International Food Research Journal 18: 373-380 (2011)

Effect of carbon and nitrogen sources on growth of *Bifidobacterium animalis* Bb12 and *Lactobacillus delbrueckii* ssp. *bulgaricus* ATCC 11842 and production of β-galactosidase under different culture conditions

Laxmi, N. P., Mutamed, M. A. and Nagendra, P. S.

School of Biomedical and Health Sciences, Faculty of Health, Engineering and Science, Victoria University, Werribee Campus, P. O. Box 14428, Victoria 8001, Australia

Abstract: In this study, the effect of various carbon sources such as lactose, glucose and galactose and nitrogen sources such as yeast extract, peptone, casein hydrolysate, tryptone, ammonium sulphate and MRS broth (control) on growth of *Bifidobacterium animalis* BB12 and *Lactobacillus delbrueckii* ssp. *bulgaricus* and production of β-galactosidase (β-gal) by these organisms were evaluated. The medium for carbon source contained 3.5% yeast extract, 0.3% K_2HPO_4, 0.1% KH_2PO_4, 0.05% $MgSO_4.7H_2O$, 0.03% L-cysteine and 4% of lactose, glucose or galactose was supplemented as a carbon source. Similarly, the medium for nitrogen source contained 4% lactose, 0.3% K_2HPO_4, 0.1% KH_2PO_4, 0.05% $MgSO_4.7H_2O$, 0.03% L-cysteine and 3.5% of yeast extract, peptone, casein hydrolysate, tryptone, ammonium sulphate or MRS broth as a nitrogen source was added. In general, lactose, glucose and galactose were found to be suitable for β-galactosidase production. The highest level of β-gal activity of 73.66 unit/mL was produced by *B. animalis* Bb12 and 48.63 unit/mL by *L. delbrueckii* spp. *bulgaricus* ATCC 11842 in the presence of galactose as the carbon source. The strains were able to utilize a wide range of nitrogen sources such as yeast extract, peptone, casein hydrolysate, tryptone and ammonium sulphate. *B. animalis* Bb12 produced the highest level of β-gal in MRS broth and yeast extract produced the highest level of β-gal by *L. delbrueckii* spp. *bulgaricus* ATCC 11842.

Keywords: β-Galactosidase, *Bifidobacterium*, *Lactobacillus*, β-galactosidase, carbon and nitrogen sources

Introduction

In the manufacture of dairy products, β-galactosidase (β-gal) has been extensively used to hydrolyse lactose into glucose and galactose (Mahoney, 1998). *Lactobacillus delbrueckii* ssp. *bulgaricus* ATCC 11842 is particularly a promising microorganism for production of β-gal (Vasiljevic *et al*., 2005), and has a commercial importance in food and pharmaceutical industries (Somkuti and Holsinger, 1997). It was shown that β-gal could hydrolyse β-galactosidic bond in β-lactose (Huber *et al*., 1981). Lactose can be hydrolysed with β-gal to avoid lactose crystallization in frozen concentrated deserts and milk consumption by lactose-intolerant individuals can be improved (Shah, 1993; Shah *et al*., 1993; Kim and Rajagopal, 2000). In addition to this, lactose acts as a galactosyl donor and an acceptor to form di-, tri-, or higher galactooligosaccharides (Wallenfels and Weil, 1972; Prenosil *et al*., 1987). Furthermore, β-gal has been found in abundant in biological systems and micro-organisms such as yeasts, molds and bacteria still remain the only commercially exploited sources (Agrawal *et al*., 1989). *Bifidobacterium* and *Lactobacillus Bifidobacterium* have been used for their probiotic properties (Shah, 2007). *Bifidobacterium* and *Lactobacillus* have become organisms of interest for commercial production of β-gal (Shah and Jelen, 1990). Moreover, most of the β-gal are not accepted for food use, are costly and many are not available in adequate quantities for industrial application (Kim and Rajagopal, 2000; Albayrak and Yang, 2002). In addition to this, not many studies have been carried out recently for economical production of β-gal. Hence, selection of micro-organisms which are safe for human use and are capable of producing high level of β-gal becomes vital. In the present study, *B. animalis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 were used for the production of β-gal under various growth conditions in various carbon and nitrogen sources.

Materials and Methods

Micro-organisms

Pure cultures of *B. animalis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 were 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 sterile MRS broth (50% w/v) containing 50% glycerol.

