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Some Factors Affecting Glucoamylase Production from *Aspergillus* sp.

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Aspergillus sp.의 Glucoamylase 생산에 미치는 요인

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The effects of carbon, nitrogen sources and culture conditions on glucoamylase production from *Aspergillus* sp. were investigated. Among tested carbon sources, soluble starch was most effective for the production of the enzyme, and the level of concentration for the optimal enzyme production was found to be 5%. For nitrogen sources, yeast extract was best for the enzyme production, with the level of 1%. The enzyme was maximally produced by cultivating the organism at medium of initial pH 6.0, and temperature of 28°C. Wheat bran was most suitable for the enzyme production from the organism in solid state culture.

Glucoamylase (EC 3.2.1.3) is an extracellular enzyme which catalyzes the stepwise hydrolysis of α -1,4 links in starch and oligosaccharides into β -glucose from non-reducing ends of the chain. The enzyme has been isolated from several species of fungi of the genera *Aspergillus* (1-5), *Rhizopus* (6, 7), *Mucor* (8), *Penicillium* (9), *Cephalosporium* (10), and *Endomyces* (11). It was also found that *Aspergillus* species contains at least two isozymes which are readily separated by electrophoresis (12, 13). Fungal glucoamylases are generally regarded as glycoprotein (13). In addition, glucoamylase was also found in some yeast (14, 15) and bacteria (16). Most commercial glucoamylase have been produced mainly from fungal sources such as *Aspergillus* and *Rhizopus*. Glucoamylase has broad application, e.g. glucose syrup production from prethinned starch, use in brewing, alcohol, baking, textile, paper, fermentation, pharmaceuticals, soft drink and confectionery (17). It is important to maintain the proper initial pH of the culture medium, culture temperature as well as medium composition for the optimal production of enzyme. We have published the isolation of *Aspergillus* sp., which produced glucoamylase highly (18), and properties of the enzyme from the species (19). Now, this paper describes the effect of medium composition and culture conditions on the production of glucoamylase from the *Aspergillus* sp.

Materials and Methods

Microorganism and cultivation

Organism used was the *Aspergillus* species which was isolated from soil (18). In case of liquid culture, 100 m*l* of E-flask containing 20 m*l* of basal medium was inoculated with 0.2 m*l* of previously activated suspension of the isolated strain. The basal medium was composed of 3% glucose, 2% peptone, 0.1% KH_2PO_4, 0.05% KCl, and 0.01% $FeSO_4$. The effect of carbon and nitrogen sources was studied by substituting glucose and peptone with various carbon and nitrogen compounds. For solid culture, 0.2 m*l* of seed culture in chapek-dox broth was inoculated into solid medium (solid, 20g: water, 20 m*l*),

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and cultivated at each temperature.

Crude enzyme preparation

200 ml of distilled water was added to each solid koji and the mixture was stirred with a glass rod to extract the enzyme completely. The mixture was filtered using Toyo No. 2 filter paper and the filtrate was centrifuged at 3,000 rpm for 30 minutes. The supernatant was used as a crude enzyme solution for solid culture. In case of liquid culture, culture broth itself was used as a crude enzyme preparation.

Enzyme assay

Since crude enzyme preparation prepared from the isolated strain produced only glucose from soluble starch (19), therefore glucoamylase activity was determined by measuring the amount of glucose released from soluble starch by dinitrosalicylic acid (20). The enzyme reaction was performed with 0.5 ml of 4% soluble starch, 0.45 ml of 0.2 M Na-acetate buffer (pH 4.5) and 50 μl of enzyme solution at 60°C for 15 minutes. One unit of glucoamylase activity was defined as the amount of enzyme to produce one μ mole of glucose per minute. The glucoamylase activity was also measured by using color formation of *p*-nitrophenol from *p*-nitrophenyl α-D-glucopyranoside (21). The reaction mixture consisted of 0.1 ml of 0.3% *p*-nitrophenyl α-D-glucopyranoside solution, 0.8 ml of 0.2 M Na-acetate buffer (pH 4.5) and 0.1 ml of the enzyme solution. After incubation for 30 minutes at 60°C, the reaction was stopped by addition of 1 ml of 1% Na-carbonate solution. The released *p*-nitrophenol was measured at 420 nm. One unit of the enzyme activity by the method of *p*-nitrophenol was defined as the amount of enzyme that release 1 μ mole of *p*-nitrophenol from *p*-nitrophenyl α-D-glucopyranoside per minute.

