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Short communication

Glucoamylase production by the marine yeast *Aureobasidium pullulans* N13d and hydrolysis of potato starch granules by the enzyme

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Abstract

Under the optimal conditions, 10 U/ml of glucoamylase was produced by the marine yeast *Aureobasidium pullulans* N13d. It was noticed that the crude glucoamylase actively hydrolyzed potato starch granules, but poorly digested raw corn starch and sweet potato starch, resulting in conversion of 68.5, 19 and 22% of them into glucose within 6 h of incubation in the presence of 40 g/l of potato starch granules and 20 U/ml of the crude enzyme. When potato starch granules concentration was increased from 10 to 80 g/l, hydrolysis extent was decreased from 85.6 to 60%, while potato starch granules concentration was increased from 80 to 360 g/l, hydrolysis extent was decreased from 60 to 56%. Ratio of hydrolysis extent of potato starch granules to hydrolysis extent of gelatinized potato starch was 86.0% and the hydrolysis extent of potato starch granules by action of the crude glucoamylase (1.0 U/ml) was 18.5% within 30 min at 60 °C. Only glucose was detected during the hydrolysis, indicating that the crude enzyme could hydrolyze both α-1,4 and α-1,6 linkages of starch molecule in the potato starch.

Keywords: *Aureobasidium pullulans*; Marine yeast; Glucoamylase; Potato starch granules

1. Introduction

Amylases have many applications in food, textile, paper and pharmaceutical industries [1]. Starch is the best substrate for production of yeast cells including single cell protein on a large scale due to its low price and easy availability of raw material in most regions of the world [2]. Because most of yeasts from environments are safe (GRAS, generally regarded as safe), interest in amylolytic yeasts has increased in recent years as their potential value for conversion of starchy biomass to single-cell protein or ethanol has been recognized [1,3]. To date, it has been noticed that the terrestrial yeasts which can produce extracellular amylolytic enzymes include *Lipomyces*, *Saccharomycopsis*, *Schwanniomyces*, *Candida japonica* and *Filobasidium capsuligenum* and extracellular amylolytic enzymes produced by them have been well characterized [1,3]. For example, it was found that *Saccharomycopsis fibuligera* could convert starch into trehalose effectively, and the trehalose yield could be over 23 g/100 g of cell dry weight [4].

Most starch digesting enzymes reported to date hardly digest ungelatinized potato starch because of the larger size of these granules [5]. It was reported that potato starch granule would be swollen at 60 °C [6]. On the other hand, next to corn, potato is the most important source of starch. Therefore, enzymes that are capable of digesting ungelatinized potato starch granules are economically attractive for they can increase the range of starch sources for direct hydrolysis [5]. Such enzymes have been reported from different fungal and bacterial strains. However, very few studies exist on the potato starch granules digesting amylase from marine yeasts [1]. It has been reported that amylase is an important enzyme in the guts of marine animals because it can help digest starchy feed and amylase activity of the guts will decide which components can be used in the compound diet, showing the stage of development in marine animals [7]. Therefore, it is very important to obtain amylase-producing marine yeasts which could be used in mariculture.

In search for amylase-producing marine yeasts, over 100 samples of marine sediment and sea water were collected from deep sea of Pacific Ocean and more than 300 strains were isolated from these samples in our laboratory. We found that strain N13d which was identified to be *Aureobasidium pullulans* by sequence analysis of its 18S rRNA gene and routine yeast identification methods could produce more

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extracellular amylase than any other strains tested and grew well in YPD medium prepared with sea water. In the present study, the crude glucoamylase production by the marine yeast and its potato starch granules digesting activity were conducted, aiming at exploring its potential applications in starch processing.

2. Materials and methods

2.1. Yeast strain and cell growth conditions

The marine yeast *A. pullulans* N13d, isolated from deep sea of Pacific Ocean, was maintained on YPD agar slant containing 20 g/l glucose, 20 g/l peptone, 10 g/l yeast extract, and 20 g/l agar at 4 °C and subcultured each month. The yeast cells were grown in the amylase production medium containing 1.0 g of peptone, 1.0 g of soluble starch, 100 ml of sea water, pH 4.0 at 28 °C for 56 h by shaking at 180 rpm.

2.2. Fermentation

The fermentation was carried out in a Biostat B2 2-l fermentor (B. Braun, Germany) with a working volume of 2 l of the production medium. The fermentor with 1800 ml of the medium was sterilized at 121 °C for 30 min. After cooling, the medium was inoculated with 200 ml of inoculum to reach an initial OD_{600nm} value of 0.6 in the culture. The fermentation was carried out at temperature of 28 °C and different aeration rates of 2.0, 4.0, 6.0 and 8.0 l/min, but with different agitation speeds of 150, 200, 250 and 300 rpm, respectively. The culture in fermentor was collected at 8 h time interval for determination of amylase activity in the supernatant and cell dry weight.

