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Lactose hydrolysing ability of sonicated cultures of *Lactobacillus delbrueckii* ssp. *bulgaricus* 11842

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Abstract — The lactose-hydrolysing ability of *Lactobacillus delbrueckii* ssp. *bulgaricus* strain 11842 (LB 11842) was tested after cultivation in MRS broth under varying conditions. After sonication for an optimum time determined to be 4 min to disrupt the cells, cultures were added to various substrates, including pH 7 buffered 2.5 and 5% lactose test solution, skim milk, or a 30% lactose or whey permeate solutions. Freezing point changes were used to measure the progress of hydrolysis at a wide range of temperatures. Cultures added to the lactose test solutions resulted in 20% and 63% of the lactose being hydrolysed after 3 h at 7 and 51 °C, respectively. Values for hydrolysis in skim milk were slightly higher. Enzyme activity was not hindered in 30% lactose or 30% whey permeate solution and the hydrolysis rate was acceptable at a low temperature (7 °C) in skim milk.

***Lactobacillus bulgaricus* / lactose hydrolysis / sonication / dairy culture / cryoscopy**

Résumé — Aptitude à l'hydrolyse du lactosérum de cultures soniquées de *Lactobacillus delbrueckii* ssp. *bulgaricus* 11842. La capacité d'hydrolyse du lactosérum par *Lactobacillus delbrueckii* ssp. *bulgaricus* souche 11842 (LB 11842) fut testé après culture sur bouillon MRS sous différents paramètres. Après rupture des cellules par sonication pour une durée optimale de 4 min, les cultures furent incorporées à divers substrats tels la solution de référence à 2,5 ou 5 % de lactose tamponée à pH 7, le lait écrémé ou une solution de lactose ou de perméat de lactosérum contenant 30 % de lactose. La variation du point de congélation fut utilisée pour suivre l'évolution de l'hydrolyse pour une game étendue de températures. Les cultures ajoutées à la solution référence de lactose produisirent après 3 h des hydrolyses respectives de 20 % à 7 °C et 63 % à 51 °C. Des valeurs légèrement supérieures ont été obtenues pour le lait écrémé. L'activité enzymatique n'est pas entravée pour les solutions à 30 % de lactose ou 30 % de perméat de lactosérum et le taux d'hydrolyse pour le lait écrémé est acceptable à basse température (7 °C).

***Lactobacillus bulgaricus* / hydrolyse du lactose / sonication / culture laitière / cryoscopie**

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1. INTRODUCTION

Lactose, the disaccharide 4-O-β-D-galactopyranosyl-D-glucopyranose, is found almost exclusively in milk. The main problem arising from the presence of lactose in food products is the prevalence of "lactose intolerance", common among the adults of many of the world's ethnic groups, which detracts from wider consumption of dairy products. The overall nutrition of the affected groups could be improved with lactose-hydrolysed dairy products.

Sources of the lactose-hydrolysing enzyme, β-galactosidase, (β-D-galactoside galactohydrolase, E.C.3.2.1.23) available commercially include extracts from yeasts, molds and bacterial cultures. Enzyme preparations for use in the dairy industry are produced with extensive purification and therefore are quite expensive. As a result, the cost of lactose-reduced milk is about 80% higher than regular unhydrolysed milk. If an economically attractive method of hydrolysis were developed, more uses for lactose and dairy products containing lactose could become feasible [2].

Most bacterial cultures commonly used in the production of fermented dairy products also produce β-galactosidase (β-gal). In an attempt to conceptualize a process for lactose hydrolysis in dairy products which would not require extensive enzyme purification, it was proposed to use a suitable common dairy bacterial culture, disrupt the cells to release the intracellular enzyme and add the disrupted culture to the dairy product with little purification other than removal of the growth medium [5, 15].

