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Extraction and characterisation of β-galactosidase produced by *Bifidobacterium animalis* spp. *lactis* Bb12 and *Lactobacillus delbrueckii* spp. *bulgaricus* ATCC 11842 grown in whey

[1]Prasad, L. N., [2]Ghosh, B. C., [3]Sherkat, F. and [4*]Shah, N. P.

[1]*School of Biomedical and Health Sciences, Victoria University, Werribee Campus, P. O. Box 14428, Victoria 8001, Australia*
[2]*National Dairy Research Institute, India*
[3]*School of Applied Sciences, RMIT University, Melbourne, Victoria 3001, Australia*
[4]*Food and Nutritional Science-School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong*

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Abstract

This study investigated the production of β-Galactosidase (β-gal) by *Bifidobacterium animalis* ssp. lactis Bb12 and *Lactobacillus delbrueckii* ssp. *bulgaricus* ATCC 11842 in whey and the effect of four different extraction methods i.e. sonication, acetone-toluene, SDS-chloroform and lysozyme-EDTA treatment on enzyme activity from these organisms. Both organisms were grown in deproteinised whey containing yeast extract (3.0 g/L), peptone (5.0 g/L) and glucose (10.0 g/L) for 18 h, at 37 ºC for *B. animalis* ssp. *lactis* Bb12 and at 45ºC for *L. delbrueckii* ssp. *bulgaricus* ATCC 11842. The optimum intracelluar β-gal activity on 15 mM o-nitrophenyl β-D-galactopyranoside (ONPG) assay was at pH 6.8 for both organisms irrespective of the method of extraction used. Also, the effect of temperature on enzyme activity was studied at various temperatures (30, 35, 40, 45, and 50°C). At 35°C and 40°C, *B. animalis* ssp. *lactis* Bb12 exhibited more intracellular β-gal activity extracted by sonication than other temperatures and methods. However, *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 showed more intracellular β-gal activity at 35°C and 45°C when extracted by lysozyme-EDTA treatment. Among the four methods used for β-gal extraction, sonication gave the best result (6.80 Unit/mL) for *B. animalis* ssp. *lactis* Bb12 while lysozyme-EDTA treatment was found to be the best (7.77 Unit/mL) for *L. delbrueckii* ssp. *bulgaricus* ATCC 11842.

Introduction

β-Gal; lactase, EC 3.2.1.23) catalyzes the hydrolysis of lactose to glucose and galactose. This enzyme is used to hydrolyse milk lactose to combat the problems of lactose intolerance by individuals who are deficient in lactase (Artolozaga *et al*., 1998). Commercial β-gal is produced from bacteria (such as *Streptococcus thermophilus* and *Lactobaccillus lactis*); yeasts (such as *Kluyveromyces lactis* and *Kluyveromyces marxianus*) and moulds (such as *Aspergillus niger*, *Aspergillus candidus* and *Aspergillus oryzae* (Panesar *et al*., 2006; Zheng *et al*., 2006). Since β-gal is an intracellular enzyme, one of the major hindrances in effective production of this enzyme is its release in sufficient quantities from cells. The use of whole cells as a source of β-gal may appear as a good alternative, however, a major drawback is the poor permeability of cell wall membrane. Therefore, different methods have been applied to increase their permeability of microbial cell walls (Panesar *et al*., 2006).

Several workers have reported on the release of β-gal through permeabilization of microbial cells by organic solvents (Flores *et al*., 1994; Numanoglu and Sungur, 2004; Panesar *et al*., 2007; Park *et al*., 2007). Flores et al. (1994) studied the permeabilization of *K. lactis* cells by chloroform, toluene and ethanol to release β-gal enzyme. They found that the effectiveness of solvents was dependent on the incubation time, incubation temperature and concentration of both cells and solvents. Mechanical methods such as sonication, high-pressure homogenizer or bead mills have been traditionally used for the disruption of microbial cells (Geciova *et al*., 2000). The method of choice should be robust enough to disrupt cell membranes efficiently but gentle enough to preserve enzyme activity (Numanoglu and Sungur, 2004).

Sonication is one of the most widely used methods for disruption of the bacterial cell walls (Engler,

*Corresponding author.
Email: *npshah@hku.hk*

1985). Among the three methods, sonication, bead milling and high-pressure homogenizer, sonication was found to be more effective for releasing β-gal (Toba *et al*., 1990; Sakakibara *et al*., 1994). Berger *et al*. (1995) compared two physical disruption methods for the extraction of intracellular β-gal enzyme from *Thermus* species and found that the sonication was superior to the glass-bead milling. Bury *et al*. (2001) studied on the disruption of cells of *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 who concluded that sonication was the least effective method on the release of β-gal.