2.3. Determination of amylase activity

The reaction mixture containing 2.0 ml of 10 g/l soluble starch in 0.2 M acetate buffer (pH 4.5) and 0.5 ml of the supernatant was incubated at 60 °C for 30 min. The amount of reducing sugar in the reaction mixture was determined by using DNS method [8]. One unit of amylase activity was defined as the amount of enzyme required to release 1 μmol of reducing sugars per min under the assay conditions.

2.4. Effects of pH and temperature on amylase activity

The effect of pH on potato starch granules digesting enzyme activity in the supernatant was determined in the range of pH 3.0–10.0. The buffers used were 0.1 M citrate-Na_2HPO_4 buffer (pH 3.0–4.0), 0.1 M acetate buffer (pH 4.0–6.0), 0.1 M Tris–HCl buffer (pH 7.0–8.0) and 0.1 M glycine-NaOH buffer (pH 9.0–10.0). The optimal temperature for potato starch granules digesting enzyme activity was determined at 35, 40, 45, 50, 55, 60, 70 and 75 °C in the same buffer as described below. Here, pre-incubated sample at 4 °C was used as reference to calculate the residual activity.

2.5. Ungelatinized- and gelatinized-starch-saccharifying activities

Potato starch granules degrading activity was assayed in a reaction mixture consisting of 0.5 μl of the concentrated supernatant (the final enzyme concentration was about 1.0 U/ml) and 1.0 ml of 10 g/l potato starch granules or 1.0 ml of 10 g/l gelatinized potato starch in 0.2 M acetate buffer (pH 4.5). The reaction was carried out by shaking at 60 °C and 180 rpm for 30 min and terminated by heating at 100 °C for 5 min and centrifugation at 10,000 rpm for 5 min. Glucose released was detected by using the DNS method [8].

2.6. Potato starch granules hydrolysis

Effect of potato starch granules concentration on hydrolysis was studied by varying their concentrations from 10 to 400 g/l in the reaction mixture containing 1.0 ml acetate buffer (pH 4.5) and 20 U/ml of the concentrated supernatant by shaking at 180 rpm and 60 °C. To determine the extent of starch hydrolysis, the released glucose was assayed after 6 h of incubation. The end products of the potato starch hydrolysis after 6 h of incubation at 60 °C were withdrawn and identified to ascertain the extent of hydrolysis by ascending thin layer chromatography (Silica gel 60, MERCK, Germany) with the solvent system of *n*-butanol–pyridine–water (6:4:3) and a detection reagent comprising 20 g/l diphenylamine in acetone–20 g/l aniline in acetone–850 g/l phosphoric acid (5:5:1 by volume).

3. Results and discussions

3.1. Amylase production during the fermentation

It was found that the optimal medium for amylase production by this yeast strain was 1.0 g of peptone, 1.0 g of soluble starch, 100 ml of sea water, pH 4.0. The optimal conditions for amylase production by this yeast strain were that temperature was 28 °C, aeration rate was 6 l/min and agitation speed was 250 rpm. Under these conditions, 10 U/ml of amylase activity was produced within 56 h of fermentation (data not shown). In order to know when the highest yields of amylase was reached during the fermentation under the optimal conditions, amylase production and cell growth of the marine yeast strain were monitored during the fermentation. The data in Fig. 1 indicate that the highest yields of amylase were achieved within 56 h of the fermentation when the cell growth reached late stationary phase. Our results also reveal that the crude glucoamylase towards soluble starch worked best at 60 °C and pH 4.5, respectively (data not shown).

3.2. Potato starch granules hydrolysis

The results in Fig. 2 shows that the crude glucoamylase could convert 68.5, 22 and 19% of potato starch granules, raw sweet potato starch and raw corn starch into glucose under the same conditions of temperature 60 °C, pH 4.5, shaking speed 180 rpm, starch concentration 40 g/l and the enzyme concentration 20 U/ml within 6 h. When the relative glucoamylase activity towards gelatinized soluble starch was regarded as 100%, the results in Table 1 indicate that the relative glucoamylase activities towards potato starch granules, raw

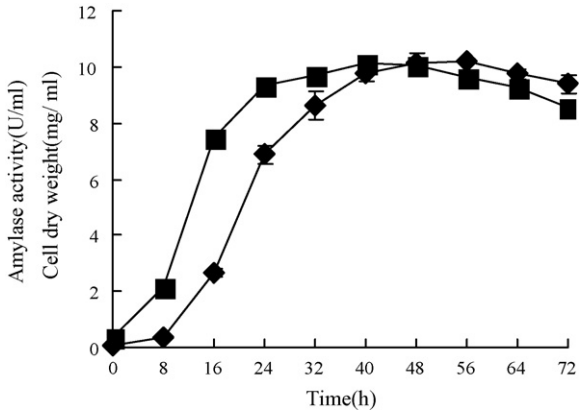

Fig. 1. The time course of amylase production (◆) and cell growth (■) during the fermentation. Data are given as means ± S.D., $n = 3$. Fermentation temperature: 28 °C; aeration rate: 6 l/min; agitation speed: 250 rpm.