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Full Length Research Paper

Optimization of glucose isomerase production by *Streptomyces albaduncus*

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The effect of environmental factors on glucose isomerase productivity of Saudi Arabia isolated *Streptomyces albaduncus* was studied. During growth of the tested microorganism in the basal medium at 30°C, the glucose isomerase production reached the maximum level (6530 U/L) after 96 h incubation period. Irrespective of the carbon source, replacement of xylose with different carbohydrate did not increase the enzyme productivity. On the other hand, the highest enzyme productivity was obtained when corn steep liquor (2.0%, w/v) and yeast extract (1%) were used as nitrogen sources. Also, the optimum initial pH for maximum growth and enzyme productivity was 7. Further improvement in the glucose isomerase production was obtained after the addition of 0.01% (w/v) DL-isoleucine to the culture medium. Accordingly, optimization of the environmental condition associated with increase in the glucose isomerase enzyme productivity from *S. albaduncus* up to 15328 U/L which is approximately 2.3 more times as compared to that obtained under the initial condition. Finally, a promising production of glucose isomerase (14100 U/L) at large scale level was achieved by cultivation of the tested strain in the optimum environmental conditions using laboratory fermentor of 14 L.

Key words: Glucose isomerase, fermentation, environmental condition, *Streptomyces albaduncus*.

INTRODUCTION

Glucose isomerase (GI) enzyme is considered one of the most important industrial enzymes (Sriprapundh et al., 2003; Rao et al., 2008). The main practical application of this enzyme stems for its ability to isomerize D-glucose to D-fructose, and hence, it is widely used in industry for production of high-fructose corn syrup (HFCS) which is used all over the world as an alternative to sucrose or invert sugar in the food and beverage industry (Fenn et al., 2004; Heo et al., 2008; Brat et al., 2009). Due to its ability to catalyze the isomerization of D-xylose to D-xylulose, the GI enzyme has also potential application in the conversion of hemicellulosic biomass to ethanol (Borgi et al., 2004; Joo et al., 2005; Gromada et al., 2008).

Although, the enzyme is widely distributed in various prokaryotes, most industrially used GI enzymes are obtained from *Streptomyces* spp (Stanbury et al., 2000a; van Maris et al., 2007; Martín and Aparicio, 2009). In a previous study, a high GI-producer, identified as *Streptomyces albaduncus*, was isolated from soil sample collected from the Western Region of Saudi Arabia (Yassien and Jiman-Fatani, 2011). Improvement of the enzyme-productivity of the selected isolate(s) could be achieved through physiological optimization, including formulation and pH of the medium, the incubation period, and temperature (Lowe, 2001).

The aim of the present study was to investigate the optimum condition of enzyme production by

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Abbreviations: GI, Glucose isomerase; **HFCS,** high fructose corn syrup; **OFM,** optimum fermentation medium.

S. albaduncus, in addition to large scale enzyme production through cultivation in a laboratory fermentor of 14 L.

MATERIALS AND METHODS

Organisms

A strain of *Streptomyces albaduncus*, a high producer microorganism of glucose isomerase (GI), was isolated previously from soil sample in West Area of Saudi Arabia (Yassien and Jiman-Fatani, 2011).

The purified isolate was maintained onto starch nitrate agar (20 g soluble starch, 2.0 g KNO_3, 1.0 g K_2HPO_4, 0.5 g $MgSO_4$, 0.5 g NaCl, 3.0 g $CaCO_3$, 0.01 g $FeSO_4$, 0.01 g $MnCl_2$, 0.01 g $ZnSO_4$, 20 g agar per liter) at 4°C and subcultured onto the same medium every 4 weeks. Storing of the obtained isolates was carried out by preparing a heavy *Streptomyces* suspension in Brain Heart Infusion containing 15% glycerol and stored at -86°C.

Quantitative determination of glucose isomerase production

The GI activities as well as the specific production of GI (GI activity/dry cell weight) were determined as described by Bok et al. (1984).

Preparation of inoculum

Sporulated cultures of *S. albaduncus* 4-5 days old on starch-nitrate agar were harvested in sterile normal saline. The final count in the spore suspension was adjusted to about 10^5 CFU/ml, as determined by the standard viable count technique. The spore suspension was used as an inoculum to give a final count of 10^3 CFU/ml in the medium.