*Corresponding author.
Email: *Nagendra. Shah@vu.edu.au*
Tel: +613 9919 8289; Fax: +613 9919 8284

© All Rights Reserved

Culture condition

The organisms were activated in two successive transfers in lactobacilli MRS broth (Difco, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) supplemented with 0.05% L-cysteine (Sigma Chemical Company, St. Louis, MO, USA) incubated at 37ºC for *B. animalis* Bb12, and 45ºC for *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 for 18 h. The activated cultures were again inoculated into MRS broth and inoculated at 37ºC for *B. animalis* Bb12 and 45ºC for *L. delbrueckii* spp. *bulgaricus* ATCC 11842 for 18 h. For production of β-gal, 1 mL of culture was transferred to a medium that contained 4% lactose, 0.3% K_2HPO_4, 0.1% KH_2PO_4, 0.05% $MgSO_4.7H_2O$ and 0.03% L-cysteine. In order to examine the effect of various nitrogen sources on β-gal production, 3.5% of each of yeast extract, peptone, casein hydrolysate, tryptone or ammonium sulphate was added individually in the medium. MRS broth was used as a control. Similarly, 1 mL of culture was transferred to a medium that contained 3.5% yeast extract, 0.3% K_2HPO_4, 0.1% KH_2PO_4, 0.05% $MgSO_4.7H_2O$ and 0.03% L-cysteine. To examine the effect of various carbon sources on β-gal production, 4% of glucose, lactose or galactose was added individually in the medium. All fermentation experiments were carried out for 12 h and culture was maintained at 37ºC for *B. animalis* Bb12 and 45ºC for *L. delbrueckii* ssp. *bulgaricus* ATCC 11842.

Production of β-galactosidase

For production of β-gal, cells of *B. animalis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 were first harvested by centrifugation (1252 x g for 20 min at 10ºC). The supernatant was discarded and cell pellets were collected. A total of 5 mL of 0.03 M sodium phosphate buffer (pH 6.8) was added to the cell pellets and vortexed thoroughly. Lysozyme at 75 µl per millilitre of cell pellet in TE buffer (1 mM EDTA and 10 mM Tris-HCl, pH 8.0) was used to release the enzyme from the organisms. β-Gal activity was determined according to the method of (Nagy *et al*., 2001). The reaction mixture consisted of 0.5 mL of crude enzyme (cells treated with lysozyme) and 0.5 mL of 15 mM o-nitrophenyl β-D-galactopyranoside (ONPG) in 0.03 M sodium phosphate buffer (pH 6.8). After 10 min at 37ºC, 2 mL of 0.1 M sodium carbonate was added to the reaction mixture to stop the reaction. Absorbance was measured at 420 nm with a spectrophotometer (Pharmacia, Biotech LKB-Novespec II, UV/VIS spectrophotometer, Ontario, Canada). A unit of β-gal was defined as the amount of enzyme that catalysed the formation of 1 µmol of o-nitrophenol from ONPG per gram of sample per min under the assay condition.

Determination of protein

Lowry method was used for protein quantification as described by (Waterberg and Mathews, 1984). Bovine serum albumin (Sigma) was used as a standard.

Enumeration of micro-organisms

For enumeration of *B. animalis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842, MRS agar supplemented with 1% D-glucose (w/v) was used. Peptone and water 0.15% (w/v) diluent was used to perform serial dilutions. Plates were incubated at 37ºC for *B. animalis* Bb12 and 45ºC for *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 for 72 h in an anaerobic jar (Becton Dickinson Microbiology System, Sparks, MD, USA) with a gas generating kit (Oxoid Ltd., Hamshire, UK). Plates showing 25 to 250 colonies were counted and results were expressed as colonies forming units (CFU) per millilitre of sample.

Determination of pH

The pH of the aliquots withdrawn every 6 h during the fermentation was monitored using a microprocessor pH meter (Merk Pty Limited, 207 Colchester Rd, Kilsyth 3137, Victoria, Australia) after calibrating with fresh pH 4.0 and 7.0 standard buffers.

Statistical analysis

All experiments were replicated three times. All analyses were performed in triplicate and data were analysed using one-way analysis of variance (ANOVA) at 5% significance level. Analyses were performed using SAS (SAS, 1995). ANOVA data with a $p < 0.05$ were classified as statistically significant.

Results and Discussion

Effect of carbon source on production of protein and β-gal by *B. animalis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842

The effect of different carbon sources on the production of protein by *B. animalis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 in presence of lactose, glucose and galactose as the sole carbon source is shown in Figure 1. In general, *B. animalis* Bb12 produced higher ($p<0.05$) protein content than *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 with various carbon source including lactose, glucose and galactose. Statistically, *B. animalis* Bb12 had significantly different protein content with lactose and glucose; however, there was no significant difference ($p>0.05$) in protein content between glucose and

galactose. However, *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 had no significant difference ($p>0.05$) in terms of protein content with various carbon source including lactose, glucose and galactose. *B. animalis* Bb12 produced the highest ($p<0.05$) amount of protein (0.28 mg/mL) with lactose; however, this organism produced similar level of protein content with galactose and glucose (0.24 mg/mL). The protein content increased ($p<0.05$) by 260.26, 105.08 and 218.42 percent in lactose, glucose and galactose, respectively at 12 h as compared with 0 h (data not shown). On the other hand, *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 produced similar level of protein (0.11 mg/mL) with lactose, glucose and galactose. At 12 h, the protein content increased by 98.25, 85.25 and 51.39 percent in lactose, glucose and galactose, respectively as compared with 0 h (data not shown). The protein concentration gradually increased during incubation. However, the level remained lowest with glucose and galactose in both organisms.

