PRODUCTION OF BETA-GALACTOSIDASE IN SUBMERGED MEDIA BY *ASPERGILLUS ORYZAE*, PTCC 5163

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Abstract

Different compositions of liquid media were used for their efficacy in the production of beta-galactosidase by *Aspergillus oryzae* in submerged fermentation. Enzyme production was improved by switching from lactose-based media to soybean meal- and wheat bran-based media. Further improvement was made when the soybean meal medium was supplemented with sodium nitrate and magnesium sulfate. Results also indicate that fungal extracellular beta-galactosidase production occurs concomitantly with growth on complex media but not on lactose- based media. The effectiveness of Tween 80 on beta-galactosidase activity was found to depend on the type of the medium used.

Introduction

Hydrolysis of lactose in milk and milk products can rease digestibility and may improve functional proper- ; of dairy products [1,2,3]. Therefore, beta-galactosidase ı be considered as an important biocatalyst in the related ɔd industries [2,4].

Beta-galactosidase has been investigated in different ɔcies of bacteria [3], yeasts [5,6], molds [7,8], plants and ımal sources [3,9]. The bacterial enzymes function op- ıally at neutral pH values (for hydrolysis of lactose in lk and sweet whey) and about 35°C [2,10], which is a ıwback with respect to microbial contamination [2]. The racellular yeast enzymes have a narrow pH stability ıge (around 7) [5,11] and their temperature of optimal ivity lies below 40°C [11,12]. The fungal enzymes wever, are stable over a broad pH range [13,14] and itable for treatment of acid whey [15,16]. Their optimum nperature is 55°C [3,17].

The extracellular beta-galactosidase of *A. oryzae* [18] has an optimum pH of 4 to 5.4 [19,20,21] and when immobilized, is suitable for hydrolysis of lactose in dairy products.

For the industrial production of enzymes, it is specially important to improve conditions so as to obtain higher yield. At present, only 5% of enzymes are produced by solid state fermentation. Submerged fermentation appears to be the method of choice where sterilization and process control are easier to achieve [10,22]. It should, however, be noted that filamentous fungi generally produce less quantities of secretory enzymes in liquid cultures than they do in solid state cultures [4,18,23].

In this paper, we report on the production of beta-galactosidase by *A. oryzae* PTCC 5163 using different liquid media.

Materials and Methods

Aspergillus oryzae PTCC 5163 was obtained from Persian Type Culture Collection (PTCC), Tehran, Iran. It was maintained on skim milk at -70°C and cultured on Potato Dextrose Agar and subcultured every two weeks.

Yeast extract, peptone, PDA (Potato Dextrose Agar)

ywords: *A. oryzae*; Beta-galactosidase

obtained from Difco Laboratories (USA), Tween 80 from Merck and the other chemicals used were of analytical grade. Corn steep liqour, cheese whey, wheat bran, soybean meal and spent grain were obtained locally.

Whey, lactose, wheat bran and soybean meal as carbon sources and corn steep liquor, spent grain, sodium nitrate, peptone and yeast extract as nitrogen sources were selected. Eleven culture media were designed and are indicated in the results (Table 1).

Production of Enzyme

100 ml of each medium was prepared in 500 ml Erlenmeyer flasks, the pH of each one was adjusted to 4.8 (± 0.2).

After sterilization, they were inoculated with 4 ml of conidia suspension of *A. oryzae* (10^7 conidia/ml). They were incubated at 30°C on a rotary shaker (150 rpm) for 160 h. Every 8 h the supernatants were checked for enzyme activity.

In one of the shake-flask experiments, Tween 80 at 2 g/L was added to media and supernatants were checked for enzyme activity. Enzyme productivity in cultures were calculated by the following formula:

$$\text{Productivity} = \frac{\text{Total Enzyme Activity (IU)}}{\text{Fermentation Time (h)} \times \text{Volume (L)}}$$

Enzyme Assay

Known amounts of culture fluid were taken during fermentation and centrifuged at 5000 rpm for 10 min (Beckman model J2-21 centrifuge). The supernatant was used directly for the determination of enzyme activity. Beta-galactosidase activity was assayed using the chromogenic substrate o-nitrophenyl β-D-galacto-pyranoside (ONPG) supplied by Sigma Chemical Co. Assay mixtures contained 6 mg ONPG in 3.5 ml of 0.1 M citrate-phosphate buffer (pH 4.5).

The reaction was started by addition of supernatants containing enzyme (0.5 ml) at 30°C for 10 min. The reaction was stopped by addition of 1 mol/L sodium carbonate (1 ml) and the absorbance was read against blank at 420 nm (UNICAM 8620 uv/vis spectrometer) [3, 4, 16, 19, 20].

One unit of enzyme activity was defined as the amount of enzyme which liberates 1 μmol of orto-nitrophenol per min. under the above assay conditions [14, 16, 20].

Results

Conidia of *A. oryzae* were inoculated into different media (Table 1) in order to screen the best medium.