Enzyme production

Erlenmeyer flasks of 250 ml containing 50 ml of basal medium (1% tryptone, 0.7% yeast extract, 1% D-xylose, 0.1% $MgSO_4$ w/v, pH 7.0) were inoculated with a spore suspension of the tested isolate to give the required final count. Then, the cultures were incubated at 30°C for 120 h in a cooling incubator shaker (S19R-2, Sheldon, USA) at 250 rpm.

Biomass determination

After incubation, the cell mass was obtained by centrifugation of a known volume of culture broth at 14,000 rpm for 20 min and washed twice with distilled water in centrifuge tubes of predetermined weights. The tubes were dried in hot air oven at 100°C to a constant weight. The dry cell weight per liter of culture broth was calculated.

Enzyme preparation

An aliquot of the culture broth obtained after fermentation was sonified on ice for 10 min at 22 V output power. In the resulting suspension of ruptured cells, the enzymatic activity of GI of the isolates was determined.

Enzyme assay

A 0.5 ml portion of each sonified culture broth was mixed with 5 ml of maleate buffer-salts solution (maleate buffer [0.2 M solution of acid sodium maleate and 0.2 M NaOH] containing 0.05% $MgSO_4.7H_2O$ and 0.003% $CoCl_2.6H_2O$.) containing 1% glucose and incubated in a water bath at 65°C for 60 min. The mixture was cooled in ice to stop the enzymatic reaction. The amount of fructose in the prepared mixture was determined as described by Kulka (1956). In brief, portions of 0.5 ml of the reaction mixture were transferred into test tubes containing 1.5 ml of distilled water. Then 6 ml of ketose reagent (1:1 ration of A [0.05g resorcinaol in 100 ml ethanol] and B [0.216 g $FeNH_4(SO_4)_2.12H_2O$ in 1000 ml 12 M HCl]) was added. The content of the tube was mixed thoroughly and covered with glass marbles, then immersed in a water bath at 80°C for 40 min, where a red color was developed in positive cases. The tube was immediately cooled in ice water at the end of the heating period and the absorbance was then measured using spectrophotometer at 480 nm. The absorbance result was converted to GI activity (U/L) using a standard curve constructed for D-fructose. One unit of GI activity (U) is defined as the amount of enzyme that produced 1 µmol of D-fructose per min under the assay conditions described above. Specific GI production is expressed as units of enzyme activity (GI activity per gram of dry cells). A blank containing 0.5 ml of distilled water and another sample containing 0.5 ml of uninoculated screening medium were used as negative control.

Optimization of glucose isomerase productivity of *S. albaduncus*

The effect of different factors on growth and GI production by *S. albaduncus* was investigated. The basal medium used for this study was started by the following ingredients; 1% tryptone, 0.7% yeast extract, D-xylose 1%, $MgSO_4$ 0.1% w/v, and the pH adjusted to 7.0. All experiments were carried out using 250 ml Erlenmeyer flasks containing 50 ml of the fermentation medium inoculated with 0.5 ml of *S. albaduncus* spore suspension. The flasks were shaken at 250 rpm at a specified temperature for the given incubation period. At the end of the incubation period, the sonified culture broth was assayed for the GI activity. Microbial growth was quantified through determination of the dry cell weight. Each sample was assayed in triplicate.

Effect of incubation period

A group of flasks containing basal medium were inoculated and incubated at 30°C. A flask was removed for determination of both growth and GI production after 12, 24, 36, 48, 60, 72, 84, 96, and 120 h incubation periods.

Effect of incubation temperature

A set of flasks containing basal medium was incubated at different temperatures (20, 25, 30, 37, and 45°C) for 96 h. At the end of the incubation period, both microbial growth and GI production at each temperature were determined.

Effect of different carbohydrate

To find out the ability of different carbohydrates to induce the formation of GI enzyme, D-xylose in basal medium was be replaced by other carbohydrates (1%).