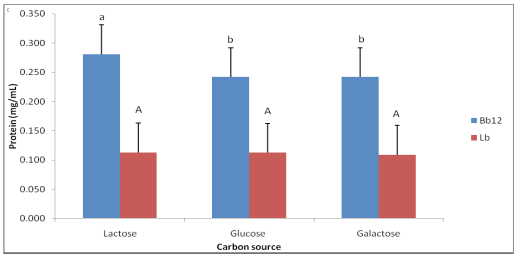

Figure 1. Effect of carbon source on the protein production by *B. animalis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842. Medium contained 3.5% yeast extract, 0.3% K_2HPO_4, 0.1% KH_2PO_4, 0.05% $MgSO_4.7H_2O$, 0.03% L-cysteine and 4% of various carbon sources including lactose, glucose and galactose. Determinations were made after a 12-h cultivation. Bars indicate standard deviations. Different letters within each type of determination indicate a significant difference ($p<0.05$)

Effect of carbon sources on the β-galactosidase production

The influence of various carbon sources including lactose, glucose and galactose on production of β-gal by *B. animalis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 is shown in Figure 2. In general, both organisms produced higher ($p<0.05$) β-gal in galactose than lactose and glucose and lower ($p<0.05$) β-gal in glucose than that in lactose and galactose. *B. animalis* Bb12 produced the highest ($p<0.05$) amount of β-gal with galactose (73.66 unit/mL) followed by lactose (57.04 unit/mL) and lowest activity with glucose (31.08 unit/mL). The β-gal production increased ($p<0.05$) by 33.39, 27.43 and 95.02 percent in lactose, glucose and galactose, respectively, at 12 h as compared with 0 h (data not shown). A similar pattern was seen with *L. delbrueckii* ssp. *bulgaricus* ATCC 11842, which produced the highest ($p<0.05$) amount of β-gal (48.63 unit/mL) with galactose followed by lactose (33.0 uni/mL) and lowest activity with glucose (28.9 unit/mL). At 12 h, the β-gal production increased ($p<0.05$) by 28.65, 33.29 and 124.32 percent in lactose, glucose and galactose, respectively, as compared with 0 h (data not shown).

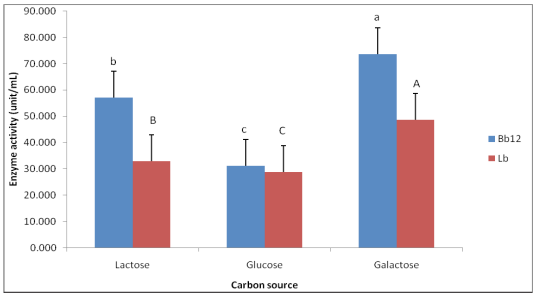

Figure 2. Effect of carbon source on the β-galactosidase production by *B. animalis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842. Medium contained 3.5% yeast extract, 0.3% K_2HPO_4, 0.1% KH_2PO_4, 0.05% $MgSO_4.7H_2O$, 0.03% L-cysteine and 4% of various carbon sources including lactose, glucose and galactose. Determinations were made after 12-h cultivation. Bars indicate standard deviations. Different letters within each type of determination indicate a significant difference ($p<0.05$)

A number of investigators have reported about the regulation of carbon source on β-gal biosynthesis in different micro-organisms (Fantes and Roberts 1973; Montero *et al*., 1989; Fiedurek and Szczodrak, 1994; Nikolaev and Vinetski, 1998; de Vries *et al*., 1999; Nagy *et al*., 2001; Fekete *et al*., 2002). All these authors have reported that the role of carbon source in the biosynthesis of β-gal may vary and depend on the micro-organisms tested. Kim and Rajagopal (2000) reported that *L. criptus* grown in MRS broth containing galactose as a carbon source showed the highest β-gal activity followed by moderate levels of enzyme production with lactose and a significant activity with glucose or maltose. The expression of β-gal by micro-organisms may be affected by the amount of carbon source in the medium (Fiedurek and Szczodrak, 1994; Incharurondo *et al*., 1998). According to Bergy's Manual of Systematic Bacteriology (John, 1986), *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 does not ferment galactose, however, it appears that increasing fermentation period resulted in an increase in β-gal in all carbon sources (lactose, glucose and galactose) in both strains at 12 h incubation period. However, galactose produced highest β-gal in both strains (Figure 2).