Estimation of microbial growth

Liquid cultured microorganism was harvested by filtration and washed with distilled water and then dried to a constant weight at 100°C. Microbial growth was expressed as milligrams (dry weight) per 20 ml of culture medium.

Table 1. Effect of carbon sources on production of glucoamylase.

Carbon source	Growth (mg/20 ml)	Enzyme activity (unit/ml)	Relative activity (%)
None	20.8	0.0	0.0
Xylose	26.0	8.3	17.4
Galactose	37.4	20.5	42.9
Fructose	46.0	27.7	58.0
Glucose	46.5	10.5	22.0
Sucrose	43.6	15.5	32.4
Lactose	25.1	16.6	34.8
Soluble starch	27.0	47.7	100.0
Glycerin	32.6	5.5	11.5
Inulin	31.0	0.0	0.0
Cellulose	29.6	5.1	10.6
Amylose	27.6	6.6	13.8
Amylopectin	21.1	19.9	41.7
Citric acid	23.2	0.0	0.0
Arabinose	34.7	0.0	0.0

Cultivation was carried out for 3 days at 28°C in basal medium with initial pH 6.0. The glucose in basal medium was replaced with the carbon sources (3%) described above.

Results and Discussion

Effect of carbon source

Production of glucoamylase with various carbon sources by the isolated *Aspergillus* strain was examined by replacing glucose in basal medium with various carbon sources. As shown in Table 1, soluble starch exhibited the highest enzyme productivity. Even though glucose was best for microbial growth, it was not suitable for the glucoamylase production. This

Table 2. Effect of soluble starch concentration on production of glucoamylase from *Aspergillus* sp.

Concentration (%)	Enzyme activity (Unit/ml)
0	37.5
1	62.0
3	72.0
5	86.5
10	62.0
15	48.4

Cultivation was carried out at 28°C for 3 days in basal medium with initial pH 6.0. Only carbon source in basal medium was replaced with soluble starch.

Table 3. Effect of nitrogen sources on production of glucoamylase.

Nitrogen source	Growth (mg/20 ml)	Enzyme activity (unit/ml)	Relative activity (%)
None	0.0	13.3	11.4
$(NH_4)_2SO_4$	16.3	43.2	37.0
NH_4Cl	9.4	33.0	28.3
KNO_3	12.0	26.6	22.8
$NaNO_3$	6.8	21.6	18.5
Urea	0.0	3.3	2.8
Yeast extract	14.0	116.5	100.0
Peptone	16.0	48.0	41.0
Casein	15.0	26.6	22.8
Gelatin	7.4	38.2	32.7
Lysine	0.0	28.3	24.2
Tyrosine	14.7	23.3	19.9
Glutamic acid	0.0	15.5	13.2

Cultivation was carried out for 3 days at 28°C in basal medium with initial pH 6.0. The peptone in the basal medium was replaced with various nitrogen sources (2%) described above. The carbon source used was soluble starch.

result is in consistent with the finding of Lewis (22). Soluble starch appears to function as an inducer for glucoamylase production for the organism. Therfore, glucose in basal medium was replaced with soluble starch. Table 2 shows the effect of soluble starch concentration on the enzyme production. As shown in Table 2, 5% level of soluble starch was optimum for glucoamylase production.

Effect of nitrogen source

Various nitrogen sources were replaced with peptone to investigate the effect of nitrogen sources on the glucoamylase production. As shown in Table 3, yeast extract was most suitable for glucoamylase production among tested nitrogen sources. The inorganic nitrogen sources were generally ineffective for the organism not only for growth, but also for production of the enzyme. The concentration of yeast extract was varied from 0 to 5% to examine the concentration effect on the enzyme production (Table 4). The optimal yeast extract concentration for the enzyme production was 1% as shown in Table 4. The variation of glucoamylase production was much higher with variable yeast extract concentration,

Table 4. Effect of yeast extract concentration on production of glucoamylase from *Aspergillus* sp.

