International Journal of
Dairy Science

ISSN 1811-9743

Academic
Journals Inc.

www.academicjournals.com

International Journal of Dairy Science 6 (2): 150-157, 2011
ISSN 1811-9743 / DOI: 10.3923/ijds.2011.150.157

Production of β-galactosidase using Novel Yeast Isolate from Whey

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ABSTRACT

The hydrolysis of lactose by enzyme β-galactosidase into glucose and galactose play an important role in biotechnology, pharmaceutical and food processing industries. The aim of present study was focused on the isolation of novel yeast strains and formulation of low-cost medium based on whey for optimal β-galactosidase activity. β-galactosidase activities of isolated strains were carried out by ONPG assay. The yeast isolate (*Kluyveromyces marxianus*) showed maximum enzyme activity (1710 IU g^{-1} DW), when grown on whey permeate at pH 5.5, temperature 30°C after incubation period of 28 h. The utilization of dairy waste (whey) was found to be economical for β-galactosidase production by using isolated yeast cells. This enzyme is also known for its trans-galactosylation reaction and synthesized lactose based derivatives including lactulose and galacto-oligosaccarides, which can be further used in probiotic foods.

Key words: Whey, yeast isolation, β-galactosidase, ONPG assay, enzyme activity, optimization

INTRODUCTION

Whey is obtained as a by-product during the production of paneer, chhana and shrikhand which is a rich source of milk protein, water soluble vitamins, lactose and minerals (Keerthana and Reddy, 2006). Most of the milk plants do not have proper treatment system for the disposal of whey and the dumping of whey constitutes a significant loss of potential food and energy as whey retains about 55% of total milk nutrients. Its disposal as waste poses serious pollution problems for the surrounding environment (Carrara and Rubiolo, 1994). It affects the physical and chemical structure of soil resulting in a decrease of crop yield and when released in water bodies, reduces the aquatic life by depleting the dissolved oxygen. To overcome this problem, a better alternative is subjecting the whey to processes through which the value added products can be manufactured which may contribute wholly or partially to the costs. Availability of carbohydrate reservoir of lactose in whey and presence of other essential nutrients for the growth of microorganisms makes the whey one of the most potent raw materials for the production of different bio-products through biotechnological means (Panesar *et al*., 2007a).

The enzyme β-galactosidase (EC 3.2.1.23) catalyzes the hydrolysis of whey lactose to glucose and galactose. The use of β-galactosidase to avoid lactose crystallization in condensed and frozen dairy products such as ice cream and condensed milk raises its industrial importance and make it suitable for avoiding whey disposal (Dagbagli and Goksungur, 2008). Sweet syrup, produced by

lactose conversion through hydrolysis of whey by β-galactosidase, can be used in dairy, confectionary, baking and soft drink industries (Panesar *et al*., 2010). In addition, β-galactosidase is also used to avoid the problems of lactose intolerance by individuals due to deficiency of enzyme (Artolozaga *et al*., 1998; Rajakala and Selvi, 2006). New applications for β-galactosidase, such as in the production of biologically active galacto-oligosaccharides (Boon *et al*., 2000; Albayrak and Yang, 2002). The activity and stability of enzyme is influenced by the type of strain, cultivation and the growth medium composition (Tari *et al*., 2007; Jurado *et al*., 2004). Commercial β-galactosidases are produced from Bacteria (such as *Streptococcus salivarius* subsp. *thermophilus* and *Lactobaccilus lacti*) yeasts (such as *Kluyveromyces lactis* and *Kluyveromyces marxianus*) and moulds (such as *Aspergillus niger, Aspergillus candidus* and *Aspergillus oryzae* (El-Sawah and Microbiology, 1999; Hassan *et al*., 2006; Panesar *et al*., 2006; Zheng *et al*., 2006; Panesar *et al*., 2007b). So it is important to search new strains, which can display higher enzyme production on industrial byproducts like whey. Keeping the above in view, the present study was performed to isolate and to optimize the process conditions for maximum β-galactosidase production using whey medium.

MATERIALS AND METHODS

This study was conducted during the period of Jan, 2010 to May, 2010.

Sampling: Whey samples were collected from different states of India like Punjab (Verka Milk Plant; Sangrur, Patiala and Ludhiana), Haryana (Vita Milk Plant; Rohtak), Madhya Pradesh (Sanchi Milk Dairy Plant; Gwalior) and Bihar (Darbhanga Dairy; Darbhanga and Sudha Dairy; Bhagalpur).