Salasbury (1989) found that lysozyme is often used for lysis of peptidoglycan layers as it catalyses hydrolysis of β 1-4-glycosidic bonds. The enzyme is commercially available at a reasonable cost, and is produced from egg-white preparations. Gram-negative bacteria are less susceptible than the Gram-positive ones as their outer layer made of peptidoglycan, is responsible for rigidity of bacterial cell wall and for determination of cell shape. It is made up of a polysaccharide backbone consisting of alternating N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) residues in equal amounts. However, combining lysozyme-EDTA treatment allows the disruption of the cell wall and subsequent attack on the peptidoglycan structure (Salasbury, 1989). Therefore, lysozyme-EDTA mixture is very efficient for releasing β-gal from Gram-negative bacteria cell walls (Andrews and Asenjo, 1987; Geciova *et al*., 2000).

Numanoglu and Sungur (2004) compared chemical (toluene, SDS-chloroform) and physical (glass bead mill) methods to facilitate the release of β-gal from *K. lactis* cells and found that the physical method was better than chemical ones. This was in agreement with Fiedurek and Szczodrak (1994) who used three methods such as solvent and detergent extraction, freezing and thawing extraction, and mechanical disintegration to release the β-gal from *K. fragilis* cells and found that the highest yield was obtained by mechanical disintegration.

The lactic acid bacteria (LAB) requires numerous growth factors such as whey, reconstituted skim milk (RSM) and MRS broth in addition to carbohydrate and nitrogen sources in a growth medium (Stiles and Holzapfel, 1997) to be used for the enzyme production. In search for a suitable and inexpensive medium is readily available components such as whey appear as an attractive alternative to RSM (Gupta and Gandhi, 1995; Bury *et al*., 2000). The β-gal activity of a given microorganism depends on the characteristics of a medium. To maximize the enzyme activity, a rich medium is necessary. Therefore, sweet whey appears highly attractive mostly due to relatively high lactose content. Lactose constitutes over 70% of the total solids in whey (Rhimi *et al*., 2007).

There are two types of whey; i) Sweet whey is produced during the producing of rennet types or hard cheeses like Cheddar or Swiss cheeses. ii) Acid whey (also known as "sour whey") is obtained during the production of acid types cheeses such as cottage cheese. Sweet whey is a rich source of whey proteins, lactose, enzymes, vitamins, bioactive compounds and minerals (Agrawal *et a*l., 1989; Joshi *et al*., 1989; Keerthana and Reddy, 2006). Many small-size cheese plants do not have proper treatment systems for the disposal of whey and the dumping of whey constitutes a significant loss of potential food as whey retains about 40-45% of total milk solids (Panesar *et al*., 2007). Its disposal as waste poses serious pollution problems for the surrounding environment (Carrara and Rubiolo, 1994; Dagbagli and Goksungur, 2008; Magalhaes *et al*., 2010a). Sweet syrup produced through lactose hydrolysis by β-gal can be used in dairy, confectionary, baking and soft drink industries (Mahoney, 1997; Rajakala *et al*., 2006). Other applications of β-gal could also include the production of biologically-active galacto-oligosaccharides from lactose hydrolysis (Boon *et al*., 2000; Albayrak and Yang, 2002).

The *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 was selected based on previous evidence as a high β-gal producer (Vasiljevic and Jelen, 2003). The *B. animalis* ssp. *lactis* Bb12 was found to possess the highest level of β-gal activity compared to others Bifidobacteria (Dechter and Hoover, 1998). Therefore, the present study was undertaken to evaluate the suitability of sweet whey as a medium for the production of β-gal from *B. animalis* ssp. *lactis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842. This study also evaluated physical and chemical methods of enzyme extraction from bacteria in terms of their efficacy and enzyme yield.

Materials and Methods

Micro-organisms

Pure culture of *B. animalis* Bb12 was obtained from Chr. Hansen, (Bayswater, VIC, Australia) and *L. delbrueckii* ssp. *bulgaricus* ATCC was obtained from Victoria University Culture Collection (Werribee, Victoria, Australia). The purity of the cultures was confirmed by Gram staining. The stock cultures were stored at -80ºC in 50/50 sterile MRS broth (Difco, Becton, Dickinson and Company, New Jersey, USA) and glycerol (MERCK Pty Ltd, Colchester Road, Kilsyth, Australia.