The hydrolysis of lactose does not correlate well with the hydrolysis of other related compounds, when working with β-gal from different sources or when data obtained under variable conditions are being compared. Measured β-gal activity can vary markedly according to what substrate is being used [9, 16]. Thus hydrolysis in simple solutions of lactose, the substrate of interest, may be preferable as the indicator of hydrolysing ability rather than the commonly-used o-nitrophenyl galactopyranoside (ONPG).

The progress of hydrolysis in lactose containing solutions may be measured by the rapid, convenient and reliable cryoscopic method. This method has been used in several experimental studies, in which highly significant linear correlations were found between the change in freezing point and results from other methods suitable to monitor lactose hydrolysis, including HPLC [1, 6, 11, 21]. Substrates used by these authors included milk, ultrafiltration permeate, acid whey, aqueous lactose solutions, and whey with added lactose. More recently, Kreft and Jelen [10] established highly significant linear correlations between freezing point depression and lactose and glucose levels during lactose hydrolysis using milk as a substrate and disrupted dairy bacterial cultures as a source of β-gal enzyme.

Previous works have shown that *Lactobacillus delbrueckii* ssp. *bulgaricus*, strain 11842 (LB 11842), a common dairy organism, as well as other strains of this species produce β-gal with notable hydrolysing ability [4, 14, 15, 17, 18]. However, optimal conditions for maximization of the β-gal production by LB 11842 or for lactose hydrolysis by the disrupted cultures have not been thoroughly studied.

The specific objectives of this study were (a) to determine the suitability of various conditions for β-gal production by LB 11842 and (b) to investigate the effectiveness of lactose hydrolysis by β-gal from LB 11842, after sonication of the cultures, in various lactose-containing test solutions as monitored by freezing point changes. The test solutions included pH 7 buffered 5% lactose solution, 30% lactose, 30% whey permeate solutions and milk.

2. MATERIALS AND METHODS

2.1. Organisms and media

Cultures of LB 11842 were obtained from the American Type Culture Collection (ATCC, Rockville, Maryland). Cultures were maintained frozen at –20 °C, and revived in sterile skim milk. At least 3 successive daily transfers were made before actual experimental runs were carried out.

The basic growth medium was a commercial brand of MRS broth [3]. Since for LB 11842, β-gal is a constitutive enzyme produced irrespective of the presence or absence of lactose in the medium [8], the absence of lactose in MRS was not considered a problem. Cultures were grown anaerobically or aerobically, as per the experimental design. All organisms were grown as static cultures for 18 or 24 h, or both, at 37 °C, as specified.

2.2. Culture treatment, cell disruption and viable counts

Growth was stopped by cooling cultures in ice water and growth medium was removed by separating the biomass by centrifugation with an International Clinical Centrifuge, Model CL 1572C, (International Equipment Co., Boston, Mass.) at $1700 \times g$ for 20 min, and decanting the growth medium. The harvested cells were not washed, as the basic measure of hydrolysis was the change in the freezing point (Δf.p.) of the test solution, resulting from the hydrolysis. In the initial experiments, the cultures were immediately resuspended in chilled 0.1% peptone water or distilled water, equal in volume to the removed growth medium. In later experiments, the cultures were resuspended directly in the specified test solution, in most cases 5% lactose in Fisher brand pH 7 potassium-sodium phosphate buffer (0.05 mol·L^{-1}).

For every replication in this whole study, the procedure was repeated from the growth of the culture to hydrolysis. Thus, each replication was carried out with freshly grown cells; therefore possible deterioration during storage was not a confounding factor. After growth, cell preparations were kept chilled at all stages except during the 20 min centrifugation.

Cellular disruption was achieved by sonication with a Braun-Sonic 2000 sonicator (Ultrasonic Power Corp., Freeport, Ill.) using a 19 mm diameter probe and output setting of 75 watts. Cultures resuspended in water or test solutions were sonicated in an ice water bath, initially for times varying between 2 and 12 min. A 4 min treatment was found to give the optimum results, and was used for the rest of the study.