All of these media were suitable for growth of *A. oryzae*. Mass production is one of the most important purposes of enzyme production, because beta-galactosidase production is growth dependent. After 48-60 h of growing *A. oryzae*, the growth was complete and 20-24 g dry mass/L was produced in all of the above-mentioned media but with various production times and enzyme activity rates (Fig. 1).

Maximum production of beta-galactosidase in the media is compared in Figure 2. The most interesting results were the poor production of beta-galactosidase on lactose-based media and the significant high production on soybean meal- and wheat bran-based media (Fig. 2).

Comparison between maximum production of beta galactosidase and production time in media is indicative of enzyme productivity (Table 2).

One possible explanation for the long production time with *A. oryzae* is the inefficient secretion of the enzyme. It is possible that an increase in productivity can be achieved using surfactants in the medium, in order to improve cell wall permeability. The results showed that Tween 80 caused a slight increase in final beta-galactosidase activity.

Discussion

Whey has been used as a suitable medium for beta galactosidase production [24]. It contains about 14, 22, 7[illegible] and 98% of the original milk fat, protein, ash and lactose respectively [2, 25]. Lactose of whey is not only a suitable

Table 1. Composition of media (g/l)

C1	Cheese Whey(70), Corn steep liquor(20), Peptone(10)
C2	Cheese Whey(20), Spent grain(10)
L	Lactose(20), Peptone(2), Yeast extract(1)
W1	Wheat bran(20), Spent grain(10)
W2	Wheat bran(20), Corn steep liquor(20)
W3	Wheat bran(20), $NaNO_3$(1), $MgSO_4.7H_2O$(0.12), KH_2PO_4(2.5)
LW	Lactose(15), Wheat bran(10), Yeast extract(1.5), KCl(0.5), $FeSO_4.7H_2O$(0.01)
S1	Soybean meal(20), $NaNO_3$(0.3), $MgSO_4.7H_2O$(0.12), KH_2PO_4(2.5)
S2	Soybean meal(20), Corn steep liquor(20)
SW	Wheat bran(10), Soybean meal(10), $NaNO_3$(0.3), KCl(2), KH_2PO_4(2.5)
Soytone	Soytone(20), $NaNO_3$(0.3), $MgSO_4.7H_2O$(0.12), KH_2PO_4(2.5)

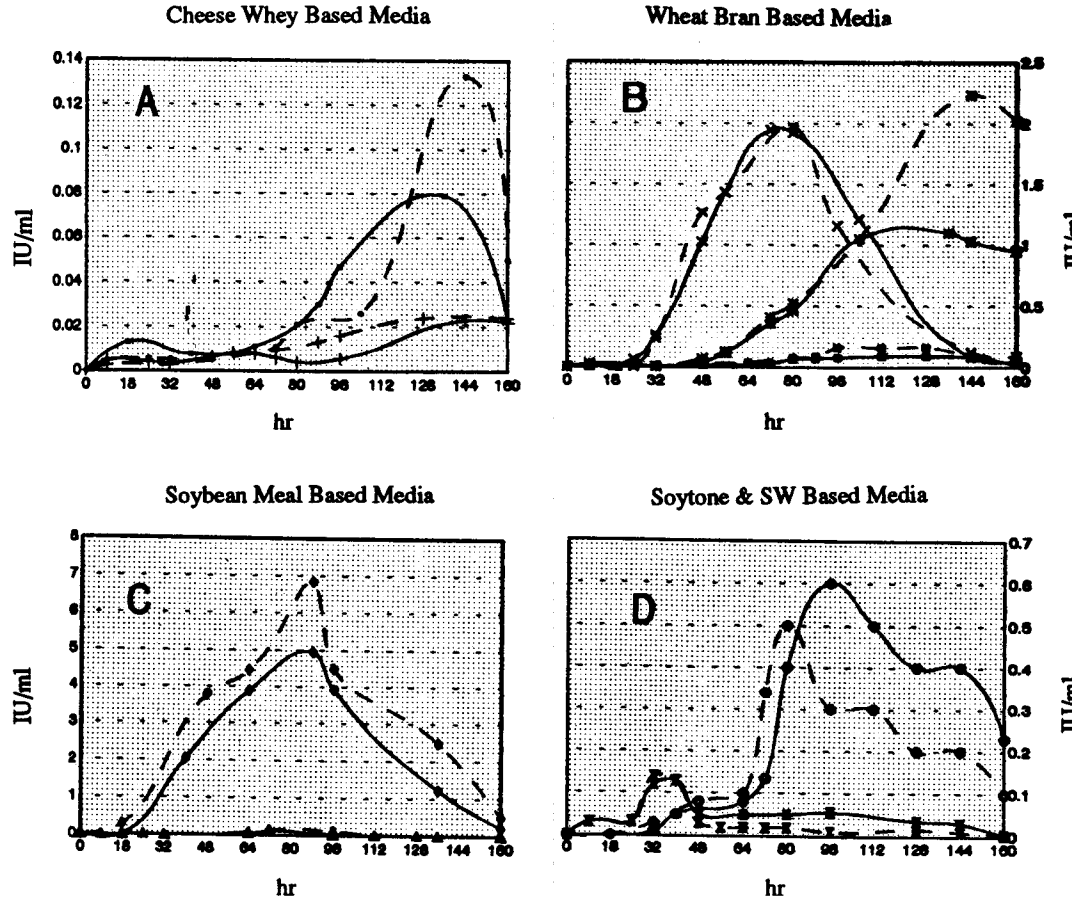
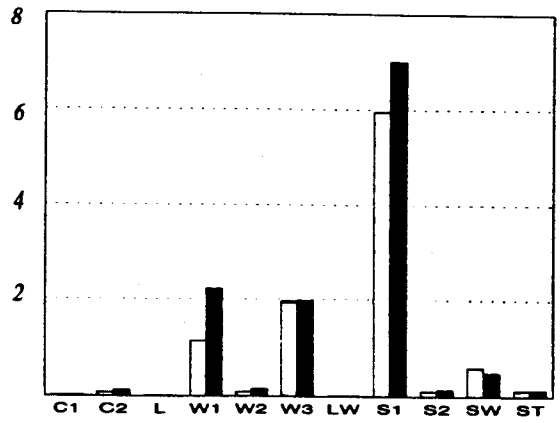


