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Production and Properties of Invertase from *Aspergillus niger*

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Abstract

Production and properties of invertase from *Aspergillus niger* were investigated. Inulin and sucrose were best carbon source and yeast extract was most suitable for the production of the enzyme among tested carbon and nitrogen sources. The enzyme was maximally produced by cultivating the organism at medium of pH 4.5 and temperature of 30℃. The optimum pH and temperature for the enzyme activity were pH 5.0 and temperature of 50℃, respectively. Among tested metal ions, Hg^{2+}, Cu^{2+} and Ag^{+} ions inhibited the enzyme activity drastically.

Introduction

The invertase(EC 3.2.1.26) is found widely distributed in plants, animals and microorganism. The enzyme was thoroughly studied from yeast. There is evidence that yeast invertase exists in at least two forms which are related to its location in the cell. The internal enzyme is devoid of detectable carbohydrate. In contrast, the external enzyme which is located in the cell wall is a glycoprotein containing about 50% carbohydrate[1-11]. Relatively few reports have been published about fungal invertase. It has been reported that *Neurospora* invertase is also a glycoprotein, containing 50% mannan and 2-3% glucosamine[12]. Madyasthat *et al.* isolated the extracelluar invertase producing fungus, *Aspergillus atheus*, and characterized the enzyme from the fungus[13]. It is important to isolate an organism which produce high activity of extracelluar invertase. Here we report that factors affecting production of extracelluar invertase form *Aspergillus niger* which was isolated from soil, and the properties of the enzyme.

Materials and Methods

Microorganism and cultivation

Organism used was *Aspergillus niger* which was isolated from soil. The modified Chapek-Dox medium[14] was used to cultivate the organism, which was composed of 3% glucose, 0.2% peptone, 0.05% potassium chloride, 0.05% magnesium sulfate, 0.1% dipotassium hydrogen phosphate, and 0.001% ferrous sulfate. The effect of carbon and nitrogen sources on the production of the enzyme was studied by substituting glucose and peptone with various carbon and nitrogen compounds. The cultivation was carried out by addition of seed culture (inoculum size : 1%) to the culture vessel(250ml E-flask) and cultivated for 7 days at 30℃.

Crude enzyme preparation

The culture broth was filtered using Toyo No. 2 filter paper and the filtrate was centrifuged at 3,000 rpm for 30 min. The supernatant was used as a crude enzyme solution.

Enzyme assay

The invertase reaction was performed in assay

mixture containing 0.1M Na-acetate buffer, pH 5.0 and 28.7 mM sucrose. The reaction was initiated by adding 20μl of the enzyme solution to the assay mixture previously equilibrated to 50℃ for 5 min. The total assay volume was 1 ml and the temperature of incubation was 50℃. The reaction was performed for 20 min, and then 1 ml dinitrosalicylic acid(DNSA) was added. It was maintained in boiling water for 5 min and the resulting color produced was measured at 540 nm after addition of 9 ml water.

Results and Discussion

Effect of carbon source

Production of invertase with various carbon sources by the *Aspergillus niger* was investigated by replacing glucose in the basal medium with various carbon sources listed in Table 1. Inulin exhibited the highest enzyme productivity, followed by sucrose. The enzyme catalyzes the hydrolysis of sugars possessing unsubstituted β-D-fructofuranosyl residue such as sucrose and inulin. Since the enzyme was mainly produced in the medium containing sucrose and inulin as a carbon source, it seems likely that invertase from the organism is an inducible enzyme. Therefore inulin and sucrose seem to function as inducer for the production of the enzyme.

Table 1. Effect of carbon sources on production of invertase

Carbon source	Absorbance at 540nm
Galactose	0.11
Fructose	0.09
Glucose	0.02
Maltose	0.14
Sucrose	0.31
Lactose	0.09
Dextrin	0.19
Soluble starch	0.18
Glycerin	0.05
Inulin	0.46
Cellulose	0.03
Amylopectin	0.11
Citric acid	0.01
Araban	0.00
Xylose	0.00
Amylose	0.13

The medium composition was same as described in Methods except carbon source. The cultivation was carried out for 7 days at 30℃.

Effect of nitrogen source

Various nitrogen sources were replaced with peptone to investigate the effect of nitrogen sources on the enzyme production. As shown in Table 2, yeast extract was most suitable for the invertase production among tested nitrogen sources. The inorganic nitrogen sources were generally ineffective for the organism to produce the enzyme.

