Effect of Yeast Extract Supplementation on β-Galactosidase Activity of *Lactobacillus delbrueckii* subsp. *bulgaricus* 11842 Grown in Whey

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Abstract

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Lactobacillus delbrueckii subsp. *bulgaricus* 11842 (LB 11842) was grown in reconstituted sweet whey supplemented with 0 to 1% yeast extract. The β-galactosidase activity was determined from the hydrolysis of *o*-nitrophenyl-β-D-galactopyranoside (ONPG) by sonicated cultures. Shake flask cultures containing 0.2–0.8% yeast extract gave 2.5 times higher β-galactosidase activity than LB 11842 grown in sole (intact) whey. When supplementation with 1% yeast extract resulted even in a threefold increase of β-galactosidase activity. The rate of acid production increased with the addition of yeast extract, while the increase in the total cell count with increasing amount of yeast extract was slight. A maximum β-galactosidase activity of 1.08 ± 0.15 μmol ONP released per min per ml of culture was obtained using a whey based growth medium supplemented with 1% yeast extract.

Keywords: β-galactosidase; *Lactobacillus delbrueckii* subsp. *bulgaricus* 11842; yeast extract; whey

Current industrially applicable methods of lactose hydrolysis involve the use of β-galactosidase (EC 3.2.1.23) purified from bacteria, yeasts, or fungi. The addition of soluble β-galactosidase to milk or whey is simple but it can be expensive due to the high costs of the enzyme purification. Other, more cost effective approaches (MAHONEY 1997) may involve immobilization of purified β-galactosidase by entrapping in cellulose acetate fibers, adsorbing to phenol-formaldehyde resin, covalently binding to porous glass beads, or using crude enzyme preparations obtained by culturing and subsequent disruption of high enzyme producing microorganism such as LB 11842 (JELEN 1993; SHAH & JELEN 1990, 1991; KREFT & JELEN 2000).

Although *Lactobacillus delbrueckii* subsp. *bulgaricus* 11842 (LB 11842) grows very well on MRS Broth (DE MAN *et al*. 1960) or other complex broths, an inexpensive and readily available growth medium, such as whey, would be desirable for fermentation on an industrial scale. LB 11842 is able to grow on sweet cheese whey or whey permeate, but the fermentation time can be substantially shortened with complex growth supplements. The rate of growth and lactic acid production by lactic acid bacteria grown in whey can be increased by addition of corn steep liquor (COX & MACBEAN 1977; REDDY *et al*. 1976), molasses (GUPTA & GANDHI 1995; CHIARINI *et al.* 1992), whey protein concentrate (BURY *et al*. 1998, 1999), meat extract (ABD EL HAFEZ *et al*. 1994), or yeast extract (GUPTA & GANDHI 1995; ABD EL HAFEZ *et al*. 1994; PARENTE & ZOTTOLA 1991; COX & MACBEAN 1977; REDDY *et al*. 1976). However, the effectiveness of the supplementation for the production of β-galactosidase has not been studied to any great extent. GECIOVA (1999), in studies with LB 11842, used a whey based cultivation media supplemented with a whey protein concentrate and a yeast extract as specified by BURY *et al*. (1999). The strain reached the maximal cell count (3.1×10^8) after 6 hours of fermentation (pH controlled, 1.5 l)

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while the maximal β-galactosidase activity (measured by freezing point depression was obtained after 8 hrs.

The purpose of the present study was to examine the production of active β-galactosidase LB 11842 grown in sweet whey supplemented with various amounts of yeast extract.

MATERIALS AND METHODS

Microorganisms and Media: LB 11842 was obtained from University of Alberta, Department of Agricultural, Food and Nutritional Science. The culture was propagated every two weeks in sterile MRS broth (Difco, Detroit, MI) enriched with 1% (w/v) α-lactose (Fisher Scientific Co., New Jersey). To prepare the culture for fermentation in the whey based broth, about 0.5 ml of refrigerated culture was added to 5 ml of the above MRS/lactose broth. After incubation at 43°C for 8 to 12 hrs, 1 ml of active culture was used to inoculate 150 ml of sterile broth containing 3% (w/v) reconstituted whey powder and 1.3% (w/v) MRS powder (WMRS broth). This culture was incubated at 43 ± 1°C with shaking at 140 ± 30 RPM in a controlled environmental incubator shaker (New Brunswick Co., USA) for 10–12 hrs.