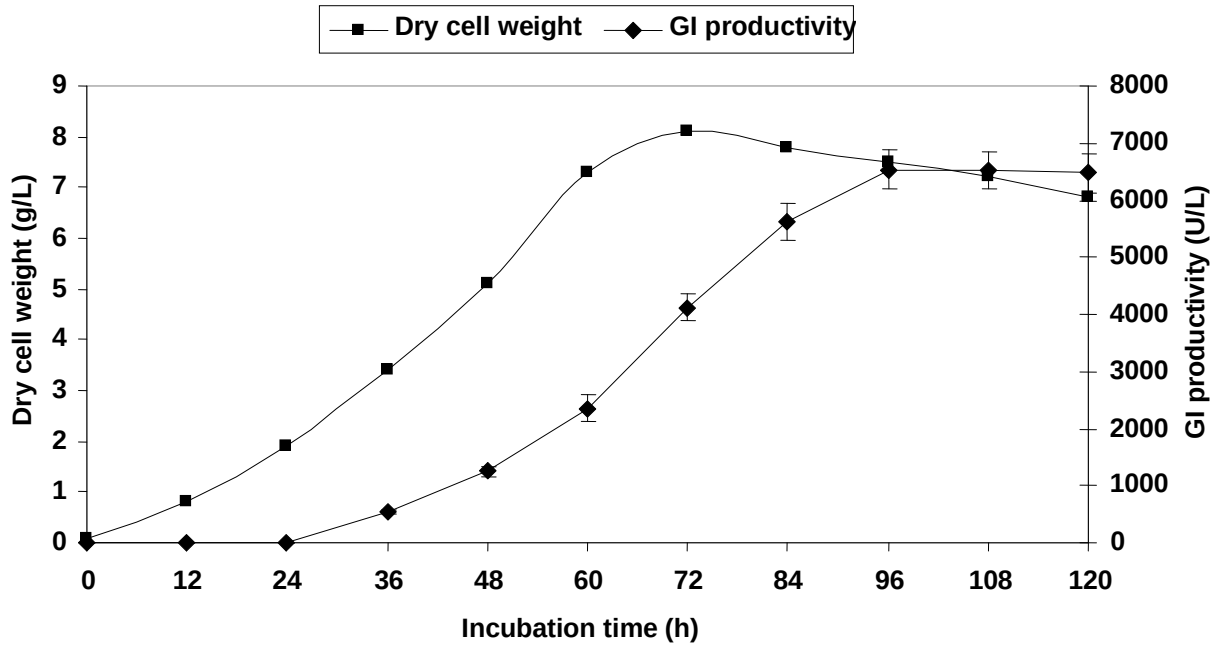

Figure 1. Effect of incubation time on growth and GI productivity by *S. albaduncus* in basal medium at 30°C and for 96 h.

Effect of addition of different protein sources and urea

To a group of flasks containing the basal medium, different protein sources were added at different concentrations. Urea was also tested at a concentration of 1% (w/v). The flasks were inoculated and incubated at 30°C for 96 h. Both microbial growth and GI activity were then determined.

The most suitable carbon and protein sources obtained from the previous results will be added to the basal medium and the modified medium will be used to continue the other experiments for reaching to the optimum cultural condition for growth and highest production of the GI enzyme.

Effect of initial pH

Different flasks containing modified basal medium with initial pH values ranging from 5.5 to 9.0 were inoculated with the tested organism and incubated at 30°C for 96 h, after which both microbial growth and activity of the GI enzyme were determined.

Effect of addition of different amino acids

Different amino acids (0.01%) were incorporated into the modified basal medium, inoculated with the tested organism, and incubated at 30°C for 96 h, after which both microbial growth and activity of the GI enzyme were determined.

Production of Glucose Isomerase at large scale level

The fermentation was conducted in a batch mode using 14 L laboratory glass fermentor (Bioflo 110, New Brunswick Scientific Co., Inc., NJ, USA). The fermentor was operated at 30°C without pH control. The agitation was carried out using two six-bladed Rushton turbine impellers, 77 mm in diameter, separated by 80 mm and rotating at 250 rpm. Air was sterilized by a resterilizable cartridge filter, 0.22 µm, and introduced into the vessel through a single orifice sparger at a flow rate of 3 vvm. Exhaust gases left the fermentor after passing through a water-cooled condenser. Foaming was controlled automatically with sterile silicone oil. Enzyme production was carried out in 8 L (working volume) of culture medium. The pH of the medium was adjusted to 7.0 before sterilization. Inoculum of spore suspension prepared as described above and used at a level of 1% (v/v). During the fermentation process, samples were aseptically withdrawn at different time intervals for monitoring microbial growth and GI-productivity.

Statistical analysis

Statistical significance between means was tested by analysis of variance (ANOVA) and student t-test using Instat-ANOVA software. The differences between means were considered statistically significant when the test yielded a value $P<0.05$.