Culture growth conditions

The organisms were activated in two successive transfers in MRS broth supplemented with 0.05% L-cysteine (Sigma Chemical Company, St. Louis, MO, USA) and incubated at 37ºC for *B. animalis* ssp. *lactis* Bb12, and 45ºC for *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 for 18 h. Activated organisms were grown in deproteinized sweet whey supplemented with yeast extract (3.0 g/L), peptone (5 g/L) and glucose (10 g/L). The sweet whey was deproteinized by heating at 85ºC for 10 min after adjusting the pH to 4.5 using lactic acid. The heat-treated whey was cooled to room temperature and filtered through Whatman no. 1 filter paper. The pH of whey medium was then re-adjusted to 7.0 and sterilized at 121ºC for 15 min then inoculated aseptically with 1% of each organism and incubated at 37 ºC for *B. animalis* ssp. *lactis* Bb12 or 45ºC for *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 for 18 h under anaerobic conditions.

Enzyme extraction

After 18 h of incubation, the cells were harvested by centrifuging at 10,000×g for 10 min at 4°C. The supernatant was considered to be containing extracellular enzymes. The cell pellet was crushed and washed twice with a 0.03 M sodium phosphate buffer (pH 6.8) and centrifuged at 10,000×g for 10 min at 4°C. The washed pellets were resuspended in 5 mL of 0.2 M phosphate buffer (pH 6.8) for intracellular enzyme extraction using four different cell disintegration methods listed below:

Sonication: The cell suspensions were sonicated for 30 min in ice bath using Sonirep 150 MSE (MSE Instruments, Crawley, UK) sonicator according to the method of Beccerra et al. (1998). The extract was then centrifuged at 15,000×g and 4°C for 10 min and the supernatant containing the crude enzyme was stored at –20°C until used for enzyme assays.

Lysozyme-EDTA treatment: Lysozyme solution was prepared by dissolving 50 mg of lysozyme (Sigma Aldrich Pty Lim, Castle Hill NSW, Australia) in 1.5 mL of TE (Tris-EDTA; Ethylenediamine Tetraacetic Acid) buffer containing 1 mM EDTA and 10 mM Tris-HCl, adjusted to pH 8.0. The lysozyme preparation was added to the cell suspension at the rate of 75 µL per mL, incubated for 30 min at room temperature then kept at -200C until enzyme activity measurement.

Toluene-acetone treatment: Ten millilitre of cell suspension was ground for 10 min in a pestle and mortar with 2.0 g alumina (Sigma Aldrich Pty Lim, Castle Hill NSW, Australia) and 0.1 mL of 9:1 mixture of toluene (BDH Chemical, Pty Limited, Kilsyth, Vic, Australia with 99.5% purity) and acetone (Merck Pty Limited Kilsyth, Vic, Australia with 99% purity) solvents. The suspension was extended in 8 mL phosphate buffer and centrifuged at 15,000×g for 10 min at 4°C (Mahoney et al., 1975). The supernatant obtained was kept at -20^{0}C until used for enzyme assay.

Sodium Dodecyl Sulfate (SDS)-Chloroform treatment: Permeabilization of cell membrane was carried out by vortexing 10 mL of the cell suspension in the presence of 100 µL chloroform and 50 µL 0.1% SDS solution for 30 min at room temperature (Mahoney et al., 1975). The suspension was centrifuged at 15,000×g for 10 min at 4 °C and the supernatant was kept at -20^{0}C until needed for the enzyme assay.

Enzyme assay

The β-Gal was determined as described by Hsu *et al*. (2005). The reaction mixture was composed of 0.5 mL of supernatant containing extracted enzyme and 0.5 mL of 15 mM o-nitrophenyl β-D-galactopyranoside (ONPG) in 0.03 M sodium phosphate buffer (pH 6.8). After incubation for 10 min at 37°C, 2.0 mL of 0.1 M sodium carbonate was added to the mixture to stop the reaction. Absorbance was measured at 420 nm with a spectrophotometer (Model Helios R, Unicam Co., Cambridge, UK). One unit of β-gal was defined as the amount of enzyme that produced one micro-mol (µM) of o-nitrophenol per min under the assay condition.