The whey based broths used for the fermentations were made by dissolving 6 g spray dried whey powder (Kraft, Montreal) per 100 ml of distilled water and adding the desired amount of powdered yeast extract (Fermtech, BDH Inc., Toronto). Flasks containing 150 ml of this whey-based broth were autoclaved at 121°C for 15 min.

Fermentation: Flasks containing 150 ml of sterile whey based broth supplemented with 0, 0.2, 0.4, 0.6, 0.8 or 1% yeast extract (YE) were inoculated with 5 ml of the culture grown in WMRS broth. The culture was grown in the incubator shaker at 40–43°C and 140 ± 30 RPM. The pH was monitored using a combination pH probe sterilized with 70% ethanol and maintained at pH 5.5 ± 0.3 by the manual addition of 2M NaOH (Fisher Scientific Co, Inc., USA). Cell growth was followed by direct microscopic enumeration and the rate of acid production was determined as the consumption of 2M NaOH.

Measuring β-galactosidase Activity: A method of measuring β-galactosidase activity using *o*-nitrophenyl-β-D-galactopyranoside (ONPG) described by MAHONEY *et al.* (1975) was adapted as follows.

Approximately 5 ml of culture was sonicated in a 15 ml polypropylene centrifuge tube using the intermediate tip of a Sonic 300 dismembrator (Artek Systems Corporation, Farmingdale, USA) at 60% intensity. The sample was cooled using an ice water bath to prevent activity loss (SHAH & JELEN 1990) during sonication. The β-galactosidase activity of the culture was measured using the sonicated suspension.

The samples were appropriately diluted using cold (0–4°C) 0.1M phosphate buffer (pH 7). The ONPG was hydrolyzed by adding 1.6 ml of cold 0.005M ONPG in 0.1M phosphate buffer (pH 7) to 0.1 ml of diluted sample. The dilutions varied to obtain the absorbance readings within the maximum sensitivity range. The mixture was incubated at 37°C for 15 min. The hydrolysis was stopped by adding 2 ml of cold 0.5M sodium carbonate. The concentration of *o*-nitrophenol was determined from the absorbance at 420 nm (Spectronic 21, Bausch & Lomb, USA). For this assay, the molar absorptivity was 4.8×10^3 M^{-1} cm^{-1}. The enzymatic activity was expressed per ml of undiluted culture. All solutions were cooled in ice water. Experiments and all analyses were carried out at least in duplicate. Unless indicated otherwise, results are expressed as average ± SD of all available data. Statistical significance of differences was determined by a *t*-test where appropriate.

RESULTS AND DISCUSSION

Significant amounts of precipitated protein were produced by autoclaving the broth, so reliable estimation of cell biomass by optical density was not possible. Instead, the cells were counted by direct microscopic examination (Fig. 1). Cells grown in whey supplemented with 1.0% yeast extract (YE) were, on average, twice as long and somewhat thicker than cells grown in whey or whey supplemented with 0.2% YE. In their experiments with *L. bulgaricus* LBR in supplemented whey ultrafiltrate, COX and MACBEAN (1977) also observed as much as a twofold variation in the average cell length.

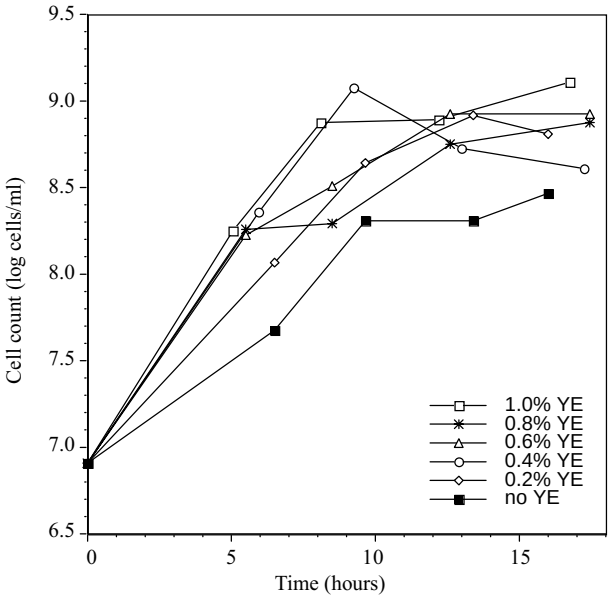

Fig. 1. Direct microscopic cell count of LB 11842 grown in 150 ml of sterilized (121°C/15 min) whey broth (60 g/l spray dried whey powder) supplemented with 0–1% yeast extract. The shake flask cultures were incubated at 40–43°C and the pH was maintained at 5.5 ± 0.3 using 2M NaOH

