Note

Production of α -Glucosidases I and II from *Bacillus cereus* NY-14[†]

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Bacillus cereus NY-14, which we isolated from soil and identified, can digest soluble starch and effectively produce maltopentaose (G_5) in culture medium.¹⁾ Of three enzymes related to the production and later disappearance of G_5 in culture medium containing soluble starch, the properties of cell-bound α -glucosidases I²⁾ and II³⁾ have been reported. α -Glucosidase I, which hydrolyzes maltose, maltotriose, maltotetraose, *p*-nitrophenyl α -maltoside, *p*-nitrophenyl β -maltoside, isopanose, and P_6 (6³-*O*- α -maltotriosylmaltotriose), corresponded to a maltase. α -Glucosidase II, which hydrolyzes isomaltose, isomaltotriose, panose, and *p*-nitrophenyl α -D-glucopyranoside, was classified as an isomaltase (oligo-1,6-glucosidase; EC 3.2.1.10). Both enzymes are important in the production and later disappearance of G_5 in culture medium containing soluble starch. This paper describes the effects of various carbohydrates on α -glucosidase production.

Maltose, maltotriose, amylose (DP=17), and isomal-

tose were obtained from Hayashibara Biochemical Research Lab., Inc. Maltotetraose, maltopentaose, maltohexaose, and maltoheptaose were purchased from Nihon Shokuhin Kako Co., Ltd. All other chemicals were obtained from the usual commercial sources.

The basal medium used contained 1% peptone (Kyokuto Pharmaceutical Industrial Co., Ltd.) and 0.5% NaCl (pH 8.0). The strain was cultivated in a test tube (2.1-cm diameter, 21-cm length) containing 10 ml of the medium at 30°C for 24 hr with shaking (250 rpm). For the study of the course of enzyme production, the bacterium was cultivated in a 5-liter Erlenmeyer flask containing 500 ml of the medium at 30°C with shaking. After incubation, the cells were harvested by centrifugation at 15,000 rpm (27,300 $\times g$) at 4°C for 20 min, suspended in 50 mM phosphate buffer (pH 6.9) containing 5 mM ethylenediaminetetraacetic acid, and disrupted by ultrasonication (model NS-250, Nihon Seiki Seisakusho Co., Ltd.) at 20 kHz for 10 min at 4°C. After centrifugation, enzyme activities in the supernatant were assayed as described previously.^{2,3)} The enzyme solution to be tested was incubated with 1% maltose (α -glucosidase I) or isomaltose (α -glucosidase II) at 30°C and the amount of glucose formed was measured by the method of Papadopoulos *et al.*⁴⁾ One unit of α -glucosidase activity was defined as the amount of enzyme that hydrolyzed 1 μ mol of maltose (α -glucosidase I) or isomaltose (α -glucosidase II) per minute under the conditions described above. In this study, the enzyme activity is shown as the amount produced in 10 ml of the culture. The amount of total sugar of the culture filtrate was measured by the phenol-sulfuric acid method⁵⁾ and that of reducing sugar by the dinitrosalicylic acid (DNS) method⁶⁾ with glucose as the standard. Growth of the cells was estimated by photometrical absorbance at 660 nm in a 1-cm cell after 10-fold dilution of the culture using deionized water. Maltooligo-

FIG. 1. Effects of Carbon Source on Enzyme Production.

Medium, basal medium + 0.5% of a carbon source. IG₂, isomaltose.

[†] Part of this work was presented at the Annual Meeting of the Agricultural Chemical Society of Japan, Sapporo, 1985. Symbols: G₁, G₂, G₃...etc., are glucose, maltose, maltotriose...etc. DP, average degree of polymerization.

FIG. 2. Course of Enzyme Production.

Medium, basal medium+5% soluble starch. $\odot$, G₁; $\blacktriangle$, G₂; $\bullet$, G₃; $\triangle$, G₄; $\circ$, G₅; $\square$, G₆; $\blacksquare$, G₇. Extracellular α -amylase activity was assayed as described previously.⁸⁾ An enzyme solution (100 μ l) was incubated with 100 μ l of 50 mm acetate buffer (pH 6.0) containing 10 mm CaCl₂ and 200 μ l of 1% soluble starch (E. Merck, A. G., W. Germany) at 30°C. The amount of reducing sugar produced was measured by the DNS method⁶⁾ with glucose as the standard. One unit of the enzyme activity was defined as the amount that liberated 1 μ mol of aldehyde group as glucose per minute under these conditions.

saccharides formed in the culture filtrate were measured by high-performance liquid chromatography as described elsewhere.¹⁾ The amount of each maltooligosaccharide was found by the peak area method.⁷⁾

The effects of various carbon sources on enzyme production are shown in Fig. 1. The bacterium was incubated in the basal medium containing 0.5% of one of the carbohydrates. α -Glucosidases I and II were produced by using G₂, G₇, amylose ($\overline{DP}$ =17), or soluble starch (Kanto Kagaku Co., Ltd.) as the carbon source. Ten times as much α -glucosidase I activity was produced as α -glucosidase II activity. Among these carbohydrates, G₇ was hydrolyzed by extracellular α -amylase yielding G₂ and G₅, and both amylose ($\overline{DP}$ =17) and soluble starch were hydrolyzed by the same enzyme to G₂~G₆ in which G₅ was preponderant.⁸⁾ α -Glucosidase II (isomaltase) was not

produced when isomaltose was used as the carbon source. Therefore, G₂ was the most efficient inducer of α -glucosidases I and II.

The effects of G₂ concentration on enzyme production were also examined. α -Glucosidases I and II were produced when the medium contained more than 0.05% (w/v) of G₂ (data not shown).

The course of enzyme production was investigated using basal medium containing 5% soluble starch to examine the enzymes in G₅ production. Figure 2 shows the amount of each enzyme produced and each maltooligosaccharide formed. During the exponential phase of growth, secreted α -amylase hydrolyzed soluble starch, yielding maltooligosaccharides in which G₅ was the main product. The G₅ content increased and reached a maximum after about 30 hr; at that time the ratio of G₅ to the residual sugar was

39.2% and to the initial sugar was 27.2%. Then the G_5 content decreased during the stationary phase of growth. α -Glucosidases I and II were induced at an early stage by G_2 that arose from soluble starch by the action of extracellular α -amylase. α -Glucosidase production reached a maximum after about 12 hr and then decreased. These results suggested that the cell-bound α -glucosidases I and II were central to the specific accumulation of G_5 , mainly during the exponential phase of growth of *B. cereus* NY-14.

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