



Research Journal of **Microbiology**

ISSN 1816-4935



Academic
Journals Inc.

www.academicjournals.com

Production of β -D-Galactosidase from Whey Using *Kluyveromyces marxianus*

Parmjit S. Panesar

Biotechnology Research Laboratory, Department of Food Engineering and Technology,
Sant Longowal Institute of Engineering and Technology, Longowal 148106, India

Abstract: β -D-galactosidase (β -D-galactoside galactohydrolase, EC 3.2.1.23), most commonly known as lactase is widely distributed in nature and has wide scope in food industry, because of its applications in the production of lactose hydrolyzed milk and low lactose dairy products. The present study reports the use of whey as the fermentation medium for the production of β -D-galactosidase using a culture of *Kluyveromyces marxianus* as the producer organism. The effect of different process parameters such as pH of the medium, temperature, inoculum size, age of inoculum, agitation and incubation time was monitored to enhance the production of β -D-galactosidase. The maximum enzyme activity was observed with pH 5.0, temperature 30°C, inoculum size 6% (v/v) having 20 h age, under shaking conditions 100 rpm after 28 h of incubation.

Key words: Whey, yeast, β -D-galactosidase, enzyme activity

INTRODUCTION

Whey is the by-product separated from milk during the cheese-making process, which consists of water, lactose, proteins, vitamins and mineral salts. The production of cheese is increasing worldwide, which results in generation of large quantities of whey. Several possibilities of cheese whey exploitation have been investigated; however, approximately half of the world cheese whey production is not treated, but is discarded as effluent. The dumping of whey constitutes a significant loss of potential food and energy. This by-product poses a serious pollution problem, if disposed untreated, as it imposes a high biochemical oxygen demand (Marwaha and Kennedy, 1988; Mawson, 1994). The problem of whey treatment demands simple and economical solutions. A better alternative is subjecting the whey to processes through which the value added products are manufactured which may contribute wholly or partially to the costs. The carbohydrate reservoir of lactose (4-5%) in whey and presence of other essential nutrients makes whey as good natural medium for growth of microorganisms and potential substrate for bio-processing through microbial fermentation (Gandhi, 1989). It can be low priced source of sugars for the fermentation process. Availability of carbohydrate reservoir of lactose in whey and presence of other essential nutrients makes whey as potential substrate for the growth of lactose utilizing microorganisms.

Lactose utilizing yeasts such as *Kluyveromyces* sp. are important sources for the β -galactosidase production. It is one of the most promising enzymes for biotechnological applications and can be used for the production of lactose hydrolyzed milk and other low-lactose dairy products to meet the dietary requirements of lactose intolerant people (Mahoney, 1997; Panesar *et al.*, 2006). The yeast *Kluyveromyces* has been reported to be the most important source for the production of β -galactosidase as the enzyme from the yeast has an optimum pH suitable for lactose hydrolysis in milk (Joshi *et al.*, 1989; Stred'ansky *et al.*, 1993; Panesar *et al.*, 2007). Enzymatic lactose hydrolysis in milk can be used to solve the problems of lactose intolerance of the individuals who are deficient in lactase (Gekas and Lopez-Leiva, 1985; Roy and Gupta, 2003; Panesar *et al.*, 2006).

Increasing demand for β -D-galactosidase requires cost-effective production methods to ensure economic feasibility of lactose hydrolysis at commercial scale. The production of β -D-galactosidase through lactose utilizing yeasts could be an alternative processing route for whey lactose utilization. The use of biotechnological means to find the suitability of whey for enzyme production can serve dual purpose while producing valuable product, β -D-galactosidase and addressing to the whey disposal environmental pollution problem. Keeping in view the availability and quantity of the lactose rich dairy by-product whey, the possibility of developing a technology for the production of β -D-galactosidase from whey can be explored to overcome the problems encountered in its disposal.

MATERIALS AND METHODS

Procurement of Micro-Organism

Kluyveromyces marxianus NCIM 3465 was procured from National Collection of Industrial Micro-organisms, National Chemical Laboratory, Pune (India).

Maintenance of the Culture

The yeast culture was revived on maintenance medium containing (w/v) malt extract (0.3%), yeast extract (0.3%), peptone (0.5%) and glucose (1.0%). The culture was incubated under shaking conditions at 30°C for 48 h and maintained for fortnightly intervals on agar slants at 4°C.

Preparation of Starter Culture

The maintenance medium (50 mL) were taken in Erlenmeyer flask (250 mL), sterilized by autoclaving at 15 psi for 20 min, cooled and inoculated with a loopful of culture from slant and incubated under shaking conditions at 30°C for 24 h.