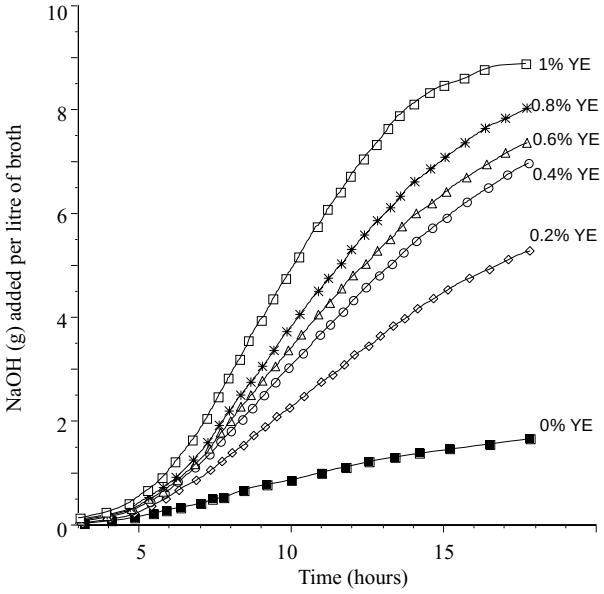

Fig. 2. Acid production of LB 11842 grown in 150 ml of sterilized (121°C/15 min) whey broth (60 g/l spray dried whey powder) supplemented with 0–1% yeast extract. The shake flask cultures were incubated at 40–43°C and the production of acid was inferred from the amount of NaOH required to maintain the pH at 5.5 ± 0.3

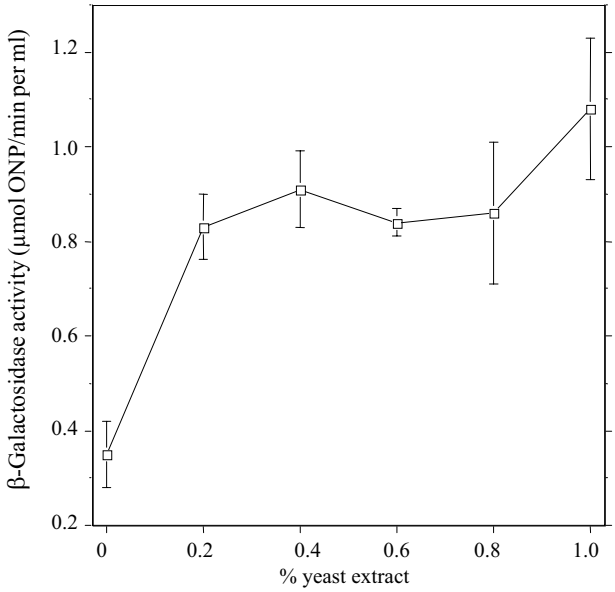

Fig. 3. The β-galactosidase activity of LB 11842 grown in 150 ml of sterilized (121°C/15 min) whey broth (60 g/l spray dried whey powder) supplemented with 0–1% yeast extract. The shake flask cultures were incubated at 40–43°C and the pH was maintained at 5.5 ± 0.3 using 2M NaOH. The β-galactosidase activity at pH 7 was determined by its ability to hydrolyze *o*-nitrophenyl-β-D-galactopyranoside (ONPG) at 37°C. Bars represent one standard deviation (n = 4)

Peak cell counts occurred approximately eight to ten hours after inoculating the shake flasks. The peak cell counts for LB11842 grown in whey ranged from 2–3 × 10^8 cells/ml while whey broths with 0.2–1.0% YE had counts of 5 × 10^8 to 1 × 10^9 cells/ml.

Addition of yeast extract (YE) had a significant effect on the rate of acid production by LB 11842 in whey (Fig. 2). Although increasing levels of supplementation increased the rate of acid production, the cell counts seemed relatively unaffected. The production of acid continued even after the peak cell count was reached which suggests that the bacteria were directing the available energy towards maintenance rather than growth (COX & MACBEAN 1977; BEAL & CORRIEU 1995; GONCALVES *et al.* 1991). Increasing the concentration of YE increases the amount of nutrients available to the bacteria, which could explain the increased rate of acid production.