Effect of pH and temperature on β-Gal activity

The intracellular β-gal extracted by four different methods were characterised for their optimum activity by incubating the enzyme in substrate of 15 mM o-nitrophenyl β-D-galactopyranoside (ONPG) adjusted at three levels of assay pH (4.5, 5.5 and 6.8) with 2N NaOH, or 3N HCL in 0.03 M sodium phosphate buffer for 10 min at 37ºC. Similarly, the effect of temperature on enzyme activity was studied by incubating the enzyme in above mentioned substrate at various temperatures (30, 35, 40, 45 and 50ºC) for 10 min at pH 6.8.

Statistical analysis

All analyses were performed in triplicate and data were analyzed using Statistical Analysis System (SAS) software (SAS, 1995) and one-way analysis of variance (ANOVA) at 5% confidence level. ANOVA data with a $P < 0.05$ were classified as statistically significant.

Results and Discussion

β-Gal production in whey and its extraction

The activity of β-gal from *B. animalis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 in whey and its extraction using various methods is shown in Table 1. *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 produced more ($p<0.05$) intracellular β-gal than *B. animalis* ssp. *lactis* Bb12 with all extraction methods, except sonication. There were significant ($p<0.05$) differences in β-gal levels extracted from each organism by the four extraction methods. Sonication method was found to be more effective for *B. animalis* Bb12 than the others methods, however, lysozyme-EDTA treatment was found to be more effective for *L. delbrueckii* ssp. *bulgaricus* ATCC 11842. The maximum intracellular β-gal activity (7.77 Unit/mL) was obtained from *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 by lysozyme treatment while the lowest activity (2.05 Unit/mL) was measured using toluene-acetone treatment. Similarly, this method resulted in the lowest activity (0.64 Unit/mL) from *B. animalis* Bb12 while the highest β-gal activity (6.80 Unit/mL) was obtained by sonication. However, lower intracellular β-gal activities (4.85 Unit/mL) and (1.58 Unit/mL) were obtained from *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 and *B. animalis* Bb12, respectively by SDS-chloroform treatment. Toluene-acetone treatment was not as effective as the SDS-chloroform method. SDS is a non-ionic detergent which works by disrupting non-covalent bonds in proteins, thereby denaturing them, causing the molecules to lose their native shape (Panesar *et al*., 2006). Chloroform is also a common solvent because it is relatively unreactive, miscible with most organic liquids, and conveniently volatile. It is an effective solvent for alkaloids in their base form and thus plant materials are commonly extracted with chloroform for pharmaceutical processing. Thus the action of SDS-chloroform mixture could be of synergistic nature resulting in efficient permeabilization of cell wall of yeast cells and subsequent release of the enzyme (Panesar *et al*., 2006).

Our findings agree with those of Berger *et al*. (1995) who found that sonication was more effective than high-pressure homogenization, bead milling and toluene-acetone treatments for the release of β-gal from *Thermus* species. However, our results are contrary to the finding by Bury *et al*. (2001) who concluded that sonication was the least effective method on the release of β-gal from *L. delbrueckii* ssp. *bulgaricus* ATCC 11842. Therefore in our study, sonication method was found to be more effective for *B. animalis* Bb12, while lysozyme-EDTA treatment was more effective for *L. delbrueckii* ssp. *bulgaricus* ATCC 11842.

Table 1. Effects of extraction methods on intracellular β-gal activity from *B. animalis* ssp. *lactis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 grown in whey for 18 h at 37 ºC

Methods	Lb ATCC 11842 (Unit/mL)	Bb12 (Unit/mL)
Sonication	3.09±0.34^{A}	6.80±0.35^{A}
Toluene-Acetone	2.05±0.35^{D}	0.64±0.06^{D}
SDS-Chloroform	4.85±1.14^{C}	1.58±0.15^{C}
Lysozyme treatment	7.77±2.78^{B}	3.96±1.05^{B}

Results are expressed as mean ± SE (n=3). Data were analysed by means of 1-way ANOVA. Mean values in the same row with the same lowercase superscripts are not significantly different ($P > 0.05$). Mean values in the same column for a particular organism with the same uppercase letter are not significantly different ($P > 0.05$)