Viable plate counts were obtained using standard serial dilution techniques with 0.1% peptone (Bacto-peptone, Difco Laboratories, Detroit, MI) in distilled water. The plates were poured with MRS plus 1.5% agar prepared according to the manufacturer's instructions. Plates were counted after 3 days at 39 °C.

2.3. Lactose hydrolysis

The disrupted cultures were added to the buffered lactose test solutions, in a 1:1 ratio unless specified otherwise. Thus, when the sonication liquid was water, the final concentration of the lactose solution was 2.5%. Commercial skim and 2% fat milk were also used in some experiments. Additionally, the effectiveness of hydrolysis in 30% lactose and 30% whey permeate solutions was evaluated. The initial freezing point reading was taken immediately after addition of the sonicated culture, and the mixtures were held at a given temperature, as specified by the experimental design; for most of the experiments 7, 25 and 51 °C were used.

Freezing point measurements were taken with an Advanced Cryomatic Milk Cryoscope (Advanced Instruments Inc., Norwood, Mass.), model 4C2. Aqueous solutions of 2.5% lactose (w/v) are 0.0731 mol·L^{-1}. Thus, complete hydrolysis, with no other reaction influencing the solution molality, would theoretically give a Δf.p. of 141 points or milli-degrees Hortvet (m^{o}H), as per the scale of the cryoscope; complete hydrolysis of a 5% solution would theoretically give a change of 281 m^{o}H, from which percent hydrolysis can be easily calculated.

In the initial experiments, the effectiveness of hydrolysis was followed by measuring the freezing point before adding the culture and after 1 h under the given conditions. Later, it was measured at approximately 15 min intervals for the first hour, then at 30 min intervals for at least a total three to four hour period. All runs with a specified set of conditions were replicated at least twice (sometimes up to 5 times).

2.4. Effect of biomass concentration and hydrolysis temperature

The centrifuged mass of cells produced with 100 mL of MRS broth was, on average, 1 × *g* on a wet basis. For this series, the concentrated pellet from 100 mL of growth medium was sonicated directly in 100 mL of 5% lactose solution or skim milk (rather than water as previously), giving 1% cell concentration (wet weight /volume). In comparison, the concentration of sonicated cells in previous experiments was approximately 0.5%, with 2.5% final lactose concentration. Appropriate volumes of 5% lactose solution or skim milk containing the sonicated cells were added to volumes of solution or milk with no organisms, to give final dilutions of cells ranging from 0.05% to 1%, and the mixtures were held at 51, 25 and 7 °C. A final dilution of 2% cells was obtained by sonicating the pellet from 100 mL of growth medium in 50 mL of lactose solution or milk. Freezing points were measured for at least 3 h.

2.5. Hydrolysis in concentrated lactose and permeate solutions

In the final set of experiments, the β-gal activity of sonicated cultures of LB 11842 was determined in solutions of 30% lactose in pH 7 buffer, rather than the usual 5% solution, and in whey permeate reconstituted from an industrial powdered permeate (Maple Leaf Foods Internat., Toronto, Ont.) to the 30% concentration in the same buffer. Cultures were added to both solutions at a rate of 1%, sonicated in these highly concentrated solutions and held at 25 and 51 °C. In order to bring the solution within the range of the cryoscope, which was calibrated to measure the f.p. of milk, 1 mL was withdrawn and diluted with 5 mL of distilled water.

The mean and average standard deviations were calculated for each set of replicates. The t-test for difference in means [12] was performed where appropriate. Initial reaction rate was taken as the rate of change of freezing point (m^{o}H·min^{-1}) during the first 15 min of hydrolysis.

3. RESULTS AND DISCUSSION

3.1. Controls and preliminary tests

To verify the correlation between change in freezing point and lactose hydrolysis as well as the variability of the monitoring procedure in our conditions, a commercially available preparation of β-gal enzyme in liquid form (Lactaid® drops) was added at the rate of 0.2 mL of enzyme preparation to 250 mL 2% fat milk, an addition rate very similar to the manufacturer's recommended rate for conversion of all the lactose in 1 L milk in 24 h at 6 °C. Mixtures were held at 3, 7, and 25 °C.