Production of β -D-Galactosidase

Clarified whey was used as the media for the production of enzyme and was supplemented with peptone (0.5%), yeast extract (0.3%), ammonium sulphate (0.2%) and potassium dihydrogen orthophosphate (0.1%). The fermentation medium (50 mL in 250 mL flask) was inoculated with 24 h old inoculum (unless otherwise mentioned), incubated at specified times under shaking conditions. After specific time intervals, the samples were drawn from flasks and assayed for enzyme activity.

Enzyme Assay

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 (μ M) of 2-nitrophenol liberated per min under standard assay conditions. The biomass was washed twice with phosphate buffer (0.1 M, pH 7.0) and resuspended in the same buffer. The appropriately diluted cell suspension (0.1 mL) was taken in a test tube and to this 0.9 mL of Z buffer was added. The cells were lysed by adding chloroform (50 μ L) and sodium dodecyl sulphate (20 μ L). The reaction mixture was incubated at 30°C for 10 min. Then, 0.2 mL of o-nitrophenyl- β -D-galactopyranoside was added and incubated for 5 min at the same temperature. The reaction was stopped by adding 1 mL of sodium carbonate. The liberated colour was measured at 420 nm using a spectrophotometer. All the enzyme assays were performed in triplicate and the mean values are reported.

Optimization of Process Parameters

Different process parameters such as pH, temperature, age of inoculum, inoculum size, agitation and incubation time were optimized by varying the respective parameters.

RESULTS AND DISCUSSION

The effect of following process parameters was observed to enhance the β -D-galactosidase activity during the course of the present investigation.

Effect of pH

The effect of hydrogen ion concentration on β -D-galactosidase production was monitored by using fermentation medium having a pH range of 4.0-6.0. The results of the enzyme activities showed maximum activity (1490 IU g^{-1} dry weight) at pH 5.0 (Table 1), however, at higher and lower pH levels, a decrease in the enzyme activity was observed. At pH 4.0, minimum enzyme activity of 1370 IU g^{-1} was observed. The pH of a system affects at least two aspects of microbial cells i.e., functioning of its enzymes and the transport of nutrients into the cell. The pH values also affect the RNA and protein synthesis (Klovrychev *et al.*, 1979). When micro-organisms are grown on either side of their optimum pH range, there may be an increased lag phase. The pH 5.0 has also been used for β -D-galactosidase production using different yeast strains by earlier researchers (Inchaurredo *et al.*, 1994). However, a pH 5.5 has been used for the enzyme production using *K. marxianus* (Artolozaga *et al.*, 1998), whereas, a pH 4.68 has been found optimum for β -D-galactosidase production using *K. lactis* (Ramirez-Matheus and Rivas, 2003).

From these observations, pH 5.0 was considered optimal for maximum β -D-galactosidase activity and was used in the subsequent experiments.

Effect of Temperature

To find out the optimum temperature for β -D-galactosidase production, whey medium, after inoculation, was incubated at temperatures in the range of 20-40°C (Table 1). The enzyme activity increased with increase in the temperature up to 30°C, however, a decrease in the activity was observed at higher temperature with a sharp decrease at temperature range of 35-40°C. The maximum enzyme activity of 1490 IU g^{-1} dry weight was observed at 30°C.

It has been established fact that enzymes are most active at optimum temperature and enzymatic reactions proceed at maximum rate. However, below and above optimal temperature reaction rate is decreased which causes the problems in cell metabolism. The temperature of 28-30°C has been used for β -D-galactosidase production by many workers (Ku and Hang, 1992; Hewitt and GrootWassink, 1984; Artolozaga *et al.*, 1998). Ramirez-Matheus and Rivas (2003) have reported 30.3°C as an optimal temperature for β -D-galactosidase production by *K. lactis*.

From the above observations, a temperature of 30°C was considered optimal for achieving maximum enzyme activity and it was adopted for further experimentation.

Effect of Inoculum Age

To find the effect of inoculum age on β -D-galactosidase production, whey fermentation medium was inoculated with 12-28 h old cultures. The results displayed progressive increase in the enzyme activity between 12-20 h inoculum age and the maximum enzyme activity (1530 IU g^{-1} dry weight)

Table 1: Effect of pH and temperature on β -D-galactosidase production by *K. marxianus*

pH	Enzyme activity (IU g^{-1} dry weight)	Temperature ($^{\circ}\text{C}$)	Enzyme activity (IU g^{-1} dry weight)
4.0	1370	20	1330
4.5	1460	25	1470
5.0	1490	30	1490
5.5	1480	35	1370
6.0	1440	40	250

Table 2: Effect of inoculum age and size on β -D-galactosidase production by *K. marxianus*

Inoculum age (h)	Enzyme activity (IU g ⁻¹ dry weight)	Inoculum size (% v/v)	Enzyme activity (IU g ⁻¹ dry weight)
12	1300	4	1380
16	1440	6	1540
20	1530	8	1530
24	1490	10	1530
28	1470	12	1500