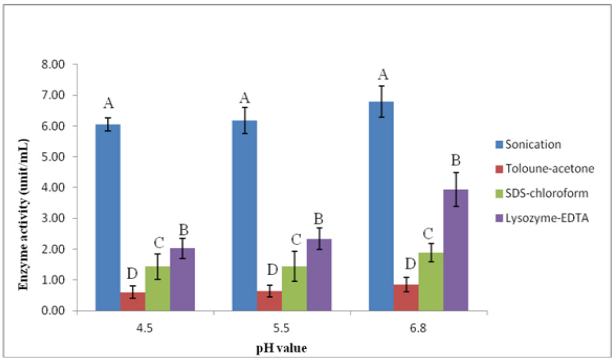

Figure 1. Effect of growth medium pH at 37 ºC on the activity of intracellular β-gal enzyme extracted by four extraction methods from *B. animalis* ssp. *lactis* Bb12. Bars indicate standard deviations. Different letters within each type of treatment indicate a significant difference ($p<0.05$). Mean values for a particular extraction method with same uppercase letters are not significantly different ($P>0.05$)

Effect of pH on the activity of intracellular enzyme extracted from B. animalis Bb12 and L. delbrueckii ssp. bulgaricus ATCC 11842

The optimum activity of the intracellular β-gal from *B. animalis* ssp. *lactis* Bb12 as extracted by four different methods and various assay pH levels ranging from (4.5, 5.5 and 6.8) is shown in Fig 1. The pH 6.8 was selected based on previous evidence as a high β-gal enzyme activity (Hsu *et al*., 2007). Among the four extraction methods employed for *B. animalis* ssp. *lactis* Bb12, sonication resulted in significantly ($p<0.05$) higher enzyme activity followed by lysozyme-EDTA treatment at pH 6.8. Enzyme from *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 also showed (Fig 2) its maximum activity at pH 6.8 where lysozyme and SDS-chloroform treatments extracted more ($p<0.05$) enzyme than the other two methods. The enzyme activity at pH 6.8 was significantly higher ($p<0.05$) than at other pH levels for the both organisms. Any drop in pH value of assay medium resulted in a reduction on β-gal enzyme activity by test organisms.

The maximum enzyme activity (7.77 Unit/mL) was obtained when *L. delbrueckii* ssp. *bulgaricus*

Table 2. Effect of assay temperature at pH 6.8 and extraction methods on intracellular β-gal activity extracted from *B. animalis* ssp. *lactis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842

	Lb ATCC 11842		Bb12	
Incubation Temp.	**Sonication** (Unit/mL)	**Lysozyme** (Unit/mL)	**Sonication** (Unit/mL)	**Lysozyme** (Unit/mL)
30 ˚C	1.23 ± 0.01^{Ca}	4.29 ± 0.06^{Cb}	3.00 ± 0.05^{Ca}	2.01 ± 0.02^{Cb}
35˚C	2.36 ± 0.02^{Aa}	7.35 ± 0.19^{Ab}	6.68 ± 0.11^{Aa}	2.13 ± 0.05^{Bb}
40˚C	2.22 ± 0.03^{Ab}	5.86 ± 0.13^{Ba}	5.67 ± 0.13^{Aa}	2.27 ± 0.02^{Ab}
45˚C	1.93 ± 0.02^{Bb}	7.45 ± 0.08^{Aa}	3.82 ± 0.05^{Ba}	1.34 ± 0.05^{Db}
50˚C	1.40 ± 0.01^{Cb}	7.25 ± 0.04^{Aa}	2.87 ± 0.04^{Ca}	1.33 ± 0.02^{Db}

Results are expressed as mean ± SE (n=3). Data were analysed by means of 1-way ANOVA. Means values in the same row with the same lowercase superscripts are not significantly different (P > 0.05). Mean values in the same column for a particular organism with the same uppercase letter are not significantly different (P > 0.05)

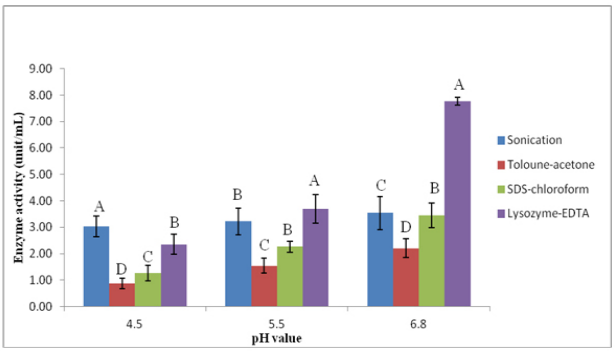

Figure 2. Effect of growth medium pH at 37 ºC on the activity of intracellular β-gal enzyme extracted by four extraction methods from *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 Bars indicate standard deviations. Different letters within each type of treatment indicate a significant difference (p<0.05). Mean values for a particular extraction method with same uppercase letters are not significantly different (P>0.05)

ATCC 11842 was treated with lysozyme-EDTA mixture (Fig 2). Lower enzyme activities were found when SDS-chloroform (4.85 Unit/mL), sonication (3.09 Unit/mL) and toluene-acetone (2.05 Unit/mL) were used. Therefore, our results revealed at pH 6.8, β-gal activity was found to be at its peak for both organisms.

