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RESEARCH ON THE STRONG TRANSGLYCOSYLATION ACTIVITY IN *Aspergillus niger*

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Aspergillus niger *M-1 strain shows strong transglycosylation activity. A gene of it was introduced into* Escherichia coli*, and isomalto-oligosaccharides were isolated by a chemical enzymatic method in order to measure the transglycosylation activity.*

Key words: *Aspergillus niger*, transglycosylation activity, protein, *E. coli* transformant, isomalto-oligosaccharides.

The isomaltooligosaccharides IMOs are of special interest to the food industry as an advanced substitute for sugar [1, 2]. Besides employing amylase and (neo) pullulanase acting in starch to produce IMOs [3, 4] and synthesis by dextranase and dextransucrase [5], the most common enzymatic method of production of IMOs in industry involves α-glucosidase with transglycosylation and hydrolyzing activity, which can form α-1,6 glucosidic linkages in addition to catalyze liberation of glucose from nonreducing ends of the substrates [6]. The transglycosylation activity is the key to IMOs bioconversion.

Aspergillus niger M-1 strain possesses an intracellular α-glucosidase (agdM) with strong transglycosylation activity. A maltose concentration of 25% was used for agdM to achieve an IMOs content of 90% in the final reaction products, which showed higher transglycosylation activity than the other α-glucosidases that had been reported [7]. The goal of our study was to investigate the strong transglycosylation activity of *A. niger* M-1.

Figure 1 shows the result of electrophoresis of purified agdM, which reveals that the molecular weight of the enzyme protein was about 110 kDa.

The gene was amplified from RNA of *A. niger* M-1 and introduced into *E. coli* BL21. The electrophoretic separation of protein of the cell extracts of *E. coli* transformant shows that *E. coli* transformant had a prominent new band about 110 kDa (Fig. 2), which is consistent with the result of electrophoresis of purified agdM (Fig. 1).

The transglycosylation activity in the cell extracts of *E. coli* transformant was detected by TLC. The cell extracts of *E. coli* transformant were found to convert maltose to glucose and a series of transglycosylation products (Fig. 3). In order to identify the transglycosylation activity in *E. coli* transformant, HPLC analysis of the reaction products was carried out. The result of HPLC shows that the components of the reaction products were the same as the sample (Fig. 4).

Based on the results, it can be confirmed that the strong transglycosylation activity of agdM observed by us earlier is due to the presence of a protein about 110 kDa which possesses enzymatic activity for transforming maltose into IMOs. The results lead to the conclusion that the cloned gene encodes a protein with strong transglycosylation activity.

The effects of the following parameters on transglycosylation activity in *E. coli* transformant were studied: metal ion and fed-batch fermentation of glucose.

The effect of metal ion on the tansglycosylation activity in *E. coli* transformant was studied by adding different ions (Cu^{2+} (19.5%), Mn^{2+} (168.8), Ca^{2+} (101.1), Mg^{2+} (87.6), Co^{2+} (0), Fe^{3+} (45.0), Zn^{2+} (33.9), and Li^{+} (121.9%)) into the medium. The results were shown in Table 1, which shows that Co^{2+} totally inhibited the transglycosylation activity of *E. coli* transformant but Mn^{2+} could enhance the transglycosylation activity significantly in comparison to the control (without addition). The results indicated that the expression of the gene was induced by the addition of Mn^{2+} in the medium. We supposed that the stability and high transglycosylation activity of the agdM were related to the existence of Mn^{2+}.

