5.0 are encouraging as this is the natural pH environment for most of the long aged cheese, however, formic acid showed its effect at maximum concentration (100 mM) which might not be naturally present in all the cheese at all the times. The results with Citric acid are somewhat promising and further experiments will be required to test that if these results stand true for the actual cheese environment (in cheese product) as well.

Future Research Recommendations
It would be interesting to look at the possibility of the connection of Lb. wasatchensis evolution with the rise in cheese industry’s practice of increasing the use of S. thermophilus culture in aged cheese production. Various researchers have explored the use of host specific phage as a selective agent in growth media (Ripp 2010, Smartt and Ripp, 2011, Schofield et al. 2012) and we suggest exploring this technique for selective enumeration of Lb. wasatchensis. Phage can also be used as potential to selectively lye the DNA only from Lb. wasatchensis in case of direct DNA extraction methods. Considering optimum growth temperature (23 to 25°C) and pH range (5.0 to 6.5) it would be interesting to investigate the ability of this organism to form a biofilm in milk/cream pasteurizer regeneration section specifically in the 23 to 25°C temperature region. Understanding the role of Lb. wasatchensis within diverse biofilm microflora will provide crucial information to tackle biofilm related contamination. There is also a need to test the effectiveness of different cleaning and sanitizing agents in removing/preventing biofilms formed/dominated by Lb. wasatchensis. Gandhi et al. (2019) in their study reported about Lactobacillus casei expressing methylglyoxal synthase causes browning and heterocyclic amine formation in Parmesan cheese extract, however, to our knowledge similar study on understanding the ability of Lb. wasatchensis to generate methylglyoxal and heterocyclic amine responsible for browning has not been done yet. It would be interesting to learn if Lb. wasatchensis possesses the ability to cause browning defect along with the gas and textural defects in aged cheese. Since, ribose and galactose are the key growth nutrients used by Lb. wasatchensis it would be interesting to study the conversion of ribose and galactose in some type of non-utilizable form for the Lb. wasatchensis.
CONCLUSION

*Lactobacillus wasatchensis* is a novel organism recently isolated from an aged Cheddar produced in Logan, Utah. The ability of this organism to survive and grow in the cheese environment to cause a gassing defect has raised concern among cheese manufacturers. The recent attention on this organism is attributed to its impact in affecting the quality of long aged cheeses (such as cheddar and parmesan) and economic impact due to low-quality cheese as well as limiting the duration of accelerated ripening. It can grow at pH 4 and robustly at the pH range of 5.0 to 6.5 and it can tolerate salt concentration up to 5% which naturally occurs in the cheese environment. *Lb. wasatchensis* can grow in the temperature range 6 to 30°C with an optimum temperature range of 23 to 25°C. *Lb. wasatchensis* utilize ribose as a primary source for its growth, however, it can slowly utilize galactose and produce gas as one of the metabolite products. The optimum growth temperature of 23°C and ribose as a key growth nutrient are used in development of plate count testing method for enumeration of *Lb. wasatchensis*. The PRC based methods can provide accurate detection of *Lb. wasatchensis*, however, further work is required for improving DNA extraction and detection limits. Recent research developments have shown some promises for controlling the growth of *Lb. wasatchensis* by using selective NSLAB organisms, bio-protective cultures and NSLAB produced organic acids in growth media and it would be interesting to test these concepts in actual cheese product as well. The study of biofilm forming ability as well as its interaction with other microorganisms within biofilm community will provide new knowledge with concerned to post pasteurization contamination. Also, the study on testing the effectiveness of different cleaning and sanitizing agent will help provide information to develop strategies to fight against biofilm formation. The commercial cheese manufacturer’s practices of using *St. thermophilus* for short cheese making process (acting a source of galactose and ribose) and accelerated ripening to shorten the ripening time, aids in increasing the probability of gas production by *Lb. wasatchensis*. The further research in developing galactose negative strains of *St. thermophilus* and also looking at converting ribose and galactose in some type of non-utilizable form for *Lb. wasatchensis* will be interesting.

Funding

The authors received no direct funding for this research. This review work is an independent work of authors mentioned above and it has received no funding from any institution or individual.

Competing interests

The authors declare no competing interests

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