Effect of initial pH

Variation of the initial pH of the medium can

Table 2. Effect of nitrogen sources on production of invertase

Nitrogen source	Absorbance at 540nm
$(NH_4)_2SO_4$	0.15
NH_4Cl	0.12
$NaNO_3$	0.00
Urea	0.01
Yeast extract	0.66
Peptone	0.21
Casein	0.18
Gelatin	0.11
Glycine	0.18
Lysine	0.12
Asparagine	0.12
Glutamic acid	0.16

The medium composition was same as described in Methods except carbon and nitrogen sources. The carbon source used was sucrose(3%). The cultivation was carried out for 7 days at 30℃.

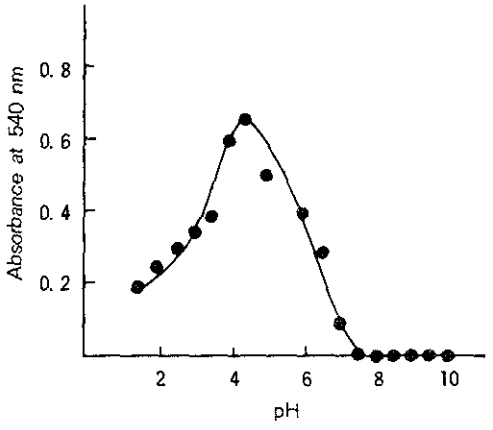

Fig. 1. Effect of initial pH of medium on production of the enzyme.
The cultivation was carried out for 7 days at 30℃.

induce metabolic changes to the organism, which might result in shift in metabolic activities. It may be either favorable or unfavorable for the desired enzyme production. The initial pH of the medium was adjusted to various pH values from 2.0 to 10.0, and the cultivation was carried out for 7 days at 30℃. As shown in Fig. 1, maximal yield of the enzyme was achieved when initial pH of the medium was adjusted to 4.5.

Effect of cultivation temperature

For determination of the optimal temperature for the enzyme production by the *Aspergillus niger*, cultivation was carried out at various temperatures ranging from 25℃ to 35℃ for 7 days. The optimal temperature for the enzyme production was 30℃ as shown in Fig. 2.

Effect of pH on activity and stability of the enzyme

The effect of pH on activity of the enzyme was examined over pH 4.0 to 10.0. The buffers(0.1M) used were as follow : pH 4.0−5.5, Na-acetate ; pH 6.0−7.5, Na-phosphate ; pH 8.0−8.5, Tris/HCl ; pH 9.0−10.0, Na-borate. The enzyme exhibited the pH optimum of 5.0 as shown in Fig. 3.

The pH optimum of the enzyme from *Aspergillus atheus* was identified as 5.7[13]. The effect of pH on stability of the enzyme was investigated by standard assay conditions after preincubation of the enzyme at given pH values at 70℃ for 5 min. The enzyme was maximally stable at pH 6.0 as shown in Fig. 4.

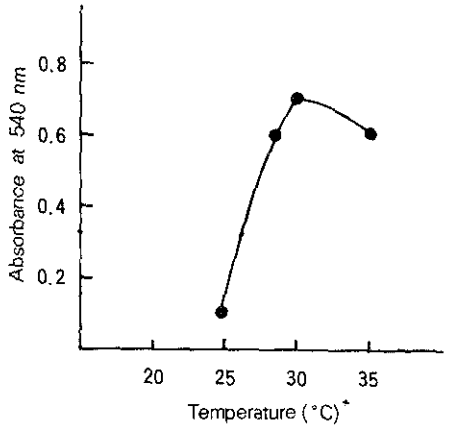

Fig. 2. Effect of cultivation temperature on production of the enzyme.
The cultivation was carried out for 7 days. The initial pH of the medium was 4.5.

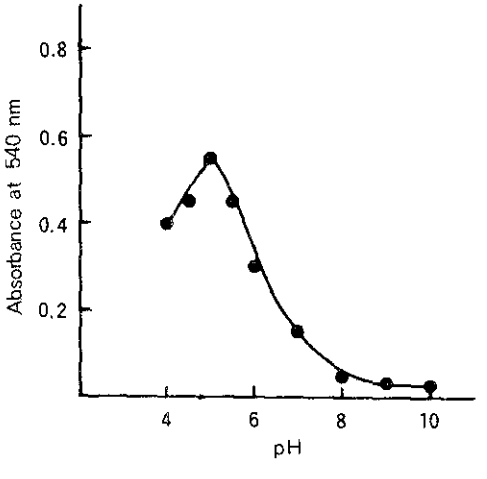

Fig. 3. Effect of pH on activity of the enzyme.
The enzyme reaction was performed as described in Methods except pH of the assay mixture.