Concentration (%)	Enzyme activity (Unit/ml)
0	2.8
0.25	7.6
0.5	38.0
1.0	82.4
2.0	37.2
5.0	38.6

Cultivation was carried out at 28°C for 3 days in basal medium with initial pH 6.0. The carbon source used was 5% soluble starch, and nitrogen source was replaced with yeast extract.

Table 5. Effect of initial pH on production of glucoamylase from *Aspergillus* sp.

pH	Enzyme activity (Unit/ml)
3	28.0
4	46.2
4.5	42.4
5	56.7
5.5	62.5
6	69.2
6.5	64.3
7	53.5
8	29.3
9	21.4

Cultivation was carried out in basal medium for 3 days at 28°C. The carbon and nitrogen sources used were 5% soluble starch and 1% yeast extract.

while it was relatively lower in case of carbon source. Therefore, it seems critical to determine optimal yeast extract concentration for maximal glucoamylase production.

Effect of initial pH

Adjustment of initial pH of the culture medium is critical in cultivation of organism since it is difficult to adjust the pH in the medium. Furthermore, it is well established that initial pH of the growth medium can ıduce various physiological changes to the organism. It may result in shift in metabolic activity, which may be either favorable or unfavorable for the desired product formation (23). The initial pH of the medium was adjusted to various pH values

Table 6. Effect of temperature on production of glucoamylase from *Aspergillus* sp.

Temperature (°C)	Enzyme activity (Unit/ml)
25	28.4
28	96.6
30	58.8
32	46.3
35	44.3

Cultivation was performed in basal medium for 3 days with initial pH 6.0. The carbon and nitrogen sources used were 5% soluble starch and 1% yeast extract.

Table 7. Effect of temperature on production of glucoamylase from *Aspergillus* sp. in wheat bran solid medium.

Temperature (°C)	Enzyme activity (Unit/ml)
25	30.2
28	42.5
30	50.7
32	42.5
35	38.7

Cultivation was done for 4 days.

from 3.0 to 9.0, and cultivation was carried out for 3 days at 28°C. The medium composition used was composed of 5% soluble starch, 1% yeast extract and same inorganic salts described in Methods. As shown in Table 5, maximal yield of glucoamylase was achieved when initial pH of the medium was 6.0.

Effect of temperature

For determining the optimal temperature for the production of glucoamylase by *Aspergillus* sp., cultivation of the inoculated medium was carried out at various temperatures ranging from 25°C to 35°C for 3 days. The optimal temperature for the enzyme production was 28°C (Table 6). It was also observed although there was considerable amount microbial growth at 35°C, less enzyme was produced at that temperature.

Solid culture

Fungal strains generally grow well and produce high level of glucoamylase at solid state medium. Therefore, several agricultural products were investigated as a substitute for liquid medium. Solid state culture was performed by inoculating 1% seed culture broth to the solid media (agricultural product, 20g: water, 20 ml). After cultivation for 4 days at 28°C, they were harvested by addition of 200 ml of water to extract the extracellular glucoamylase. The glucoamylase activities, determined by *p*-nitrophenol method, produced from each solid medium were 9.3 unit/ml (rice), 9.3 unit/ml (barley), 18.0 unit/ml (wheat bran, 8g: rice, 6g: barley, 6g), and 20.7 unit/ml (wheat bran). Wheat bran koji exhibited the highest enzyme production among tested solid state media. The effect of cultivation teperature on the glucoamylase production in wheat bran solid culture is shown in Table 7. The optimal temperature for solid culture was slightly higher than that of liquid culture. Due to ease of handling and cheap price, wheat bran can be easily used as a complete natural medium to produce the glucoamylase from the *Aspergillus* strain.

요 약

Aspergillus sp. 로부터 glucoamylase 를 생성키 위한 조건을 검토하였다. 곰팡이는 탄소원으로 soluble starch, 질소원으로 yeast extract 를 사용했을 때 최대 효소생성을 얻을 수 있었다. 그리고, 탄소원 및 질소원의 농도에 따른 효소생성은 soluble starch 를 5%, yeast extract 를 1% 수준으로 사용했을 때 효소생성은 극대화하였다. 또한 배지의 초기 pH 를 6.0, 그리고 배양온도를 28℃로 유지했을 때 효소생성이 높았다. 고체배지를 사용했을 때에는, 밀기울 배지가 glucoamylase 생성을 위해서 가장 효과가 좋았다.

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