RESULTS

Effect of incubation period

A kinetic of growth and GI production by *Streptomyces albaduncus*, using the basal medium, was plotted (Figure 1) in order to determine the optimum incubation period for GI productivity by the selected isolate. The results showed that GI production was observed only after 24 h of cultivation. Cell growth reached its maximum at around 72 h of incubation, whereas maximum enzyme activity (6530 U/L) was obtained after 96 h. Therefore, the optimum incubation period used in the subsequent experiments was 96 h.

Table 1. Effect of incubation temperature on growth and GI production by *S. albaduncus* in basal medium at pH 7.0.

Incubation temperature (°C)	Dry cell weight (g/L)	GI production (U/L)	Mean specific GI production (U/g dry cell weight)
20	4.6	1260 ± 145	274
25	5.9	3920 ± 238	664
30	7.4	6610 ± 415	893
35	6.6	5130 ± 325	777
40	3.9	1940 ± 210	497
45	2.4	980 ± 85	408

Effect of incubation temperature

On studying the effect of incubation temperature on the microbial growth and GI production, it was observed (Table 1) that the organism could efficiently grow and produces suitable level of GI enzyme in the range of incubation temperatures 25, 30, and 35°C. However, maximum cell growth and the highest levels of GI (6610 U/L with specific GI productivity 893U/g dry weight cell) were obtained at 30°C incubation temperature. Accordingly, 30°C was selected as incubation temperature for enzyme production in the subsequent experiments.

Effect of different carbohydrates

The obtained results (Table 2) showed that, none of the tested carbon sources produced higher level of GI than that obtained by using 1% xylose added in the basal medium.

Effect of addition of different protein sources and urea

According to the obtained results (Table 3), the combination of 2% corn steep liquor and 1% yeast extract produce the highest level of GI production (12450 U/L with specific GI productivity 1596 U/g dry weight cells) by the selected isolate.

Therefore, the formula of the basal medium was modified by replacing tryptone with corn steep liquor (2.0%) and increasing the yeast extract concentration to 1% w/v. This modified basal medium was routinely used in the subsequent experiments.

Effect of initial pH

It was observed that with all the initial pH values tested (5.5 to 9.5); the final pH of the spent media was in the alkaline range (8.0-8.5). The highest values of cell growth and enzyme production were obtained after 96 h of incubation at an initial pH between 6.5 - 7.5. However, maximum GI production (12980 U/L with specific GI productivity 1708 U/g dry weight cells) was obtained with initial pH 7.0 (Table 4). Accordingly, adjustment of the pH of the medium to 7.0 using ammonia solution was continued in the subsequent experiments.

Effect of addition of different amino acids

A significant increase in the GI production by *S. albaduncus* was only observed in the presence of L-proline, DL-isoleucine, L-gluamic acid, L-aspartic acid, DL-ornithine, L-aspargine, L-glutamine, and Methionine (Table 5). The highest level of GI was obtained in the presence of 0.01% DL-isoleucine (15328 U/L with specific GI production 1965 U/g dry cell weight).

Accordingly, the final formula of the optimum fermentation medium (coded OFM) used for the highest GI productivity of *S. albaduncus* formed of corn steep liquor 2.0%, yeast extract 1%, 0.01%DL-isoleucine, 1% D-xylose, 0.1% $MgSO_4$ w/v, and adjustment pH to 7.0 with ammonium hydroxide.

Growth and GI productivity of *S. albaduncus* using OFM medium

The OFM was used to test the growth and GI productivity by *S. albaduncus* along 120 h of incubation at 30°C. Cellular growth reached its maximum at around 72 h of incubation. The enzyme production was detected after 24 h of incubation and reaching the maximum level (15240 U/L) after 96 h (Figure 2).

Production of glucose isomerase by the selected strain in a laboratory fermentor

The GI productivity of the tested strain was further studied at large scale level by cultivating the tested organism in OFM medium using 14 L laboratory fermentor. According to the obtained results (Figure 3), the GI activity could be first detected after 24 h of

Table 2. Effect of different carbohydrates on growth and GI production by *S. albaduncus* at pH 7.0, 30°C and 96 h.