Effect of carbon sources on the growth

Figure 3 demonstrates the effect of carbon source including lactose, glucose and galactose on the growth of *B. animalis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842. In general, *B. animalis* Bb12 as well as *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 produced higher ($p<0.05$) final viable population in galactose than in lactose and glucose and lower ($p<0.05$) final viable population at glucose than the

lactose and galactose. It was found that *B. animalis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 had a significant difference ($p<0.05$) in the final viable population in various carbon sources. The amounts of carbon source in the medium may affect the expression of β-gal by micro-organisms (Fiedurek and Szczodrak, 1994; Inchaurrondo *et al*., 1998). The pattern showed an initial increase in viable population at the commencement of the fermentation followed by slower increasing trend towards the end of this process. A maximal population of 8.4 log CFU/mL, 7.8 log CFU/mL, 7.7 log CFU/mL in galactose, lactose and glucose, respectively, was reached in *B. animalis* Bb12 (Figure 3). Similarly, maximal population at 8.6 log CFU/mL, 7.8 log CFU/mL and 7.5 log CFU/mL in galactose, lactose and glucose, respectively, was reached in *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 (Figure 3). The final viable population of the *B. animalis* Bb12 ranged from 6.92 to 8.43 log CFU/mL and the organism showed the highest ($p<0.05$) viable population of 8.43 log CFU/mL with galactose followed by lactose (7.82 log CFU/mL) and lowest with glucose (7.75 log CFU/mL). The viable count increased ($p<0.05$) by 13.42, 11.82 and 17.65 percent in lactose, glucose and galactose, respectively, at 12 h as compared with 0 h (data not shown). Similarly, *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 showed a similar trend. The final viable population of *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 ranged from 6.67 to 8.6 log CFU/mL and the organism exhibited the highest ($p<0.05$) viable population of 8.6 log CFU/mL with galactose followed by lactose (7.87 CFU/mL) and lowest with glucose (7.5 log CFU/mL). At 12 h, the viable count increased ($p<0.05$) by 16.08, 12.44 and 26.84 percent in lactose, glucose and galactose, respectively, as compared with 0 h (data not shown).

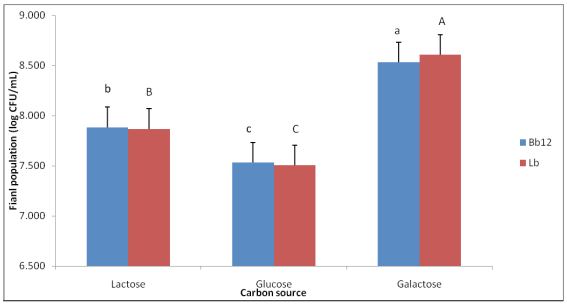

Figure 3. Effect of carbon source on the final population by *B. animalis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842. Medium contained 3.5% yeast extract, 0.3% K_2HPO_4, 0.1% KH_2PO_4, 0.05% $MgSO_4.7H_2O$, 0.03% L-cysteine and 4% of various carbon sources including lactose, glucose and galactose. Determinations were made after a 12-h cultivation. Bars indicate standard deviations. Different letters within each type of determination indicate a significant difference ($p<0.05$)

Effect of carbon sources on pH

The effect of carbon source on the pH value by *B. animalis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 is shown in Figure 4. In general, pH value in glucose was lower ($p<0.05$) as compared with other carbon sources. The pH value of *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 was significantly ($p<0.05$) higher in lactose than that with glucose and galactose. However, the pH value in glucose and galactose were not significant different ($p>0.05$). On the other hand, *B. animalis* Bb12 showed no significant difference ($p>0.05$) in terms of pH values in various carbon sources. The decrease in pH by *B. animalis* Bb12 was lowest with glucose at 4.63 followed by galactose (4.80) and lactose (4.83). The pH value decreased ($p<0.05$) by 23.82, 25.20 and 19.60 percent in lactose, glucose and galactose, respectively, at 12 h as compared with 0 h (data not shown). On the other hand, decrease in pH by *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 was lowest with galactose (pH 4.59) followed by glucose (pH 4.63) and lactose (pH 5.13). At 12 h, the pH value decreased ($p<0.05$) by 18.70, 25.32 and 26.79 percent in lactose, glucose and galactose, respectively, as compared with 0 h (data not shown). The drop in pH correlated with an increase in population of the two organisms.