At 3 h hydrolysis, mixtures held at 3 and 7 °C showed approximately 50% hydrolysis, and that at 25 °C showed approximately 70% hydrolysis (data not shown). The change in freezing point values indicated a steady increase in the molality of the solution, with a high degree of reproducibility.

Cultures sonicated in distilled water and added immediately to 5% solutions of sucrose, fructose, and glucose, and held at 7 and 51 °C produced almost no change in freezing point over 24 h, whereas addition of the same cultures to lactose solutions resulted in an increase in freezing point equivalent to approximately 60% at 7 °C; 85% hydrolysis was reached in lactose solutions within 5 h at 51 °C. According to Bergey's Manual [7], *Lactobacillus delbrueckii* ssp. *bulgaricus* does not ferment sucrose, but does ferment glucose and fructose, when the temperature is conducive to its growth. Some strains can tolerate temperatures as high as 52 °C but lactobacilli growing above 55 °C are as yet unknown. Thus the change in freezing point due to microbial or enzymatic breakdown of sugars other than lactose was deemed to be negligible for LB 11842.

Cultures sonicated in distilled water and then added to commercially available 2% fat lactose-hydrolysed milk at the ratio of 1:1 showed small changes in freezing point when held at 7, 25 or 51 °C for 3 h, compared to the same cultures held in regular unhydrolysed 2% fat milk at the same temperatures. For cultures in the lactose-hydrolysed milk the mean change in freezing point for two replicates was 5, 13, and 34 m°H respectively, and for unhydrolysed milk the respective changes were 40, 69, and 145 m°H, indicating a theoretical percent hydrolysis of over 99%, for the milk held at 51 °C. It is claimed by the manufacturer of the lactose-hydrolysed milk that the level of hydrolysis is 99%. Thus virtually no lactose would be available to be hydrolysed by the free enzyme, but any microbes not disrupted by sonication could possibly grow and/or keep producing lactic acid in the stationary stage, using the available glucose and other necessary nutrients. This might be the cause of the slight Δf.p. in the lactose hydrolysed milk.

The freezing point of plain skim milk and plain lactose solution, with no sonicated cultures added, showed very little change over 48 h when held at 51 °C; it can be concluded that there was no change in molality due to evaporation or uncontrolled microbial activity. All these results showed that the change in freezing point due to biochemical changes other than lactose hydrolysis by the free β-gal was negligible compared to that caused by the lactose hydrolysis, when hydrolysis conditions were chosen to inhibit microbial growth.

3.2. Determining conditions for optimum hydrolysis

The highest levels of hydrolysis as measured by Δf.p. after 1 h incubation at various temperatures were reached with either 4 or 8 min sonication. However, a decline in level of hydrolysis with more than 4 min sonication was noticed in some cases, therefore a 4 min interval was selected as overall optimum sonication time.

Four culture growth times between 18 and 24 h, tested in order to assess the effect of age of cultures within this time span, indicated that there was little difference in hydrolysing ability of cultures from these growth phases. Thus, subsequent experiments were generally carried out with 2 growth times, either 18 or 24 h.

Viable plate counts for cultures sonicated in lactose solutions buffered to pH 7, and then held for 3 h at 51 °C, showed no colonies at dilutions as low as $1:10^3$, indicating at least a 5log cycle reduction in viable bacteria under these conditions. For cultures sonicated in milk and then held at 51 °C, in all cases there was a decrease in viable count of up to 10 fold after 3 h; in no

case did the viable count increase. Thus, an added advantage of using this relatively high temperature for the hydrolysis was that the growth of remaining, viable cells was effectively eliminated.