Carbohydrates (1% w/v)	Dry cell weight (g/L)	GI production (U/L) ± SD	Mean specific GI production (U/g dry cell weight)
Xylose	7.6	6717 ±324	884
Glucose	7.3	874 ± 67	120
Lactose	6.5	920 ± 71	153
Maltose	6.4	1048 ± 84	164
Mannitol	7.2	764 ± 59	106
Fructose	7.1	762± 63	107
Sucrose	6.9	824 ± 68	135
Inositol	6.1	766 ± 51	126
Galactose	7.3	780 ± 62	107
Arabinose	7.8	844 ± 74	108

Table 3. Effect of different protein sources and urea on growth and GI production by *S. albaduncus* at pH 7.0, 30°C and 96 h.

Source of protein and urea	Dry cell weight (g/L)	GI Production (U/L) ± SD	Mean specific GI production (U/g dry cell weight)
Basal medium	7.5	6760 ± 294	901
Tryptone 1%	3.1	708 ± 84	228
Tryptone 2%	3.5	780± 67	223
Peptone 1%	2.7	650 ± 76	243
Peptone 2%	3.4	854 ± 72	251
Yeast extract 1%	5.6	2300 ± 224	411
Yeast extract 2%	6.4	3180 ± 289	497
Corn steep liquor 1%	5.7	1640 ± 175	288
Corn steep liquor 1.5%	5.9	1960 ± 153	332
Corn steep liquor 2.0%	6.3	3020 ± 226	479
Corn steep liquor 2.5%	7.4	3280 ± 233	443
Corn steep liquor 3%	8.1	2900 ± 234	358
Urea	2.1	506 ± 78	241
Yeast extract 1.0% and tryptone 1%	7.7	3340 ± 210	434
Yeast extract 1% and peptone 1%	4.3	1040 ± 78	242
Corn steep liquor 2% and Yeast extract 1%	7.8	12450 ± 460	1596
Corn steep liquor 2% and tryptone 1%	7.1	4750 ± 208	669
Corn steep liquor 2% and peptone 1%	6.2	4060 ± 224	654

cultivation. The maximum cellular growth and enzymatic activity (about 14100 U/L) were obtained after 72 h and 108 h, respectively. The pH has also increased gradually during fermentation to finally reach 8.7. The fermentation process was continued for 120 h where the total GI productivity reached a plateau.

DISCUSSION

A strain identified as *S. albaduncus*, a major producer of GI enzyme was isolated previously from West Area of Saudi Arabia (Yassien and Jiman-Fatani, 2011). The present study focused on improving the productivity of GI enzyme by *S. albaduncus* through physiological parameters optimization. Medium formulation is an essential stage in the design of successful laboratory experiments, pilot scale development and manufacturing processes (Stanbury et al., 2000b; Jing, 2010). The basal medium used in this study contains tryptone and yeast extract as nitrogen source; xylose, as a source of carbon and inducer for enzyme production; and $MgSO_4$, as a mineral source. Both cellular growth and GI productivity of *S. albaduncus* were monitored over a period of 120 h. Maximum cellular growth and enzyme productivity (6530 U/L) of tested isolate, were obtained after 72 and 96 h of incubation, respectively. The optimum incubation period for GI production was previously reported to be 24 h for

Table 4. Effect of pH on growth and GI production by *S. albaduncus* in modified basal medium and incubated at 30°C for 96 h.

pH	Dry cell weight (g/L)	GI production (U/L) ± SD	Mean specific GI production (U/g dry cell weight)
5.5	4.4	340 ± 45	77
6	6.1	5340 ± 235	875
6.5	7.5	7680 ± 327	1024
7	7.6	12980 ± 442	1708
7.5	8.1	8540 ± 287	1054
8	7.4	4260 ± 234	576
8.5	7.2	340 ± 43	47
9	6.7	300 ± 22	45

Table 5. Effect of different amino acids on growth and GI production by S. albaduncus in modified basal medium, with initial pH 7.0, 30°C and 96 h incubation period.