Figure 1. Production curves of beta-galactosidase on media: without Tween 80 —, with Tween 80 – –, +C1, ⁻°C2, *W1, ■W2, × W3, ◆S1, ΔS2, ○SW, ⧖ Soytone



gure 2. Maximum production of beta-galactosidase in cultures th Tween 80■, without Tween 80□

substrate, it is also the cheapest inducer of beta-galactosidase production. Supplementation of media by whey has been studied by Gomez and Castillo [26]. They claimed that cheese whey is not a repressing carbon source for beta-galactosidase production in yeasts. In contrast, our experiments with *A. oryzae* showed low yields of beta-galactosidase on whey-based media (Fig. 1A), indicating catabolite repression.

Comparing production time of pure lactose with whey lactose for enzyme induction, it was found that the former has a faster inductive activity than the latter, but the productivity by whey lactose is higher than pure lactose (Table 2).

The low yields obtained using lactose-based media is an interesting result of this work (Table 2). It can probably be explained by the effects of catabolite repression due to

Table 2. Enzyme productivity in media (IU/hr.l)

Medium	Productivity	Fermentation Time(hr)	Productivity	Fermentation Time(hr)
	Without Tween 80		With Tween 80	
C1	0.16	144	0.195	128
C2	0.56	144	0.92	144
L	0.25	24	0.45	8
W1	7.6	144	15.97	144
W2	0.80	112	1.54	104
W3	27.1	72	24.63	80
LW	0.188	32	0.194	32
S1	62.5	80	87.5	80
S2	1.53	72	2.1	72
SW	6.25	96	6.25	80
Soytone	2.71	48	4.38	32

the readily-available glucose and galactose at the intracellular level after production of only small amounts of beta-galactosidase [22].

Wheat bran contains galactan-based hemicellulose (30%) which is susceptible to hydrolysis by beta-galactosidase [27, 28]. Therefore, we tried wheat bran as a carbon source for producing beta-galactosidase. In such media, enzyme production was significantly increased (Fig. 1B). The results showed that production in W1 medium, supplemented with Tween 80 (wheat bran and spent grain), is slightly greater than that in W3 medium (wheat bran and $NaNO_3$) (Fig. 1B). However, the time required to reach maximum enzyme production was dramatically reduced by switching from medium W3 to medium W1 (Table 2).

The media containing corn steep liquor and peptone supported growth better than spent grain. In contrast, a higher enzyme production was achieved using spent grain, an observation similar to a previous report [22].

Induction of enzyme in spent grain is more efficient than in others (Figs. 1 A, B) due to a high content of hemicellulose [22]. Media bearing corn steep liquor are not suitable due to the fact that they contain large amounts of readily fermentable carbohydrates such as glucose [29]. Accordingly, enzyme production decreases because of catabolite repression.

Using soybean meal-based media of different compositions, the best production was obtained when $NaNO_3$ was added (Fig. 1C). It is interesting that the production of enzyme on mixed media containing wheat bran and soybean meal is lower than media containing soybean meal or wheat bran alone (Fig. 1). It is possible that some components of soybean meal and wheat bran combine together thus inhibiting enzyme production.

Ueno *et al.* used only soybean meal as a fermentation medium for production of beta-galactosidase by *A. oryzae* [23]. It was concluded that *A. oryzae* could not produce a significant amount of beta-galactosidase in liquid medium. They suggested that soybean meal could not induce enzyme production, but *A. oryzae* PTCC 5163 did produce high levels of beta-galactosidase on soybean meal supplemented with $NaNO_3$ (Fig. 1C).

In the final experiment, we substituted soytone for soybean meal and found that enzyme production was significantly decreased. This was probably due to the clumping together of fungal mycelia in the soluble medium, resulting in diffusion limitations.

In conclusion, the present report indicates that the highest production of beta-galactosidase by *A. oryzae* PTCC 5163 is obtained using a medium containing soybean meal and $NaNO_3$ in the form of submerged fermentation.

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