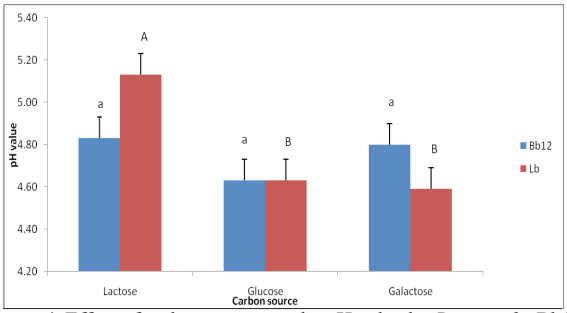

Figure 4. Effect of carbon source on the pH value by *B. animalis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842. Medium contained 3.5% yeast extract, 0.3% K_2HPO_4, 0.1% KH_2PO_4, 0.05% $MgSO_4.7H_2O$, 0.03% L-cysteine and 4% of various carbon sources including lactose, glucose and galactose. Determinations were made after a 12-h cultivation. Bars indicate standard deviations. Different letters within each type of determination indicate a significant difference ($p<0.05$)

Effect of nitrogen source on production of protein and β-gal by *B. animalis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842

Effect of nitrogen sources on the protein content

Effect of nitrogenous substrates including yeast extract, peptone, casein hydrolysate, tryptone, ammonium sulphate and MRS broth on production of protein by *B. animalis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 is shown in Figure 5. In general, *B. animalis* Bb12 produced higher ($p<0.05$) protein in yeast extract, ammonium sulphate and MRS broth MRS broth compared with other nitrogen sources. Similarly, *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 produced higher ($p<0.05$) protein in

MRS broth, tryptone and peptone than the other nitrogen sources. *B. animalis* Bb12 had significantly different protein content in yeast extract, peptone, casein hydrolysate and ammonium sulphate and there was no significant difference ($p>0.05$) in protein content between tryptone and MRS broth. Likewise, *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 had a significantly different protein content in yeast extract, peptone, tryptone and MRS broth and there was no significant difference ($p>0.05$) between casein hydrolysate and ammonium sulphate. Moreover, *B. animalis* Bb12 produced the highest amount of protein (0.17 mg/mL) with yeast extract followed by ammonium sulphate (0.15 mg/mL) and tryptone, casein hydrolysate gave the lowest protein with MRS broth (Figure 5). The protein content increased in 12 h ($p<0.05$) by 172.13, 34.55, 133.33, 58.46, 161.36 and 156.82 percent in yeast extract, peptone, casein hydrolysate, tryptone, ammonium sulphate and MRS broth, respectively, as compared with 0 h (data not shown). Similarly, *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 produced the highest amount of protein (0.13 mg/mL) with MRS broth followed by tryptone (0.11 mg/mL) and peptone. (Figure 5). At 12 h, the protein content increased ($p<0.05$) by 176.92, 177.42, 127.78, 322.22, 100.00 and 329.03 percent in yeast extract, peptone, casein hydrolysate, tryptone, ammonium sulphate and MRS broth, respectively as compared with 0 h (data not shown). The protein concentration gradually increased during incubation. However, the level remained lowest with ammonium sulphate. MRS broth is usually used to grow *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 due to its nutrient contents, hence the organism grew best in MRS broth.

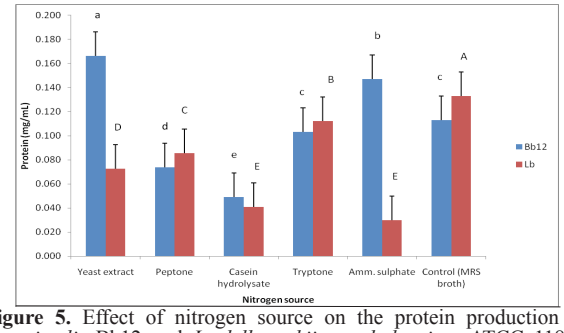

Figure 5. Effect of nitrogen source on the protein production by *B. animalis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842. Medium contained 4.0% lactose, 0.3% K_2HPO_4, 0.1% KH_2PO_4, 0.05% $MgSO_4.7H_2O$, 0.03% L-cysteine and 3.5% of various nitrogen sources including yeast extract, peptone, casein hydrolysate, tryptone, ammonium sulphate and MRS broth. Determinations were made after a 12-h cultivation. Bars indicate standard deviations. Different letters within each type of determination indicate a significant difference ($p<0.05$)