Amino acids	Dry cell weight (g/L)	GI production (U/L) ± SD	Mean specific GI production (U/g dry cell weight)
Control	7.9	13360 ± 376	1691.1
L-proline	8.1	14200 ± 410	1753.1
DL-tyrosine	7.8	12980 ± 315	1664.1
L-leucine	7.2	660 ± 76	91.6
DL-serine	6.9	680 ± 55	98.6
DL-phenylalanine	6.8	695 ± 68	100.7
DL-isoleucine	7.8	15328 ± 456	1965
L-glutamic acid	7.7	14646 ± 397	1902
L-aspartic acid	7.4	13210 ± 322	1785.1
glycine	7.5	668 ± 89	89.1
L-cystine	7.3	9780 ± 225	1339.7
L-alanine	5.9	710 ± 75	120.3
DL-valine	7.2	740 ± 72	102.8
D-alanine	6.9	748 ± 65	108.4
DL-ornithine	7.8	14050 ± 289	1801.2
L-asparagine	8.1	14680 ± 445	1812.3
L-arginine	8.1	9320 ± 185	1150.6
methionine	8.2	14460 ± 325	1763.4
L-glutamine	8.0	14380 ± 410	1797,5
glutathione	7.6	12340 ± 285	1623.6

several acidophilic *Streptomyces* strains (Bok et al., 1984), 36 h for *S. phaeochromogenes* (Sanchez and Quinto, 1975), 48 h for *S. flavogriseus* (Chen et al., 1979) and a mutant strain of *S. coelicor* (Hafner and Jackson, 1985) and 72 h for *S. murinus* (Skoet and Guertler, 1987). The longer time required for maximum enzyme GI production and maximum cellular growth by *S. albaduncus* compared with the above microorganisms, could be attributable to the use of a spore inoculum.

For GI production, *Streptomyces* spp. are usually grown at around 30°C (Bhosale et al., 1996). In the present study, the suitable microbial growth and GI production by *S albaduncus* is found to be in the range of 25-35°C. While, the maximum growth and GI production is 30°C.

Medium formulation is an essential stage in the design of successful laboratory experiments for production of the desired microbial products (Stanbury et al., 2000b). In case of GI, the presence of D-xylose significantly increases the enzyme productivity of *Streptomyces* spp (Wong et al., 1991). Replacement of D-xylose, in this study, with glucose or another carbohydrate significantly lowers the specific GI production. This could be attributed to the repressor effect of glucose or other carbon source on enzyme synthesis. Generally, the presence of glucose in the growth medium of *Streptomyces* spp. induces

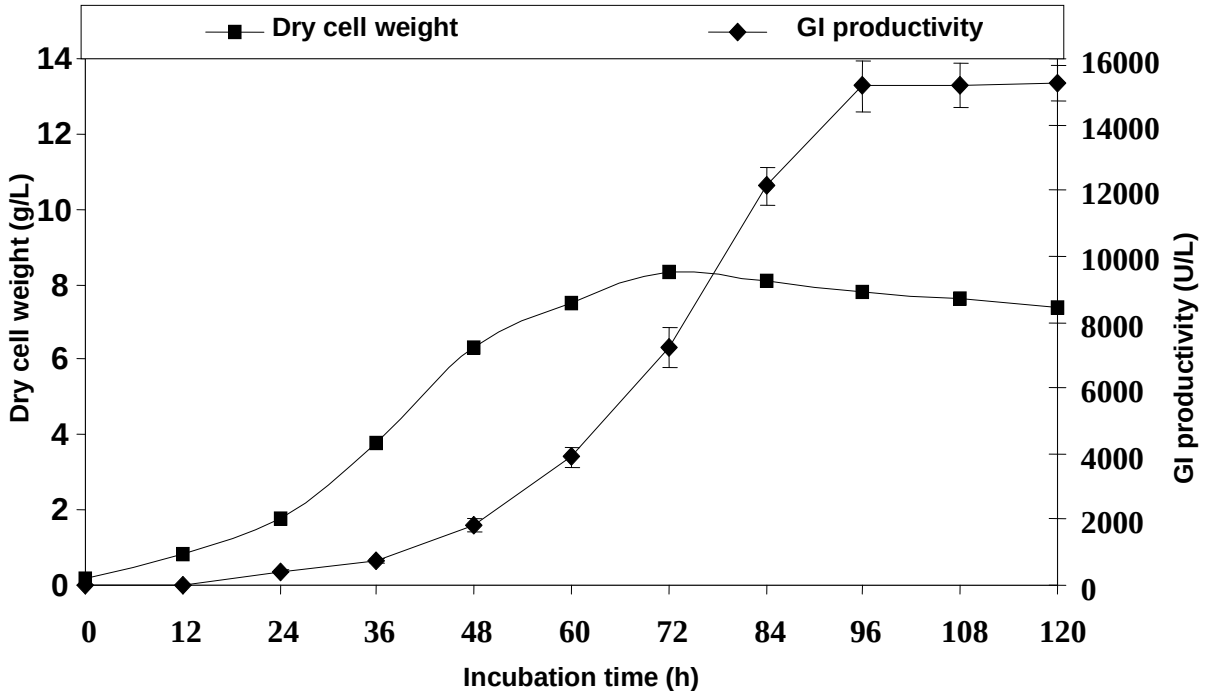

Figure 2. Kinetics of cellular growth and GI productivity by *S. albaduncus* using optimum fermentation medium along 120 h incubation period at 30°C.