Isolation and Identification of yeast cells for the production of β-galactosidases: For yeast enrichment in samples, 5 mL of different samples were inoculated in 50 mL of Malt Extracts Broth (MEB) containing 0.1 g 1000 mL chloramphenicol (Nahvi and Moeini, 2004) and were incubated at 30°C for 24 h with constant shaking at 100 rpm. Yeasts strains were then isolated on spread agar plates containing (0.3% w/v) yeast extract, peptone (0.5%), lactose (2.0%), chloramphenicol (0.1%) and agar-agar (2.0%). Incubation was done at 30°C for 48 h after making serial dilutions. Distinct colonies with morphological differences were selected from different samples and were purified by streaking on agar slants containing (w/v) malt extract (0.3%), yeast extract (0.3%), peptone (0.5%), glucose (1.0%) and agar (2.0%) and incubated at 3°C for 24 h. The yeast displaying maximum enzyme activity was got identified from Banglaore Genei, Merck Biosciences, Bangalore, India.

Fermentation condition of yeast cells for β-galactosidases production: The isolated yeast cells were inoculated into the whey based media containing different nitrogen sources (ammonium nitrate, urea, ammonium sulphate, sodium nitrate, L-aspartic acid an L-glutamic acid) and salts (calcium chloride, magnesium sulphate and potassium dihydrogen orthophosphate) with varying concentrations. Process parameters such as pH, incubation time and temperature were also optimized by varying their respective values for maximal enzyme activity.

Measurement of β-galactosidases activity: The assay for measurement of enzyme activity was followed as per the method of Miller (1972). One unit of enzyme activity is defined as one micromole

(μmol) of 2-nitrophenol liberated per min under standard assay conditions. All the enzyme assays were performed in triplicate and the mean values are reported.

RESULTS AND DISCUSSION

Identification of isolated yeast strains: Isolation of yeast cells was done from different samples. The activity of different strain has been given in Table 1. Among different isolates, yeast strain (Whey Isolate, WI-15) with maximum enzyme activity (1.129 IU mg^{-1} DW) was identified as *Kluyveromyces marxianus*.

Effect of nitrogen source and its concentration on β-galactosidase production: Various inorganic nitrogen supplements were examined to determine their effect on enzyme production. The increase in enzyme production has been observed with the addition of nitrogen into the media, which may be due an important role of nitrogen (major component of protein and nucleic acids) in growth of microbes. The maximum enzyme activity (1.632 IU mg^{-1} DW) was observed with 0.1% (w/v) of urea (Fig. 1, 2). However, it has been also observed that cell growth and ethanol yield increased with urea addition and 150 ppm urea concentration gave maximum ethanol content of 8.3% v/v (Mukhtar *et al*., 2010).

Effect of salt and its concentration on extracellular β-galactosidase production: Production of β-galactosidases production also affected by various salt (calcium chloride, magnesium sulphate and potassium dihydrogen orthophosphate). Figure 3 and 4 indicated that the optimum enzyme activity (1.656 IU mg^{-1} DW) was observed with magnesium sulphate (0.05%, w/v) which may be due to its activity as physiological ion that enhance the catalytic activity and stability of enzyme (Craig *et al*., 2000; Sutendra *et al*., 2007). Similarly, magnesium sulfate heptahydrate (0.05% w/v) has also been used for the optimal production of β-galactosidases by *E. coli* (Hsu *et al*., 2005).

Table 1: Screening of yeast isolates

Sampling/yeast isolate	Enzyme activity (IU mg^{-1} DW)
WI1	0.675
WI2	0.768
WI3	0.567
WI4	1.114
WI5	0.821
WI6	0.662
WI7	0.752
WI8	0.762
WI9	0.564
WI10	0.675
WI11	0.955
WI12	0.848
WI13	0.955
WI14	1.094
WI15	1.129
WI16	1.120
WI1	0.955

WI: Whey isolate, DW: Dry weight

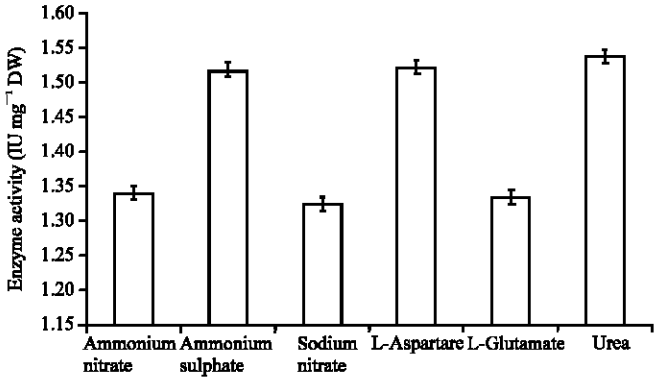

Fig. 1: Effect of nitrogen sources on β-galactosidase production by yeast isolates