The genus *Lactobacillus* is considered to be microaerophilic [7]. In order to assess the effect of gaseous atmosphere during growth on subsequent hydrolysing ability, 5 separate cultures were grown aerobically and 7 were grown anaerobically in MRS broth (Tab. I). After 4 min sonication, followed by 1 h hydrolysis test in 2.5% lactose in pH 7 buffer at 51 °C, the mean Δf.p. produced by the aerobically grown cultures was higher, though not significantly different than those grown anaerobically. There was also no significant difference between the means of the plate counts before sonication. Thus, all following experiments were carried out with aerobic growth, because the methodology is simpler, faster and less expensive than anaerobic growth.

As a measure of the effectiveness of sonication, viable plate counts were also obtained after 4 min sonication in the above experimental trials, as shown in Table I. The average reduction in count was 0.97 log cycles for the aerobically grown cultures and 0.90 log cycles for the anaerobically grown cultures. The average log count reduction observed throughout the course of this project, for 44 separate cultures, was 1.62 log cycles. A greater reduction would not measurably change the amount of released enzyme; however, metabolic activities of the high number of bacteria still remaining alive could compromise the monitoring procedure, if conditions conducive to growth of the bacteria remaining viable were to be used.

3.3. Effect of biomass concentration and hydrolysis temperature

The effects of concentration of enzyme-containing biomass on the progress of lactose hydrolysis in 5% lactose solution and skim milk are shown in Tables II and III, respectively. As expected, for sonicated cells in both substrates at the 3 temperatures of 7, 25 and 51 °C the level of hydrolysis achieved after 3 h increased with the sonicated cell concentration (wet weight/volume). For 7 and 51 °C, the initial reaction rates appeared roughly proportional to the enzyme concentration except for the 2% addition rate, where the increase over the 1% rate was small. For both substrates at 25 °C the initial reaction rates at 2% enzyme addition rate were very close to twice the 1% addition rate.

Table I. The effect of sonication and gaseous atmosphere on growth and subsequent hydrolysing ability of cultures of LB 11842 sonicated 4 min, followed by 1 h in 2.5% lactose in pH 7 buffer.

	Aerobic (n = 5)	Anaerobic (n = 7)	p-value
log (plate count) (cfu·mL^{-1}) (before sonication)	8.67 ±0.12	8.64 ±0.12	0.369
log (plate count) (after sonication)	7.69 ±0.21	7.77 ±0.49	0.373
Δf.p. (m°H)	78.8 ±6.7	72.4 ±13.0	0.149

Table II. Initial reaction rates (initial rate of change of freezing point) and calculated degree of hydrolysis reached after 3 h with different proportions of sonicated cells of *Lactobacillus delbrueckii* ssp. *bulgaricus* strain 11842 in 5% lactose solution at 3 different hydrolysis temperatures, as measured by change in freezing point. Each data point is the mean of 2 replicates.

5% Lactose solution

	Addition rate (%)					
	2	1	0.50	0.25	0.10	0.05
°C	Initial reaction rate, m°H·min^{-1}					
7	1.19	0.75	0.33	0.15	0.04	0.02
25	1.81	0.99	0.65	0.32	0.12	0.07
51	4.05	3.22	1.90	1.00	0.49	0.24
°C	Degree of hydrolysis at 3 h (%)*					
7	28.20 ±3.69	20.13 ±3.05	13.24 ±2.21	6.68 ±0.35	2.82 ±0.39	1.41 ±0.54
25	44.83 ±1.57	34.89 ±2.05	26.35 ±0.64	16.17 ±0.44	7.79 ±0.53	4.49 ±0.20
51	74.68 ±2.36	63.00 ±1.76	49.96 ±1.22	36.78 ±1.35	21.81 ±1.02	11.81 ±0.79

* ±1 standard deviation

Table III. Initial reaction rates (initial rate of change of freezing point) and calculated degree of hydrolysis reached after 3 h with different proportions of sonicated cells of *Lactobacillus delbrueckii* ssp. *bulgaricus* strain 11842 in skim milk at 3 different hydrolysis temperatures, as measured by change in freezing point. Each data point is the mean of 2 replicates.