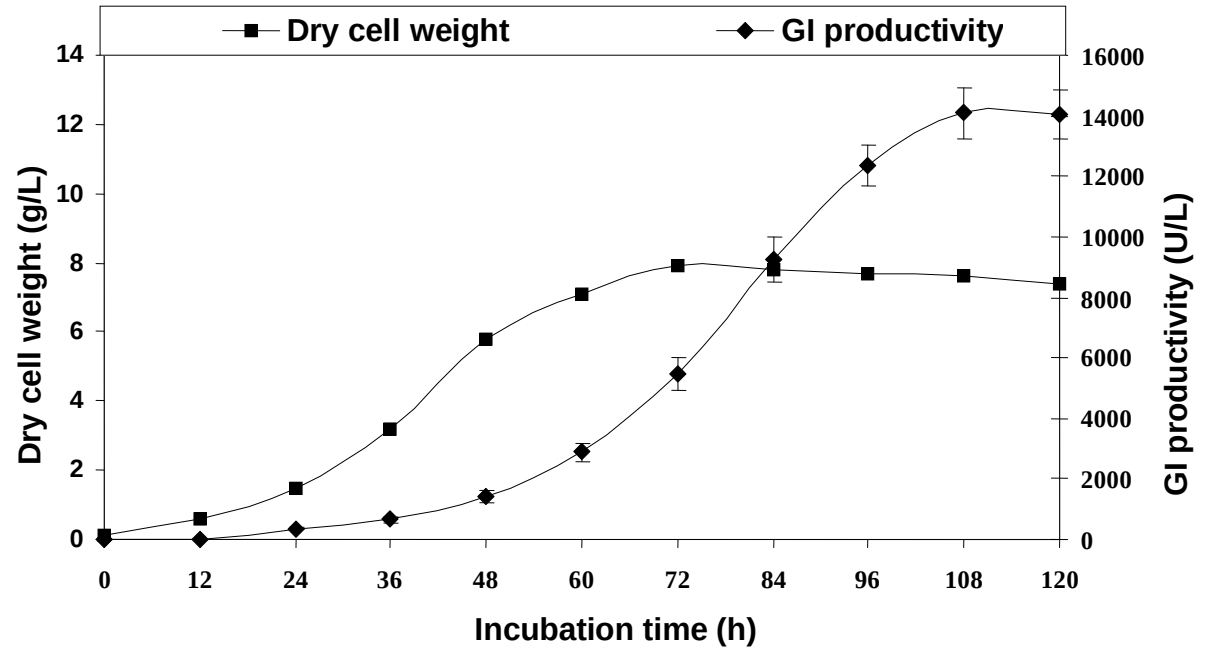

Figure 3. Kinetics of cellular growth and GI productivity by *S. albaduncus* along 120 h incubation period at 30°C in optimum fermentation medium, using a Laboratory Fermentor of 14 L.

reduction in the specific activities of the enzymes that are involved in the catabolism of other carbon sources (Kwakman and Postma, 1994). The glucose repressive effect on of xylose utilization genes was previously observed in *S. phaeochromogenes* (Sanchez and Quinto, 1975) and *S. rubiginosus* (Wong et al., 1991) as well as *Bacillus subtilis* (Kraus et al., 1994; Jacob et al., 1991), *B. megaterium* (Rygus and Hillen, 1992) and

Pseudomonas putida (Holtel et al., 1994). In *B. subtilis*, glucose repression is, in part, dependent on the concentrations of glucose and xylose in the medium (Kraus et al., 1994).