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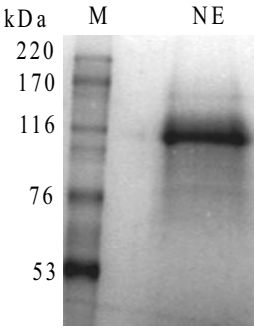

Fig. 1

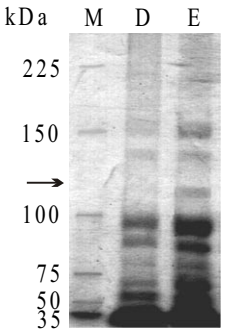

Fig. 2

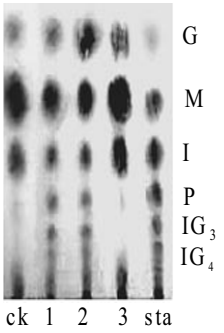

Fig. 3

Fig. 1. Electrophoresis of purified agdM (NE) from *A. niger* M-1 and molecular weight markers (M) in SDS-PAGE.
Fig. 2. Electrophoresis of the cell extract of control (without agdM gene, D), the cell extract of *E. coli* transformant (E), and molecular weight markers (M) in SDS-PAGE.
Fig. 3. TLC analysis of transformation products by *E. coli* transformant 1, 2 and control 3 (without agdM gene) from 25% maltose (CK). A mixture of glucose (G), maltose (M), isomaltose (I), panose (P), isomaltotriose (IG_3), and isomaltotetraose (IG_4) was used as sample (Sta).

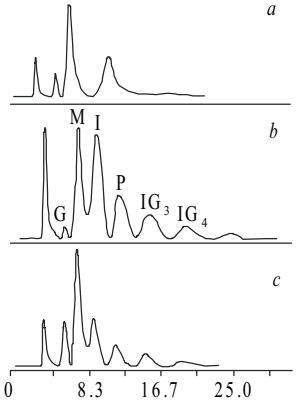

Fig. 4. HPLC of the substrate (25% maltose, *a*), the transformation products (*b*), and the sample (*c*).

The effect of fed-batch fermentation of glucose on transglycosylation activity in *E. coli* transformant was studied by adding 5% glucose into the medium when the isopropyl-*β*-thiogalatoside (IPTG)-induced activity had started for 1 hour (297.5%), 2 hours (131.2), and 3 hours (102.1%). Interestingly, we found that the transglycosylation activity of *E. coli* transformant can be enhanced by about 2-fold by adding glucose into the medium when the cells were induced by IPTG for 1 hour. We supposed here that the expression of the gene was regulated by glucose, which had not been reported before.

EXPERIMENTAL

Microorganism: *A. niger* M-1 strain was provided by the Food Fermentation Institute of Guangxi University.

***E. coli* transformant:** The agdM gene was amplified from RNA of *A. niger* M-1 using two pairs of primers:5′-ATT GAA TTC ATG GCC GGT CTA AAA AGC TTC CTT GCC AGT TC-3′, 5′-ATT GGA TCC TCA CCA TTT CAG TAC CCA GTC CTT CGC CCA-3′). Recombinant cells were induced by 1.0 mmol/L IPTG for 4 h [8].

Purification of agdM from *A. niger* M-1. The protein was eluted by ammonium sulfate fractionation, DEAE-cellulose 52 column chromatography, DEAE-sepharose CL-6B ion exchange column chromatography, and Sephadex G-100 column chromatography. The peaks of the protein were detected by the Bradford method at 280 nm [9].

SDS-PAGE. SDS-PAGE under reducing conditions was performed on 7 acrylamide separating gel and 4% staking gel [8], and stained with Coomassie Blue R250. High molecular weight markers (Promega) were used as standard proteins.

Transglycosylation Activity Assays. The cell extract was reacted with 25% maltose in sodium acetate (50 mmol/L pH 5.5) at 30° for 20 h. The production of IMOs was measured by TLC and HPLC.

TLC. Thin-layer chromatography (TLC) was carried out according to Woo-Jin Jung [10].

HPLC. For high performance liquid chromatography, a Shimadzu Class-LC10 chromatography system was used with a LC10AT pump, an RID-6A differential refractive index detector, a Spherisorb NH_2 column (10 μm, 4.0 × 300 mm), and a mobile phase of acetonitrile–water (74:26, vol/vol) at a flow rate of 1 mL/min; the injected amount of reaction products was 20 μL.

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