

Optimization of Variables for Lactase Production from Isolated *Bacillus subtilis* strain VUVD001 Through Submerged Fermentation

T.C.Venkateswarulu^{1*}, K. Vidya Prabhakar², R.Bharath Kumar¹ and S.Krupanidhi¹

^{1, 1} Department of Biotechnology, Vignan's Foundation for Science, Technology and Research University, Vadlamudi- 522213, Andhra Pradesh, India

² Department of Biotechnology, VikramaSimhapuri University, Nellore-524320, Andhra Pradesh, India

*For Correspondence - venki_biotech327@yahoo.com

Abstract

Lactase enzyme is commercially important and is generally used for lactose hydrolysis in milk and whey. To date, it has been isolated from various sources. In this study, different strains of isolated bacteria were evaluated for their lactase productivity, but *Bacillus subtilis* VUVD001 resulted with the highest production. Therefore, optimal physical conditions were determined in batch fermentation process using one-variable-at-a-time approach for the production of lactase. The influence of some physical conditions namely pH, incubation temperature and time, inoculum size on enzyme production were studied for higher yield. Maximum activity of lactase in shake flask culture was found 15.27 U/ml at optimized conditions of incubation period 36 h, temperature 37 °C, pH 7.0 and inoculums size of 5%.

Keywords Lactase, *Bacillus subtilis* VUVD001, Shake Flask Culture

Introduction

Lactase is widely used in food and pharmaceutical industries due to its ability to hydrolyze lactose in milk and its by-products. This enables reduction of lactose intolerance problem (1, 2). Previously it has been reported that different bacterial species were used for production of lactase because of various factors such as simple fermentation, high enzyme activity and stability

(3, 4). It is also reported that various microbes such as *Bacillus circulans*, *Bifidobacterium adolescentis*, *Lactobacillus reteri*, *L. plantarum* and *B. infantis* have shown lactase producing activity (5-7). The dairy industry uses bacterial genus *Lactobacillus* and *Bifidobacterium* for the production of lactase enzyme (8, 9). These *bacilli* are generally regarded as safe (GRAS) and thus, the lactase secreted by them can be consumed without excessive purification by lactose intolerance patients. Reduction in lactase activity may cause lactose intolerance (10). The yeast species, *Kluyveromyceslactis*, *K. marxianus* and *Saccharomyces fragilis* and molds such as *Aspergillus* and *Rhizomuor* species were also used in commercial production of lactase (11-13). Enzyme production was influenced by various factors namely strain type and cultivation conditions like temperature, pH, agitation speed and incubation time (14, 15). The aim of this study was to assure the production of high yield of lactase from *Bacillus subtilis* VUVD001, through optimization study. The physical conditions such as fermentation time, pH, temperature and inoculum percent were studied for improving production.

Material and Methods

Microorganism and shake flask fermentation

The organism *B. subtilis* strain VUVD001 was isolated from the effluents of Sangam Dairy,

Vadlamudi, Guntur District. India. The culture was maintained in the laboratory at room temperature and preserved at 4°C on nutrient agar medium. The original fermentation medium consisted of 4 g/L of lactose, 4 g/L of yeast extract, 1g/L of MgSO₄·7H₂O and 0.1g/L of Tryptophan. The shake-flask fermentation was performed with 100mL of production medium.

Effect of culture conditions on enzyme production

Inoculum size : The influence of inoculum size on production of lactase was studied by changing the percent of inoculum (1-6%, v/v) to production medium.

Incubation time : The effect of incubation time on production of lactase was determined by incubating the bacterial strain at 37 °C in broth medium at different time intervals.

Temperature : Optimum temperature for the production of enzyme was determined by incubating inoculated culture medium at different temperatures namely 27°C, 37°C and 47°C for 36 h.

pH : The best possible pH for production of lactase was optimized by using broth medium and determined by adjusting pH (5-8) of medium using

1M NaOH and 1M H₂SO₄. The inoculated culture flasks were kept for incubation for a period of 36h.

Lactase assay : Lactase activity was determined using ortho-Nitrophenyl- β-galactoside (ONPG) as substrate. The ONPG solution was prepared with phosphate buffer and used for assay. The assay system contained 0.5mL of enzyme source with 2.0mL of substrate and incubated for 30 min. The reaction was stopped with the addition of 0.5 mL of 1 M Na₂CO₃ and absorbance was recorded at 420 nm. Activity of lactase was determined from ONP standard graph. One unit of activity is defined as amount of enzyme that liberates 1 micromole of ONP from the substrate per minute under assay conditions (16).

Results and Discussion

Effect of incubation time : The changes in enzyme activity were observed during incubation periods from 12 to 48 h. This represents the change in state of population number from log to stationary phase. The maximum enzyme activity of 15.13 U/ml was found after 36 hours of incubation beyond this the activity declined due to depletion of nutrients (Fig.1). Qian et al (17) reported that the highest lactase activity was found at 36 h of incubation time through fermentation in