These findings agree with those of Greenberg and Mahoney (1982); Nagy *et al*. (2001) who reported that β-gal enzyme activity was found to be higher at pH 6.5 to 7.5 at 37˚C from *B. animalis*, but it appeared to be detrimental effect as enzyme is rapidly loose its activity at lower and higher of this range. Various workers reported that β-gal activity was affected by metallic ions (Hung and Lee, 2000; Kim *et al*., 2003). Moreover, Wang *et al*. (2004) also reported that the highest enzyme activity was observed in the pH range of 6.7 to 7.5.

Effect of temperature on intracellular enzyme activity extracted from B. animalis ssp. lactis Bb12 and L. delbrueckii ssp. bulgaricus ATCC 11842

Based on maximum enzyme activity results obtained for *B. animalis* ssp. *lactis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 (Table 1), only the sonication and lysozyme-EDTA methods were chosen for the study on the effect of temperature on intracellular β-gal enzyme activity extracted from these organisms (Table 2). Subsequently, the enzyme extracted from each organism was incubated at various temperatures (30, 35, 40, 45 and 50ºC) for 10 min at pH 6.8.

Intracellular β-gal enzyme extracted by sonication and lysozyme-EDTA treatment from *B. animalis* ssp. *lactis* Bb12 showed significantly (p<0.05) higher activity at 35°C and 40°C than other temperatures (Table 2), whereas, β-gal extracted from *L. delbrueckii* ssp. *bulgaricus* showed its maximum activity (p<0.05) at 35 to 45°C (Table 2). There was a significant difference (p<0.05) in β-gal production by *B. animalis* Bb12 assay temperatures at 30°C, 45°C and 50°C using sonication method while no such difference was observed at 35°C and 40°C. However, lysozyme treatment showed a significant difference (p>0.05) in β-gal production by *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 at 30°C, 35°C and 40°C but no difference at 45°C and 50°C.

The maximum enzyme activity of 6.68 Unit/mL from *B. animalis* ssp. *lactis* Bb12 was obtained by sonication at 35 ºC whereas the maximum enzyme activity of 7.45 Unit/mL from *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 was obtained by lysozyme-EDTA treatment at 45 ºC (Table 2).

Many workers have reported 37 to 45ºC as the optimum temperature range for maximum enzyme activity with different organisms (Tzortzis *et al*., 2005; Splechtna *et al*., 2006; Searle *et al*., 2009). The maximum β-gal enzyme activity from *S. thermophilus* (Somkuti and Steinberg, 1979), *B. infantis* HL96 (Hung and Lee, 2002) and *Penicillium chrysogenum* (Nagy *et al*., 2001) was obtained at 35-50 ºC. Our results also revealed that β-gal extracted by sonication and lysozyme-EDTA treatment showed higher activity at temperature range of 35 to 45°C. Further increase in temperature beyond 50°C resulted in reduction in enzyme activity. Most enzymes denatured rapidly at temperatures above 55˚ C (Bryan and Keith, 1981). Itoh *et al*. (1992); Cho *et al*. (2003) have shown that the activity of the enzyme reduced rapidly at or above 50 ºC with no activity detected beyond 60 ºC for 10 min.

Conclusion

Among the four extraction methods, sonication was found to be more effective for *B. animalis* ssp. *lactis* Bb12, whereas lysozyme-EDTA treatment was found to be more effective for *L. delbrueckii*

ssp. *bulgaricus* ATCC 11842. The enzyme activity at pH 6.8 was significantly higher ($P<0.05$) than at other pH levels for both the organisms. The optimum temperature for the activity of enzyme obtained from *B. animalis* ssp. *lactis* Bb12 was found to be at 35ºC whereas for *L. delbrueckii* ssp. bulgaricus ATCC 11842 it was 45ºC. Deproteinised sweet whey was found to be a suitable medium for β-gal production, it should be possible to produce commercial amounts of β-gal using the two organisms reported in this study, however the enzyme extraction method need to be adapted to the strain used.

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