Effect of nitrogen sources on the β-galactosidase production

The influence of various nitrogen sources including yeast extract, peptone, casein hydrolysate, tryptone, ammonium sulphate and MRS broth on production of β-gal by *B. animalis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 is shown in Figure 6. In general, *B. animalis* Bb12 produced higher ($p<0.05$) β-gal in MRS broth and tryptone compared with other nitrogen sources. Similarly, *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 produced higher ($p<0.05$) β-gal production in casein hydrolysate and yeast extract than the other nitrogen sources. Statistically, *B. animalis* Bb12 had significantly different β-gal production in yeast extract, peptone, casein hydrolysate and MRS broth and no significant difference ($p>0.05$) in ammonium sulphate, casein hydrolysate and tryptone. Likewise, *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 had significantly different β-gal production in yeast extract, casein hydrolysate, ammonium sulphate and MRS broth and this was no significant difference ($p>0.05$) in β-gal production between peptone and tryptone. *B. animalis* Bb12 produced the highest amount of β-gal (51.69 unit/mL) with MRS broth followed by tryptone (45.48 unit/mL). The β-gal production increased ($p<0.05$) by 45.32, 44.20, 81.86, 106.43, 94.51 and 104.03 percent in yeast extract, peptone, casein hydrolysate, tryptone, ammonium sulphate and MRS broth, respectively at 12 h as compared with 0 h (data not shown). MRS broth provided optimum nutrients for this organism. Hence, the organism produced the highest level of β-gal. However, *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 produced the highest amount of β-gal (50.7 unit/mL) with casein hydrolysate followed by yeast extract (42.0 unit/mL) and lowest activity (23.15 unit/mL) with peptone (Figure 6). At 12 h, the β-gal production increased ($p<0.05$) by 106.30, 14.14, 122.17, 24.97, 60.07 and 43.93 percent in yeast extract, peptone, casein hydrolysate, tryptone, ammonium sulphate and MRS broth respectively as compared with 0 h (data not shown). This may be attributed to the peptides and amino acids present in casein hydrolysate. According to Rao and Dutta (1979) and Shaikh and *et al*., 1997), nitrogen sources may affect microbial biosynthesis of β-gal.

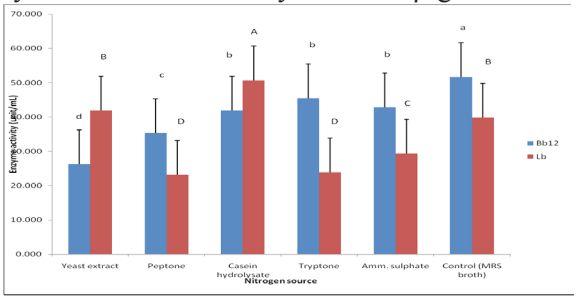

Figure 6. Effect of nitrogen source on the β-galactosidase production by *B. animalis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842. Medium contained 4.0% lactose, 0.3% K_2HPO_4, 0.1% KH_2PO_4, 0.05% $MgSO_4.7H_2O$, 0.03% L-cysteine and 3.5% of various nitrogen sources including yeast extract, peptone, casein hydrolysate, tryptone, ammonium sulphate and MRS broth. Determinations were made after a 12-h cultivation. Bars indicate standard deviations. Different letters within each type of determination indicate a significant difference ($p<0.05$)

Effect of nitrogen sources on the growth

Figure 7 demonstrates the effect of nitrogen source including yeast extract, peptone, casein hydrolysate, tryptone, ammonium sulphate and MRS broth on the growth of *B. animalis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842. In general, the viable counts were higher ($p<0.05$) in MRS broth and casein hydrolysate than other nitrogen source including yeast extract, peptone, tryptone and ammonium sulphate in both organisms (Figure 7).

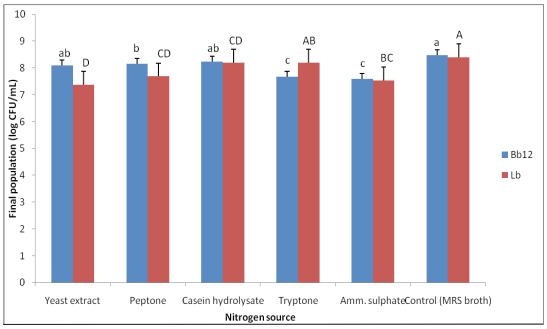

Figure 7. Effect of nitrogen source on the final population by *B. animalis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842. Medium contained 4.0% lactose, 0.3% K_2HPO_4, 0.1% KH_2PO_4, 0.05% $MgSO_4.7H_2O$, 0.03% L-cysteine and 3.5% of various nitrogen sources including yeast extract, peptone, casein hydrolysate, tryptone, ammonium sulphate and MRS broth. Determinations were made after a 12-h cultivation. Bars indicate standard deviations. Different letters within each type of determination indicate a significant difference ($p<0.05$)