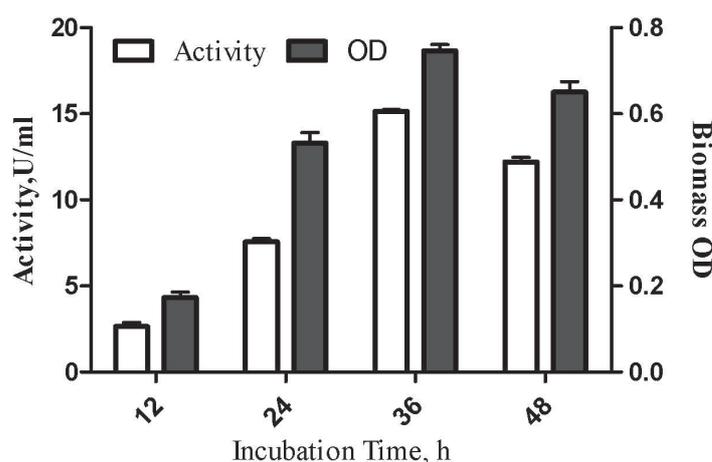


Fig. 1. Effect of incubation time on enzyme production

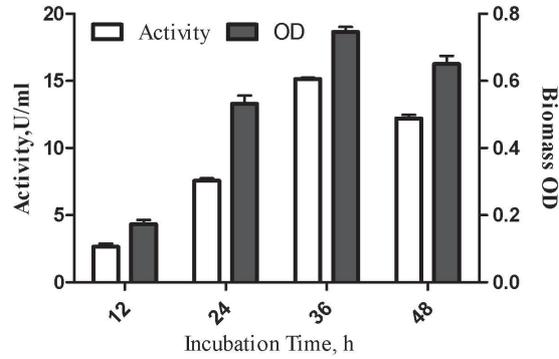


Fig. 2. Effect of temperature on production of enzyme

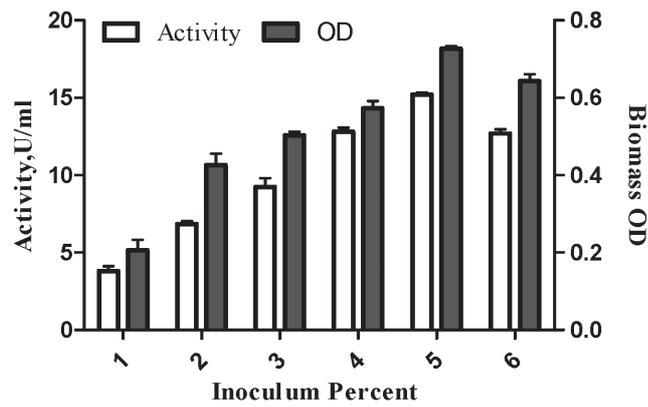


Fig. 4. Effect of size of inoculum on production of lactase

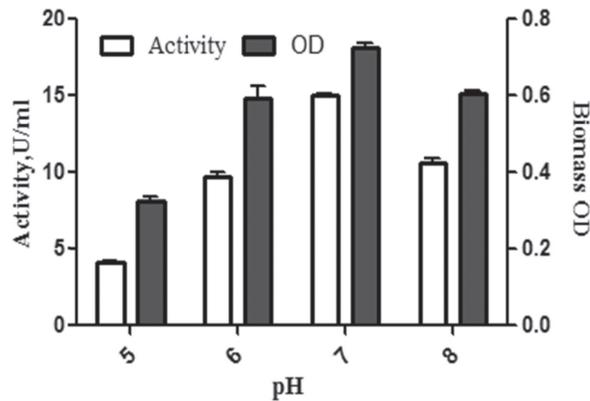


Fig. 3. Effect of pH on enzyme production

shake flasks with a thermotolerant strain. Similarly, Mukesh Kumar et al (18) observed the maximum lactase activity with 48 hours of incubation period in submerged fermentation process by using *Bacillus* sp. MPTK121. Findings of Jayashree Natarajan et al (19) also show that the optimum incubation period for lactase production was 48 h.

Effect of temperature : The submerged fermentation process showed that the enzyme activity increases with an increase in incubation temperature up to 37 °C and then enzyme activity slows down beyond this temperature. Thus, 37 °C was found optimum temperature for lactase production by *B. subtilis* strain VUVD001 (Fig.2). Murad et al (20) stated that lactase production was increased by *Lactobacilli* strain when the cultivation temperature was maintained at 30 °C. Tryland and Fiksdal (21) reported that 35 °C was the maximum temperature for lactase activity and beyond this temperature the enzyme activity was decreases up to 44 °C. Roopashri and Varadaraj (22) reported that highest lactase activity of 10.6 U/ml was obtained with *Lactobacillus plantarum* MTCC5422 in the soy whey based medium at 37 °C.

Effect of pH on enzyme production : Enzyme activity gradually increased with changes in the pH of the medium from 5.0 to 7.0 and the activity slowly decreased with an increase in pH. The highest enzyme activity was found 15.12 U/ml at pH 7.0 (Fig.3). The intracellular lactase activity was high at pH 6.8 through cultivation of *L. delbrueckii* spp.*bulgaricus* ATCC11842 and *B. animalis* spp.*lactis* Bp12 (23). Cherabarti et al (24) proved that relative activity of lactase was higher at pH 7 through submerged fermentation using the strain *B. poymyxa*. Sangwan et al (25) reported that the pH 6.8 is the optimum for the production of lactase through the submerged fermentation from *Streptococcus thermophilus*.

Effect of inoculum size : Inoculum size of bacterial culture has an important effect on the production of enzyme quantity. Maximum lactase activity of 15.20 U/ml was observed with an

inoculum size of 5%, and minimum with 1% inoculum, respectively (Fig.4). Gowdhami et al (26) observed the highest lactase production by *L. bifementans* at 2% v/v inoculum. Anisha and Prema (27) found the highest lactase activity when the fermentation medium was inoculated with 10% (v/v) of 72 h grown inoculum. Mahalakshmi et al (28) reported that the higher lactase enzyme production was achieved with 1% inoculum of *L. amylophilus* GV6.

Conclusion

It is concluded that the highest biocatalytic activity could obtained by using *B. subtilis* strain VUVD001. The maximum lactase activity was found 15.27 U/ml at optimum conditions of 5% (v/v) inoculum size, pH 7, 37 °C temperature and incubation time 36 h. Based on the results reported in this paper, it is predicted that the crude enzyme extract lactase from *B. subtilis* will have possible application in the food and pharmaceutical industries.

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