Table 3: Effect of agitation on β -D-galactosidase production by *K. marxianus*

Agitation (rpm)	Enzyme activity (IU g ⁻¹ dry weight)
0	1290
60	1430
80	1510
100	1540
120	1530

Process conditions: Inoculum age: 20 h; pH: 5.0; Inoculum size: 6% (v/v) Temperature: 30°C; Incubation period: 32 h

was observed with 20 h old yeast culture (Table 2). However, suppression in enzyme activity was observed with 24 and 28 h old inoculum. The low enzyme activity with inoculum age of 12 h may be attributed to the fact that yeast culture may have not yet entered the log phase of growth. The maximum enzyme activity observed with inoculum of 20 h, which may be due to the exponential phase of the yeast culture used as an inoculum. Similarly, 20 h old culture of *K. fragilis* for β -D-galactosidase production has been used (Chen *et al.*, 1992). However, Ku and Hang (1992) have used 24 h old culture of *K. marxianus* strains for β -D-galactosidase production.

Since, 20 h yeast culture displayed maximum β -D-galactosidase activity, it was selected for further studies.

Effect of Inoculum Size

Different inoculum levels (4-12%, v/v) were added to the whey medium to study the influence of inoculum size on the β -D-galactosidase activity (Table 2). The enzyme activity increased with the increase in inoculum size up to 6% (v/v), thereafter no improvement in enzyme activity was observed. The maximum enzyme activity of 1540 IU mg⁻¹ dry weight was observed with 6% (v/v) inoculum of yeast culture. The low enzyme activity at 4% (v/v) inoculum level may be attributed to the low density of starter culture. Therefore, an inoculum of 6% (v/v) can be considered optimal for achieving maximum enzyme activity using 20 h old yeast culture.

Effect of Agitation

To study the effect of agitation on the β -D-galactosidase production by the yeast culture, the cultivation was carried under stationary conditions in an incubator and shaking condition (60-120 rpm) on a rotary shaker (Table 3). The agitation mode of cultivation supported an increase in enzyme activity as compared to the stationary conditions. During the experimentation, a progressive increase in the enzyme activity with increase in the agitation rate up to 100 rpm was observed. However, no improvement in the enzyme activity was observed at higher agitation rate. The increase in enzyme activity with agitation mode may be attributed to the uniform distribution of the yeast culture in the medium resulting in better nutrient availability and oxygen transfer rate. The earlier studies have also supported the agitation mode for β -D-galactosidase production (Pedrique and Castillo, 1982; Champluvier *et al.*, 1988; Chen *et al.*, 1992).

Since, maximum β -D-galactosidase activity was observed with agitation rate of 100 rpm, it was selected for further investigations.

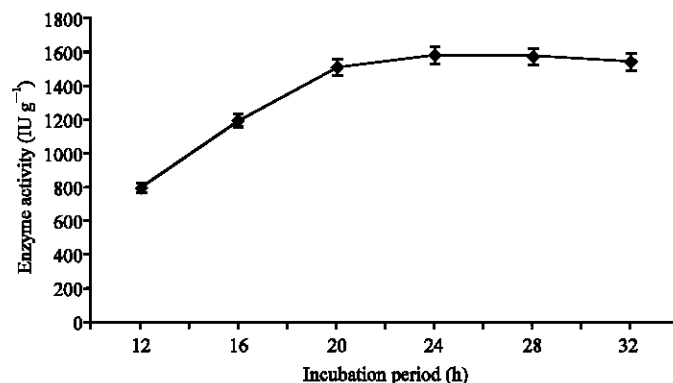


Fig. 1: Effect of incubation period on β -D-galactosidase production by *K. marxianus*. Bars indicate the standard deviation from triplicate determinations

Effect of Incubation Period

To find out the optimal incubation time for the maximal β -D-galactosidase activity, the whey medium inoculated with yeast cultures was incubated for 36 h under the above optimized conditions (Fig. 1). The results displayed an increase in enzyme activity up to 24 h and thereafter no improvement in this function was observed. However, a decrease in the enzyme activity was observed with further increase in the incubation time (28-36 h), which may be attributed to the growth of the culture having reached the stationary phase and as a consequence of metabolism. A maximum enzyme activity of 1580 IU g⁻¹ dry weight was observed after 24 h of incubation. The earlier workers also reported that maximum enzyme yield is obtained at the beginning of the stationary phase of growth, after which the yield of the enzyme decreases (Pinheiro *et al.*, 2003). Ku and Hang (1992) have reported 24 h as an optimal incubation period, however, a fermentation time of 18.5 h has also been reported optimal using *K. lactis* (Ramirez-Matheus and Rivas, 2003). Keeping the above observations in view, the incubation time of 24 h was considered optimal for maximum β -D-galactosidase activity under the above optimized conditions.