For selection of the most suitable organic nitrogen source, yeast extract and tryptone in the used fermentation medium were replaced by urea or other sources. The results revealed that the highest GI productivity of *S. albaduncus* is obtained by replacing the tryptone with corn steep liquor (2%) and increasing the concentration of yeast extract to1%. Corn steep liquor is one of the most suitable organic nitrogen sources for GI production (Bok et al., 1984; Hafner and Jackson, 1985; Skoet and Guertler, 1987). Corn steep liquor is a by-product after starch extraction from maize and hence, it is inexpensive and suitable for commercial-scale production of the enzyme (Stanbury et al., 2000b). Combination of corn steep liquor (2.0%) with yeast extract (1%) resulted in an increase in the specific GI production by about 3 to 4 times more than that obtained with corn steep liquor or yeast extract used as sole nitrogen source. With the latter combination, the GI production is approximately doubled as compared to that obtained in the basal medium. These results confirmed that, nitrogen source is a very critical factor and needs to be optimized in each fermentative process for enzyme production.

With the exception of GI production by acidophilic *Streptomyces* strains and alkaliphilic *Bacillus* strains, most other GI-producing fermentations are carried out between pH 7.0 and 8.0 (Bok et al., 1984; Bhosale et al., 1996). A suitable growth of *S. albaduncus* could be obtained at a relatively wide pH range (6.0 to 8.6), while the optimum initial pH for GI production by the tested strain was 7. Regardless of the initial pH employed, the final pH of the spent media was in the alkaline range; a characteristic result in cultures of *Streptomyces* (Chen et al., 1979).

Regarding the phosphate buffering effect, there were no difference in microbial growth or GI productivity in the presence and absence of standard phosphate buffer (data not shown) which might be due to the presence of other source of phosphate ions in the medium. In addition, the proteins, peptides, and amino acids that constitute the major part of corn steep liquor and yeast extract usually provide a good buffering capacity (Stanbury et al., 2000b). Consequently, it is irrelevant to increase the buffering capacity of the culture medium.

Proteinaceous nitrogen compounds, such as corn steep liquor and yeast extract, usually serve as sources of amino acids. However, the addition of pure amino acids to the fermentation medium is occasionally required to improve the yield of some biotechnological products (Stanbury et al., 2000b). Concerning GI production, the addition of certain amino acids was previously reported to improve the yield of enzyme in *S. violaceoniger* (Vaheri and Kauppinen, 1977). The same observation was obtained in the present study with the maximum GI level in the presence of 0.01% DL-isoleucine.

According to the obtained results, an optimum physiological condition for GI production by *Streptomyces albaduncus* was achieved by modifying the fermentation medium (1% xylose, 2% corn steep liquor, 1% yeast extract, 0.01% DL-isoleucine, 0.1% $MgSO_4$, with initial pH 7). The fermentation process was carried out at 30°C incubation temperature for 96 h. Under this condition, the GI productivity of *S. albaduncus* is increased to 15240 U/L that is about 2.3 more times as compared with that produced by the basal medium.

The results of a fermentation process carried out in shake flasks cannot usually be extrapolated to indicate possible performance in a fermentor (DeWitt et al., 1989). Controls on the reaction in a shake flask are extremely limited when compared to the possible continuous control in a fermentor. Also, many problems may arise during scaling up due to the different ways in which process parameters, such as inoculum development, sterilization, and environmental parameters are affected by the size of the unit (Banks, 1979). Accordingly, results obtained in a shake flask should only be taken as preliminary indicators for the conditions necessary for successful industrial-scale production and must be verified in studies carried out in a fermentor. In this study, the small scale process of GI production by the tested strain was scaled up from the 50 ml scale in shake flasks to a 14 liters laboratory fermentor. The fermentation medium had the same composition as that used in shake flasks while the working volume was 8 liters. Under this condition, the tested organism produce a promising level of GI enzyme (14100 U/L) which may be slightly lower than that produce at small scale (50 ml in shaking flask). This decrease in enzyme productivity is a common practice encountered during scaling up, and is usually significant (Gerritse et al., 1998); nevertheless, the scale-up losses in our case could not be considered such dramatic. Many environmental parameters; such as nutrient availability, pH, temperature, shear conditions as well as dissolved O_2 and CO_2 levels; are changed during scaling up (Banks, 1979) and hence may be responsible for the decrease in yield. Higher yields of GI are, therefore, expected to be obtained in the fermentor upon identification of the exact factors that gave rise to that decrease and rectifying them. Therefore, further studies should be continued to readjust the environmental condition to achieve the optimum condition for GI production by *S. albaduncus*.

The results obtained from this study can be used as an initial step for application at industrial level through further studies on the GI productivity of *S. albaduncus* at large scale level.

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