The viable counts of *B. animalis* Bb12 were significantly ($p<0.05$) lower in ammonium sulphate and tryptone; however, yeast extract and ammonium sulphate showed significantly ($p<0.05$) lower viable counts for *L. delbrueckii* ssp. *bulgaricus* ATCC 11842. The final viable population of the *B. animalis* Bb12 ranged from 6.8 to 8.5 log CFU/mL and the organism showed the highest viable population of 8.5 log CFU/mL at 12 h with MRS broth followed by casein hydrolysate at 8.2 log CFU/mL and lowest with ammonium sulphate at 7.6 log CFU/mL. At 12 h, the viable count increased ($p<0.05$) by 7.57, 18.63, 17.09, 3.23, 2.29 and 11.33 percent in yeast extract, peptone, casein hydrolysate, tryptone, ammonium sulphate and MRS broth, respectively, compared with 0 h (data not shown). However, the final viable population of *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 ranged from 6.6 to 8.4 log CFU/mL and the organism showed the highest viable population of 8.4 log CFU/mL at 12 h with MRS broth followed by casein hydrolysate at 8.2 log CFU/mL and lowest with peptone at 6.6 CFU/mL. At 12 h, the viable count increased ($p<0.05$) by 7.67,14.78, 22.24, 18.70, 11.94 and 23.53 percent in yeast extract, peptone, casein hydrolysate, tryptone, ammonium sulphate and MRS broth, respectively, as compared with 0 h (data not shown). This may be attributed to the nutrients in addition to the nitrogen compounds present in MRS broth and casein hydrolysate.

Effect of nitrogen sources on the pH

The effect of nitrogen source including yeast extract, peptone, casein hydrolysate, tryptone, ammonium sulphate and MRS broth on the pH value by *B. animalis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 is shown in Figure 8. In general, pH value of MRS broth was lower ($p<0.05$) compared with other nitrogen sources for both organisms. The pH value of *B. animalis* Bb12 was significantly ($p<0.05$) higher in ammonium sulphate among other nitrogen sources. Similar pH values were higher in ammonium sulphate, tryptone, casein hydrolysate and peptone. The decrease in pH value by *B. animalis* Bb12 was lowest with MRS broth (4.24) followed by yeast extract (5.56) and highest with tryptone (6.69. The pH value decreased ($p<0.05$) by 33.85, 15.50 and 2.81 percent in MRS broth, yeast extract and tryptone, respectively, at 12 h as compared with 0 h (data not shown). Similarly, the decrease in pH by *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 was lowest with MRS broth (pH 4.26) followed by yeast extract (pH 5.27) and highest with tryptone (pH 6.18). The pH value decreased ($p<0.05$) by 35.16, 20.15 and 8.08 percent in MRS broth, yeast extract and tryptone, respectively, at 12 h as compared with 0 h (data not shown). The drop in pH is correlated with increase in population of the two organisms in MRS broth.

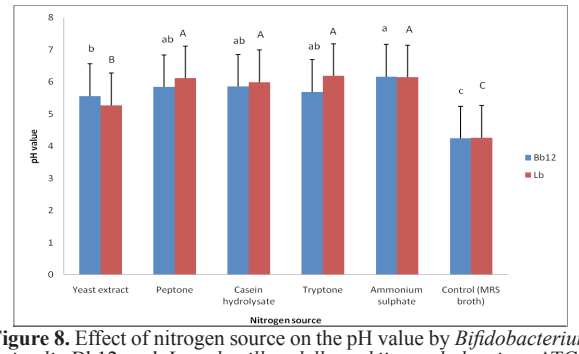

Figure 8. Effect of nitrogen source on the pH value by *Bifidobacterium animalis* Bb12 and *Lactobacillus delbrueckii* ssp. *bulgaricus* ATCC 11842. Medium contained 4.0% lactose, 0.3% K_2HPO_4, 0.1% KH_2PO_4, 0.05% $MgSO_4.7H_2O$, 0.03% L-cysteine and 3.5% of various nitrogen sources including yeast extract, peptone, casein hydrolysate, tryptone, ammonium sulphate and MRS broth. Determinations were made after a 12-h cultivation. Bars indicate standard deviations. Different letters within each type of determination indicate a significant difference ($p<0.05$)

Conclusion

The results of this study demonstrated that *B. animalis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 are capable of producing high level of β-gal. A maximum of β-gal of 73.66 unit/mL and 48.63 unit/mL was produced by *B. animalis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842, respectively in carbon source including lactose,

glucose and galactose. Similarly, maximum β-gal at 51.6 unit/mL and 50.7 unit/mL was produced by *B. animalis* Bb12 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842, respectively in nitrogen source including yeast extract, peptone, casein hydrolysate, tryptone, ammonium sulphate and MRS broth. Considering the high yield of β-gal with *B. animalis*, this organism may be a potential useful industrial strain for the production of β-gal.

References

Agrawal, S., Garg, S. K. and Dutta, S. M. 1989. Microbial β-galactosidase: production, properties and industrial applications. Indian Journal of Dairy Science 42: 251-262.