Fig. 3 shows the β-galactosidase activity after 15–17 hrs of growth in whey + YE media. The β-galactosidase activities of cultures grown in flasks supplemented with 0.2–0.8% YE were approximately 2.5 times higher than for cultures grown without YE. Since the β-galactosidase activity did not increase ($P > 0.05$) with supplementation in the range of 0.2–0.8% YE, it appears that the hydrolysis of lactose was not the rate limiting factor for growth or acid production in this range. Cultures grown in whey supplemented with 1% YE showed a significantly ($P <$ 0.1) higher β-galactosidase activity of 1.08 ± 0.15 μmol ONP released per min per ml of culture which is three times that obtained from cultures grown in unsupplemented whey. In this case, the hydrolysis of lactose may have become rate limiting at some point resulting in the increased production of β-galactosidase. Negligible amounts of β-galactosidase were released into the growth medium during fermentation (data not shown).

SHAH and JELEN (1991) cultured LB 11842 in sterile 12% non-fat dry milk at 45°C for 18 hrs and reported a β-galactosidase activity of 4.22 μmol ONP/min per g of culture after sonication. In a study by FRIEND *et al.* (1983), the β-galactosidase activity of three strains of *Lactobacillus bulgaricus* ranged from 0 to 4.45 μmol ONP/min per g of culture at 30°C. Both studies showed much higher β-galactosidase activity than that obtained in the shake flask fermentations with whey supplemented with YE. In contrast, the maximum β-galactosidase activity in parallel experiments using 1.5 l fermentations under similar conditions (BURY 2000) was almost double that reported by SHAH and JELEN (1991). The strict pH control in the 1.5 l fermentations may have been one factor responsible for the greater activity.

It can be concluded that the addition of yeast extract to whey can greatly increase not only the rate of acidification by LB 11842 but also the β-galactosidase activity produced. The total cell count seemed relatively insensi-

tive to the amount of yeast extract added while the cell length increased with the level of supplementation.

Cultures grown in shake flasks with whey containing 0.2–0.8% YE produced 2.5 times the β-galactosidase obtained from cultures grown and sonicated in unsupplemented whey. The addition of 1% YE resulted in three fold increase of the β-galactosidase activity (1.08 μmol ONP/min per ml) in comparison to that produced using unsupplemented whey.

Environmental factors such as pH and temperature should be carefully controlled in order to increase the production of β-galactosidase by LB 11842. Addition of one or more complex growth supplements may result in greater β-galactosidase activity, as the hydrolysis of lactose would tend to be rate limiting with an excess of other nutrients.

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Souhrn

BURY D., GECIOVA J., JELEN P. (2001): **Vliv přídavku kvasničného extraktu na β-galaktosidasovou aktivitu kmene** *Lactobacillus delbrueckii* subsp. *bulgaricus* 11842 **při kultivaci v syrovátkových médiích**. Potravinářské vědy, **19**: 166–170.

Kmen *Lactobacillus delbrueckii* subsp. *bulgaricus* 11842 (LB 11842) byl kultivován v obnovené syrovátce obohacené o 0 až 1% kvasničného extraktu. β-Galaktosidasová aktivita byla určena z hydrolýzy chromogenního substrátu *o*-nitrophenol-β-D-galaktopyranosidu (ONPG) u sonikované kultury. Při obohacení syrovátky o 0,2–0,8 % kvasničného extraktu byla u testované

kultury zjištěna 2,5krát vyšší β-galaktosidasová aktivita než u kultury rostoucí v baňkách se samotnou syrovátkou. Jednoprocentní přídavek kvasničného extraktu do syrovátky způsobil až trojnásobný nárůst β-galaktosidasové aktivity. Zatímco rychlost produkce kyseliny mléčné se s rostoucím přídavkem kvasničného extraktu do syrovátky zvyšovala, nárůst počtu JTK (jednotky tvořící kolonie) byl s přídavkem kvasničného extraktu jen nepatrný. Maximální β-galaktosidasová aktivita 1,08 ± 0,15 mmol ONP uvolněných za min na ml kultury byla dosažena při kultivaci v médiu obsahujícím 1% přídavek kvasničného extraktu.

Klíčová slova: β-galaktosidasa; *Lactobacillus delbrueckii* subsp. *bulgaricus* 11842; kvasničný extrakt; syrovátka

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