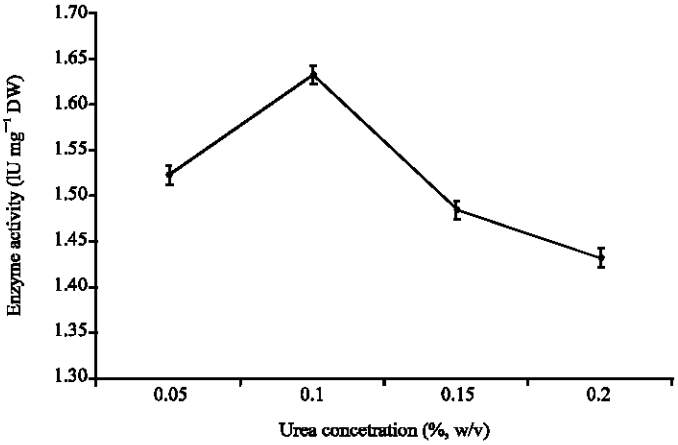

Fig. 2: Effect of urea concentration on β-galactosidase production by yeast isolates

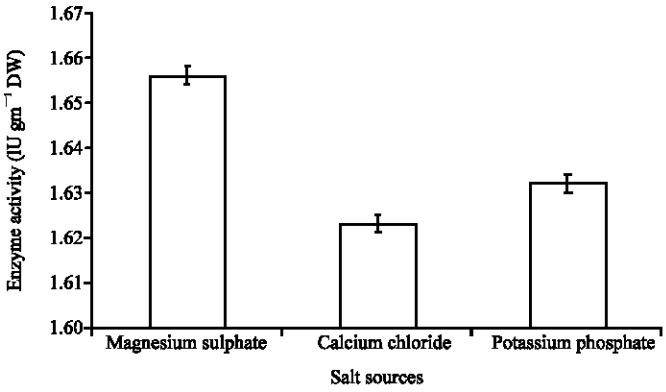

Fig. 3: Effect of salt on β-galactosidase production by yeast isolates

Effect of pH on extracellular β-galactosidase production: β-galactosidase production increased with the increase in urea concentration upto a range of 5.5, however, the decrease in β-galactosidase activity was observed by further increase and decrease in pH which is probably due

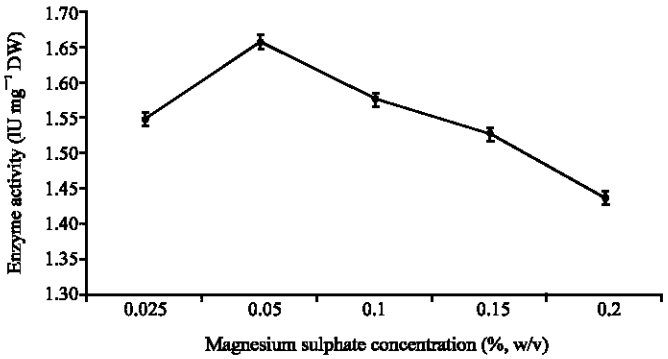

Fig. 4: Effect of magnesium sulphate concentration on β-galactosidase production by yeast isolates

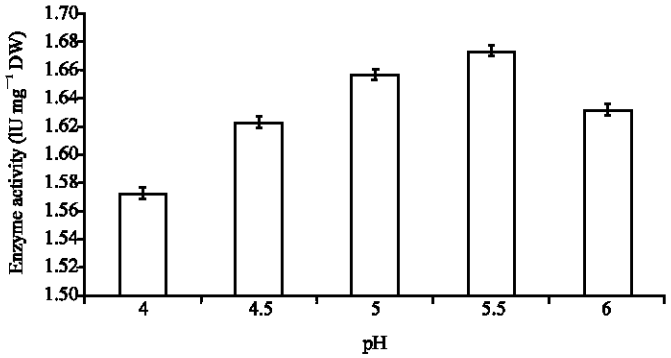

Fig. 5: Effect of pH on β-galactosidase production by yeast isolates

to the non-functioning of enzyme or transport of nutrients into the cell. The maximum enzyme activity (1.673 IU mg^{-1} DW) was observed at range of pH 5.5 (Fig. 5). Similarly, a pH 5.5 had also been observed for β-galactosidase production from *Kluyveromyces marxianus* (Castillo *et al*., 1979). Whereas, a pH 5.0 was found to be optimum for β-galactosidase production from *Kluyveromyces lactis* (Rajoka *et al*., 2003).