Albayrak, N. A. and Yang, S. T. 2002. Production of galacto-oligosaccharides from lactose by *Aspergillus oryzae* β-galactosidase immobilized on cotton cloth. Biotechnology Bioengineering 77: 8-19.

de Vries, R. P., van den Broek, H. C., Dekkers, E., Manzanares, P., de Graff, L. H. and Visser, J. 1999. Differential expression of three a-galactosidase genes and a single β-galactosidase gene from *Aspergillus niger*. Applied Environmental and Microbiology 65: 2453–2460.

Fantes, P. A. and Roberts, C. F. 1973. β-Galactosidase activity and lactose utilization in *Aspergillus nidulans*. Journal of General Microbiology 77: 471–486.

Fekete, E., Karaffa, L., Sandor, E., Seiboth, B., Biro, S., Szentirmai, A. and Kubicek, C. P. 2002. Regulation of formation of the intracellular β-galactosidase activity of *Aspergillus nidulans*. Achieves Microbiology 179: 7–14.

Fiedurek, J. and Szczodrak, J. 1994. Selection of strain, culture conditions and extraction procedures for optimum production of β-galactosidase from *Kluyveromyces fragilis*. Acta Microbiology Pol 43: 57–65.

Huber, R. E., Hurlburt, K. L. and Turner, C. L. 1981. The anomeric specificity of β-galactosidase and lac permease from *Escherichia coli*. Canadian Journal of Biochemistry 59: 100-105.

Inchaurrondo, V. A., Flores, M. V. and Voget, C. E. 1998. Growth and β-galactosidase synthesis in aerobic chemostat cultures of *Kluyveromyces lactis*. Journal of Indian Microbiology Biotechnology 20: 291-298.

John, G. H. 1986. Bergy's Manual of Systematic Bacteriology Vol 2, Ist Eds, p. 1430-1433.

Kim, J. W. and Rajagopal, S. N. 2000. Isolation and characterization of β-galactosidase from *Lactobacillus crispatus*. Folia Microbiology 45: 29–34.

Mahoney, R. R. 1998. Galactosyl-oligosaccharide formation during lactose hydrolysis: a review. Food Chemistry 63: 57-65.

Montero, S., de Arriaga, D., Busto, F. and Soler, J. 1989. Induction of intracellular and extracellular β-galactosidase activity in *Phycomyces blakesleeanus.* Biochemistry International 18: 637– 645.

Nagy, Z., Keresztessy, Z., Szentirmai, A. and Biro, S. 2001. Carbon source regulation of β-galactosidase biosynthesis in *Penicillium chrysogenum*. Journal of Basic Microbiology 41: 351–362.

Nikolaev, I. V. and Vinetski, Y. P. 1998. l-Arabinose induces synthesis of secreted β-galactosidase in the filamentous fungus *Penicillium canescens*. Biochem (Moscow) 63: 1294–1298.

Prenosil, J. E., Stuker, E. and Bourne, J. R. 1987. Formation of oligosaccharides during enzymatic lactose: Biotechnology Bioengineering 30: 1019–1025.

Rao, M. V. and Dutta, S. M. 1979. An active beta-galactosidase preparation from *Streptococcus thermophilus*. Indian Journal of Dairy Science 32:187.

Shah, N.P. 1993. Effectiveness of dairy products in alleviation of lactose intolerance. Food Australia 45(6):262-265.

Shah, N.P. 2007. Functional cultures and health benefits. International Dairy Journal 17(11):1262-1277.

Shah, N. P. and Jelen, P. 1990. Survival of lactic acid bacteria and their lactases under acidic conditions. Journal of Food Science 55 (2): 506-509.

Shah, N.P., Spurgeon, KR., and Gilmore, T. 1993. Use of dry whey and lactose hydrolysis in yogurt bases. Milchwissenschaft 49(9):494-498.

Shaikh, S. A., Khire, J. M. and Khan, M. I. 1997. Production of β-galactosidase from thermophilic fungus *Rhizomucor* ssp. Journal of Indian Microbiology Biotechnology 19: 239–245.

Somkuti, G. A. and Holsinger, V. H. 1997. Microbial technologies in the production of low- lactose dairy foods. Food Science and Technology International 3: 163-169.

Vasiljevic, T., Shah, N.P., and Jelen, P. 2005. Growth characteristic of *Lactoabcillus delbrueckii* ssp. *bulgaricus* ATCC 11842 as affected by different neutralisers. Australian Journal of Dairy Technology

60(1):3-9.

Wallenfels, K. and Weil, R. 1972. β-Galactosidase. In: Boyer, P.D. (Ed.), The Enzymes, vol. 7. Academic Press, New York, p. 617.

Waterborg, J. H. and Matthews, H. R. 1984. The Burton Assay for DNA, in Methods in Molecular Biology, voL 2: Nucleic Acids (Walker, J. M., ed.), Humana, Totowa, NJ, p. 1-3.