Skim milk

	Addition rate (%)					
	2	1	0.50	0.25	0.10	0.05
°C	Initial reaction rate, m°H·min^{-1}					
7	1.05	0.90	0.40	0.19	0.12	0.05
25	2.39	1.18	0.68	0.34	0.16	0.08
51	5.42	3.58	1.90	0.89	0.44	0.13
°C	Degree of hydrolysis at 3 h (%)*					
7	32.98 ±3.39	22.16 ±3.29	14.68 ±0.94	7.80 ±0.47	4.75 ±0.98	2.82 ±1.45
25	61.11 ±2.74	43.80 ±0.82	30.57 ±0.03	19.40 ±0.64	9.02 ±0.13	5.20 ±0.68
51	>97 ±3.03	87.23 ±4.45	69.52 ±4.68	47.12 ±1.22	25.40 ±0.74	12.89 ±0.29

* ±1 standard deviation

Figure 1 illustrates the level of hydrolysis achieved by 1% sonicated culture in skim milk and in 5% lactose at the above mentioned 3 temperatures. The level of hydrolysis reached after 3 h at 51 °C was approximately twice the level reached after 3 h at 25 °C, and 4 times the level reached after 3 h at 7 °C. Changes in freezing point values produced by sonicated organisms in skim milk were higher than in 5% lactose solution. For 25 and 51 °C, both the initial reaction rate and the calculated degree of hydrolysis at 3 h were up to 40% higher in skim milk, whereas at 7 °C the values for skim milk were up to 15% higher. This could indicate some lactic acid production in the skim milk by the remaining viable bacteria not killed by the sonication procedure, thus making the freezing point determination less suitable under these conditions.

3.4. Hydrolysis in concentrated lactose and permeate solutions

Results for hydrolysis in a 30% lactose solution are given in Figure 2 in both theoretical percent hydrolysis of the 30% solution, and as a calculated change in cryoscope readings for the undiluted solution. For 30% lactose, 100% hydrolysis would give a theoretical change in freezing point of 1680 m°H. There is a clear upward trend in theoretical percent hydrolysis of the concentrated lactose solution, reaching 18% after 3 h. However, the standard deviations were much greater than seen throughout this work in hydrolysis of a 5% solution, possibly due to interference caused by production of oligosaccharides. It is well known that the β-gal enzyme exhibits transgalactosidation activity resulting in production of trisaccharides and other

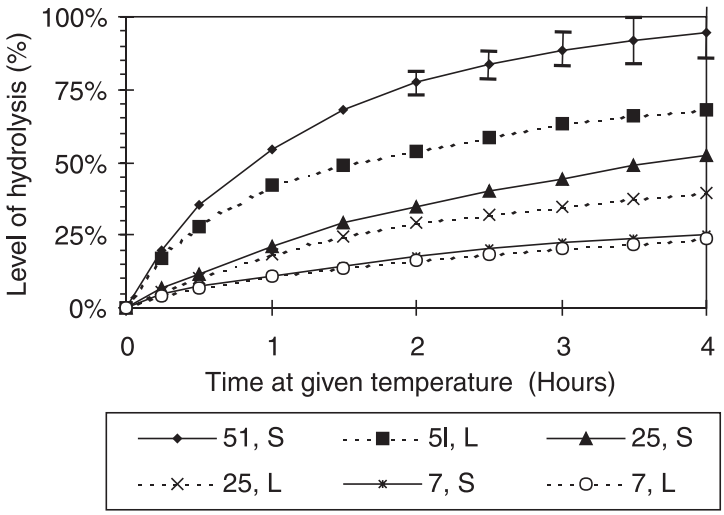

Figure 1. Lactose hydrolysis by 1% sonicated cultures of *Lactobacillus delbrueckii*, ssp. *bulgaricus*, ATCC 11842 in pH 7 buffer containing 5% lactose (L) and in skim milk (S) at 51, 25 and 7 °C temperatures. Where shown, error bars show average standard deviations (n = 2) for a given set; otherwise, they are approximately the height of the data marker point.