Effect of temperature on β-galactosidase production: The variation of enzyme activity as a function of temperature indicates that, there is an increase in β-galactosidase activity with an increase in temperature upto 30°C. However, decrease in enzyme activity was observed with an increase in temperature which might be due to the partial inactivation of enzyme or cell lysis at high temperature. The maximum enzyme activity (1.673 IU mg^{-1} DW) was observed at the temperature range of 27-30°C (Fig. 6). Similarly, the temperature of 28-30°C has also been observed for β-galactosidase production by Ku and Hang (1992) and Artolozaga *et al*. (1998). Matheus and Rivas (2003) have reported 30.0°C as an optimal temperature for β-galactosidase production by *K. lactis*.

Effect of incubation time on β-galactosidase production: β-galactosidase activity of cells increased with the increase in incubation time upto 28 h which might be due to increase in biomass, after that there is decrease of β-galactosidase production due to the further inhibition of cells

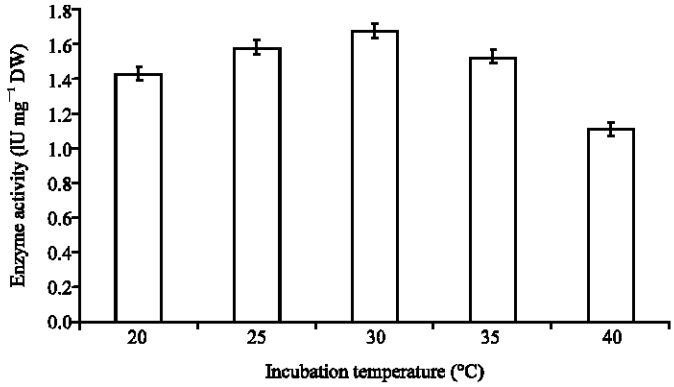

Fig. 6: Effect of temperature on β-galactosidase production by yeast isolates

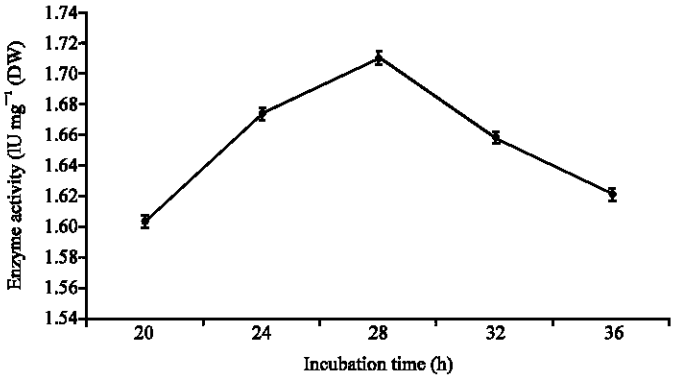

Fig. 7: Effect of incubation time on β-galactosidase production by yeast isolates

growth after the stationary phase has reached. The optimum range of yeast extract concentration and incubation time for maximum β-galactosidase activity (1.71 IU mg^{-1} DW) was observed at 26-28 h, respectively (Fig. 7). Rajoka *et al.* (2003) studied the production of β-galactosidase enzyme from *K. marxianus* and reported that the maximum activity of β-galactosidase production reached after 30-40 h and the enzyme production was apparently growth associated.

CONCLUSION

In the screening, it was observed that *Kluyveromyces marxianus* isolate presented the highest production of β-galactosidase. This strain was selected for further optimization studies of the culture medium for enzyme production. The maximum β-galactosidase activity (1.71 IU mg^{-1} DW) was observed, when yeast isolate grown on whey permeate fortified with yeast extract concentration 0.3 (%, w/v), magnesium sulphate concentration 0.05 (%, w/v), urea concentration 0.1 (%, w/v), pH 5.5, temperature 30°C and incubation time 28 h. Dairy whey utilization for β-galactosidase production can help to overcome the costs associated with enzyme production and disposal problems of the dairy industry. Beside this, the application of β-galactosidase can also be explored for lactose hydrolysis and lactulose and galacto-oligosaccharides synthesis, which further can be used as potential ingredients in functional foods.

ACKNOWLEDGMENT

The authors acknowledge the financial support given by Council of Scientific and Industrial Research (CSIR), New Delhi, India to carry out this work.

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