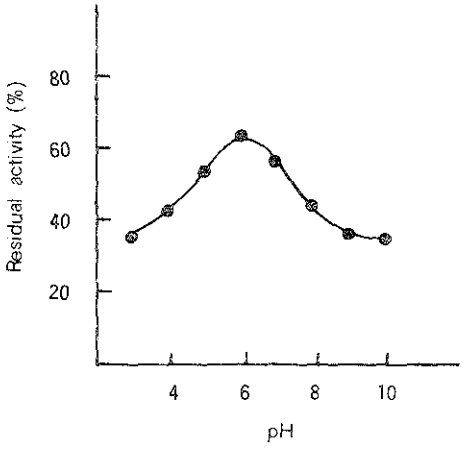

Fig. 4. Effect of pH on thermostability of the enzyme.

Effect of temperature on activity and stability of the enzyme

The effect of temperature on the activity of the enzyme was examined at various temperatures ranging from 20℃ to 70℃(Fig. 5). The enzyme exhibited the maximal activity at 50℃ when assayed for 20 min.

Above that temperature, the enzyme activity decreased, indicating the inactivation of the enzyme. The enzyme from *Aspergillus atheus* also showed the optimum temperature of 50℃[13]. Fig. 6 shows

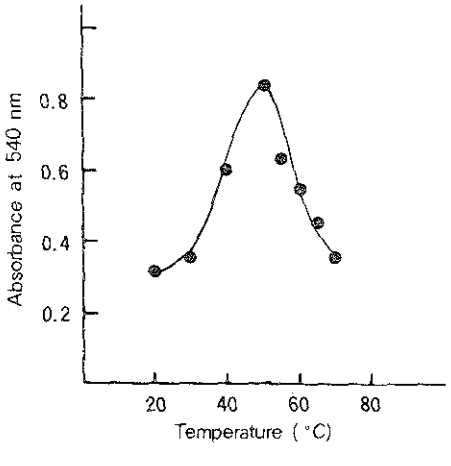

Fig. 5. Effect of reaction temperature on activity of the enzyme.

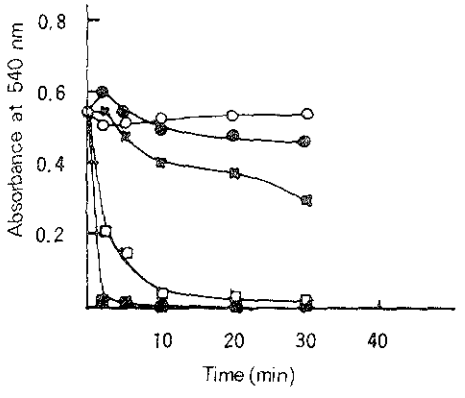

Fig. 6. Thermostability of the enzyme at various temperatures.
After heating, the residual activities were determined immediately under the standard assay conditions. 40℃(○), 50℃(●), 60℃(×), 70℃(□), 80℃(■).

the thermoinactivation of the enzyme at various temperatures in 0.1M Na-phosphate buffer(pH 7.0). The enzyme was stable for 30 min below 50℃. However, it was completely inactivated in 10 min when incubated at 70℃.

Effect of metal salts on enzyme activity

The effect of various metal salts on the enzyme activity was determined by adding 10mM of metal salts to the standard assay mixture(Table 3). The enzyme activity was completely inactivated by addition of Hg^{2+}, Ag^{+} and Cu^{2+} ions. This result coincides well with that of yeast invertase[12].

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Table 3. Effect of added metal salts on the activities of invertase

Metal salts (10mM)	Absorbance (540nm)	Relative activity(%)
Control	0.63	100.0
Mercuric chloride	0.0	0.0
Manganese chloride	0.57	90.5
Ferrous sulfate	0.41	65.5
Lead acetate	0.23	36.5
Barium hydroxide	0.33	52.3
Silver nitrate	0.07	11.1
Ammonium molybdate	0.31	49.2
Zinc chloride	0.53	84.1
Magnesium chloride	0.65	103.1
Zinc sulfate	0.59	93.6
Sodium arsenate	0.44	69.8
Sodium tungstate	0.06	9.5
Cupric acetate	0.0	0.0
Calcium chloride	0.76	120.6
Lithium carbonate	0.42	66.7

The enzyme reaction was carried out as described in Methods.

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*Aspergillus niger*에 의한 Invertase의 생성 및 특성

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요 약

*Aspergillus niger*로부터 invertase 생성의 최적조건과 생성된 효소의 특성을 조사하였다. 곰팡이는 탄소원으로 inulin과 sucrose를, 질소원으로 yeast extract를 사용했을때 최대효소 생성을 얻을 수 있었다. 또한 사용한 배지의 pH를 4.5, 배양온도를 30℃로 유지했을때 효소생성이 높았다. 효소 활성을 위한 최적조건은 pH 5.0 및 50℃였다. 효소는 pH 6.0에서 가장 안정했으며, 또한 50℃이하에서 안정하였다. 효소는 Hg^{2+}, Ag^{+} 및 Cu^{2+} 이온의 첨가에 의하여 불활성화 되었다.