CONCLUSIONS

From the foregoing account and the observations made during the present investigation, it can be concluded that whey is suitable medium for the production of β -D-galactosidase. The maximum enzyme activity can be obtained with the process conditions of pH 5.0, temperature 30°C and inoculum size 6% (v/v) of 20 h old yeast culture under shaking conditions (100 rpm) after an incubation period of 24 h. The different optimal conditions reported by various researchers for maximum β -D-galactosidase activity could be explained by the differences in the nature of the strains and medium composition used in their studies.

REFERENCES

- Artolozaga, M.J., R. Jones, A.L. Schneider, S.A. Furlan and M.F. Carvallo-Jones, 1998. One step partial purification of β -D-galactosidase from *Kluyveromyces marxianus* CDB002 using streamline-DEAE. Bioseparation, 7: 137-143.
- Champluvier, B., B. Kamp and P.G. Rouxhet, 1988. Preparation and properties of β -galactosidase confined in cells of *Kluyveromyces* sp. Enzyme Microb. Technol., 10: 611-617.

- Chen, K.C., T.C. Lee and J.Y. Houn, 1992. Search method for the optimal medium for the production of lactase by *Kluyveromyces fragilis*. Enzyme Microb. Technol., 14: 659-664.
- Gandhi, D.N., 1989. Production of some useful products of industrial importance through microbial fermentation of whey. Indian Dairyman, 45: 182-184.
- Gekas, V. and M. Lopez-Leiva, 1985. Hydrolysis of lactose: A literature review. Process Biochem., 20: 2-12.
- Hewitt, G.M. and J.W.D. GrootWassink, 1984. Simultaneous production of inulase and lactase in batch and continuous cultures of *Kluyveromyces fragilis*. Enzyme Microb. Technol., 6: 263-270.
- Inchaurredo, V.A., O.M. Yantorno and C.E. Voget, 1994. Yeast growth and β -galactosidase production during aerobic cultures in lactose limited synthetic medium. Process Biochem., 29: 47-54.
- Joshi, M.S., L.R. Gowda, L.C. Katwa and S.G. Bhat, 1989. Permeabilization of yeast cells (*Kluyveromyces fragilis*) to lactose by digitonin. Enzyme Microb. Technol., 11: 439-443.
- Klovrychev, M.F., P.N. Korolev and V.G. Bulgakova, 1979. Effect of copper ions and unfavourable pH on protein and RNA synthesis of *Candida utilis*. Microbiology, 47: 357-361.
- Ku, M.A. and Y.D. Hang, 1992. Production of yeast lactase from sauerkraut brine. Biotechnol. Lett., 14: 925-928.
- Mahoney, R.R., 1997. Lactose: Enzymatic Modification. In: Advanced Dairy Chemistry. Fox, P.F. (Ed.), Vol. 3, Chapman and Hall, London, UK., pp: 77-125.
- Marwaha, S.S. and J.F. Kennedy, 1988. Review: Whey pollution problem and potential utilization. Int. J. Food Sci. Technol., 23: 323-336.
- Mawson, A.J., 1994. Bioconversions for whey utilization and waste abatement. Bioresour. Technol., 47: 195-203.
- Miller, J.H., 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory. New York, pp: 352.
- Panesar, P.S., R. Panesar, R.S. Singh, J.F. Kennedy and H. Kumar, 2006. Microbial production, immobilization and applications of β -D-galactosidase. J. Chem. Technol. Biotechnol., 81: 530-543.
- Panesar, R., P.S. Panesar, R.S. Singh, J.F. Kennedy and M.B. Bera, 2007. Production of lactose hydrolyzed milk using ethanol permeabilized yeast cells. Food Chem., 101: 786-790.
- Pedrique, M. and F.J. Castillo, 1982. Regulation of β -D galactosidase synthesis in *Candida pseudotropicalis*. Applied Environ. Microbiol., 43: 303-310.
- Pinheiro, R., I. Belo and M. Mota, 2003. Growth and β -galactosidase activity in cultures of *Kluyveromyces marxianus* under increased air pressure. Lett. Applied Microbiol., 37: 438-442.
- Ramirez-Matheus, A.O. and N. Rivas, 2003. Production and partial characterization of β -galactosidase from *Kluyveromyces lactis* grown in deproteinized whey. Arch. Latinoam. Nutr., 53: 194-201.
- Roy, I. and M.N. Gupta, 2003. Lactose hydrolysis by Lactozym™ immobilized on cellulose beads in batch and fluidized bed modes. Process Biochem., 39: 325-332.
- Stred'ansky, M., M. Tomaska, E. Sturdik and L. Kremnický, 1993. Optimization of β -galactosidase extraction from *Kluyveromyces marxianus*. Enzyme Microb. Technol., 15: 1063-1065.