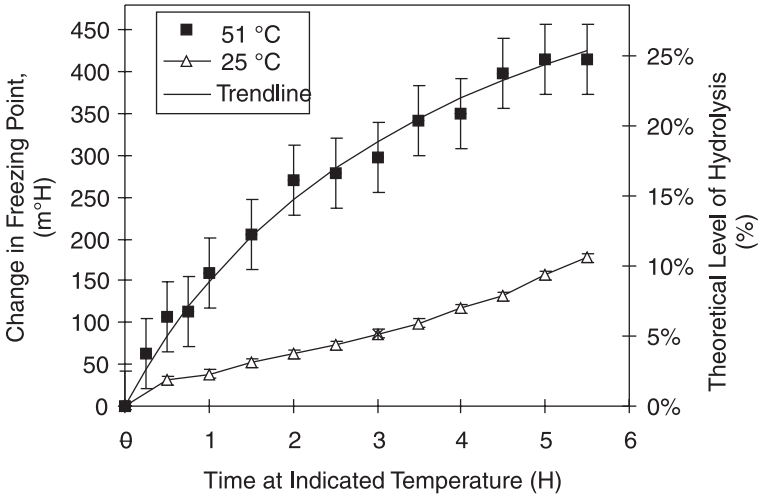

Figure 2. Lactose hydrolysis by sonicated cultures of *Lactobacillus delbrueckii*, ssp. *bulgaricus*, ATCC 11842 in 30% lactose solution at two temperatures. Error bars show average standard deviations for each set of 5 replicates.

oligosaccharides in the course of lactose hydrolysis [13, 20]. Results for hydrolysis in 30% whey permeate were very similar to hydrolysis in 30% lactose, though slightly higher (data not shown). Enzyme activity did not appear to be hindered by this high lactose concentration.

Comparing the hydrolysis by 1% sonicated cells in 5% and in 30% lactose solutions, the initial reaction rate was very similar at the two concentrations (0.99 and 1.03 $m^oH \cdot min^{-1}$, respectively) at 25 °C, whereas 30% lactose showed a much higher initial rate (3.2 vs. 4.2 $m^oH \cdot min^{-1}$, respectively) at 51 °C. The Δf.p. was about 68% higher in the 30% lactose solution than in the 5% solution at 51 °C (297.2 and 177.0 m^oH, respectively). A lactose concentration of 30% is likely near the concentration, which produces maximum activity with the present amount of enzyme. This is similar to the maximum rate of hydrolysis in acid whey with β-gal from *Aspergillus niger* obtained when lactose concentration was 21% [19].

4. CONCLUSIONS

Cryoscopy was used to evaluate the effectiveness of the β-gal in sonicated cultures of LB 11842 for lactose hydrolysis using a well defined, simple test system consisting of pH 7 buffered 5% lactose solution. The progress of hydrolysis in milk may have been confounded by the metabolic activities due to the presence of the viable bacteria surviving sonication. With 18 to 24 h aerobic growth, 4 min sonication and a sonicated cell addition rate of 1%, no microbial survival or metabolic activities

were detected in the lactose test solutions at the hydrolysis temperature of 51 °C. Initial reaction rate of lactose hydrolysis, achieved at the low temperature of 7 °C despite the organism being thermophilic, was about 4 times slower than that at 51 °C. Hydrolysis did not appear to be hindered by the high lactose concentration of 30%, but the increased variability of the experimental results might indicate interference by oligosaccharide synthesis. Aerobically-grown cultures of LB 11842 after sonication demonstrated good hydrolysing ability on various lactose-containing substrates, and over a wide range of temperatures.

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REFERENCES

[1] Baer R.J., Frank J.F., Loewenstein M., Freezing point measurement of lactose hydrolysis in acid whey and lactose solutions, J. A O A C Int. 63 (1980) 587–590.

[2] Bury D., Jelen P., Lactose hydrolysis using a disrupted dairy culture: Evaluation of technical and economical feasibility, Can. J. Agric. Eng. 42 (2000) 75–80.

[3] de Man J.C., Rogosa M., Sharpe M.E., A medium for the cultivation of *Lactobacilli*, J. Appl. Bacteriol. 23 (1960) 130–135.

[4] Hickey M.W., Hillier A.J., Jago G.R., Transport and metabolism of lactose, glucose and galactose in homofermentative *Lactobacilli*, Appl. Environ. Microbiol. 51 (1986) 825–831.

[5] Jelen P., Lactose Hydrolysis using sonicated dairy cultures, Bull. Int. Dairy Fed. 289 (1993) 54–56.

[6] Jeon I.J., Saunders S.R., Effect of oligosaccharide formation on the cryoscopic measurements of enzymatic hydrolysis of lactose in dairy products, J. Food Sci. 51 (1986) 245–246.

[7] Kandler O., Weiss N., Regular, nonsporing gram-positive rods, in: Sneath P.H.A. (Ed.), Bergey's Manual of Systematic Bacteriology, Volume 2, Williams & Williams, Los Angeles, 1986, pp. 1208–1222.

[8] Kilara A., Shahani K.M., Lactase activity of cultured and acidified dairy products, J. Dairy Sci. 59 (1975) 2031–2035.

[9] Kim S.H., Lim K.P., Kim H.S., Differences in the hydrolysis of lactose and other substrates by ?-D-galactosidase from *Kluyveromyces lactis*, J. Dairy Sci. 80 (1997) 2264–2269.

[10] Kreft M.E., Jelen P., Stability and activity of β-galactosidase in sonicated cultures of *Lactobacillus delbrueckii* ssp. *bulgaricus* 11842 as affected by temperature and ionic environments, J. Food Sci. 65 (2000) 1364–1368.

[11] Nijpels H.H., Evers P.H., Novak G., Ramet J.P., Application of cryoscopy for the measurement of enzymatic hydrolysis of lactose, J. Food Sci. 45 (1980) 1684–1687.

[12] Pellissier J.M., Two-sample hypothesis tests with numerical data, in: Exploring data using Microsoft Excel 5.0., Prentice-Hall, Inc., New Jersey, 1996, pp. 115–124.

[13] Prenosil J.E., Stuker E., Bourne J.R., Formation of oligosaccharides during enzymatic lactose hydrolysis, Part I: State of Art, Biotechnol. Bioeng. 30 (1987) 1019–1025.

[14] Shah N., Jelen P., Survival of lactic acid bacteria and their lactases under acidic conditions, J. Food Sci. 55 (1990) 506–509

[15] Shah N., Jelen P., Lactase activity and properties of sonicated dairy cultures, Milchwissenschaft 46 (1991) 570–573.

[16] Smart J., Richardson B., Molecular properties and sensitivity to cations of β-galactosidase from *Streptococcus thermophilus* with four enzyme substrates, Appl. Microbiol. Biotechnol. 26 (1987) 177–185.

[17] Smart J.B., Pilldige C.J., Garman J.H., Growth of lactic acid bacteria and bifidobacteria on lactose and lactose-related mono-, di- and trisaccharides and correlation with distribution of β-galactosidase and phospho-β-galactosidase, J. Dairy Res. 60 (1993) 557–568.

[18] Wierzbicki L.E., Kosikowski F.V., Lactase potential of various micro-organisms grown in whey, J. Dairy Sci. 56 (1972) 26–31.

[19] Wierzbicki L.E., Kosikowski F.V., Kinetics of lactose hydrolysis in acid whey by β-galactosidase from *Aspergillus niger*, J. Dairy Sci. 56 (1972) 1396–1399.

[20] Wierzbicki L.E., Kosikowski F.V., Formation of oligosaccharides during β-galactosidase action on lactose, J. Dairy Sci. 56 (1972) 1400–1403.

[21] Zarb J.M., Hourigan J.A., An enzymic, cryoscopic method for the estimation of lactose in milk products, Aust. J. Dairy Technol. 34 (1979) 184–186.