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High Acid-Stable α-Amylase Production by Milk in Liquid Culture

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Abstract : Objectives: Shochu is a popular Japanese distilled spirits. In the production of shochu, the filamentous fungus Aspergillus kawachii has traditionally been used. A. kawachii produces two types of starch hydrolytic enzymes, α-amylase (enzymatic liquefaction) and glucoamylase (enzymatic saccharification). Liquid culture system is a relatively easy microorganism to ferment with relatively low cost of production compared for solid culture. In liquid culture system, acid-unstable α-amylase (α-A) was produced abundantly, but, acid-stable α-amylase (Aα-A) was not produced. Since there is high enzyme productivity, most in shochu brewing have been adopted by a solid culture method. In this study, therefore, we investigated production of Aα-A in liquid culture system. Materials and methods: Microorganism Aspergillus kawachii NBRC 4308 was used. The mold was cultured at 30 °C for 7~14 d to allow formation of conidiospores on slant agar medium. Liquid Culture System: A. kawachii was cultured in a 100 ml of following altered SLS medium: 1.0 g of rice flour, 0.1 g of K2HPO4, 0.1 g of KCl, 0.6 g of tryptone, 0.05 g of MgSO4□7H2O, 0.001 g of FeSO4□7H2O, 0.0003 g of ZnSO4□7H2O, 0.021 g of CaCl2, 0.33 of citric acid (pH 3.0). The pH of the medium was adjusted to the designated value with 10 % HCl solution. The cultivation was shaking at 30 °C and 200 rpm for 72 h. It was filtered to obtain a crude enzyme solution. Aα-A assay: The crude enzyme solution was analyzed. An acid-stable α-amylase activity was carried out using an α-amylase assay kit (Kikkoman Corporation, Noda, Japan). It was conducted after adding 9 ml of 100 mM acetate buffer (pH 3.0) to 1 ml of the culture product supernatant and acid treatment at 37°C for 1 h. One unit of a-amylase activity was defined as the amount of enzyme that yielded 1 mmol of 2-chloro-4-nitrophenyl 6-azide-6-deoxy-b-maltopentaoside (CNP) per minute. Results and Conclusion: We experimented with co-culture of A. kawachii and lactobacillus in order to get control of pH in altered SLS medium. However, high production of acid-stable α-amylase was not obtained. We experimented with yoghurt or milk made an addition to liquid culture. The result indicated that high production of acid-stable α-amylase (964 U/g-substrate) was obtained when milk made an addition to liquid culture. Phosphate concentration in the liquid medium was a major cause of increased acid-stable α-amylase activity. In liquid culture, acid-stable α-amylase activity was enhanced by milk, but Fats and oils in the milk were oxidized. In addition, Tryptone is not approved as a food additive in Japan. Thus, alter SLS medium added to skim milk excepting for the fats and oils in the milk instead of tryptone. The result indicated that high production of acid-stable α-amylase was obtained with the same effect as milk.

Keywords : acid-stable α-amylase, liquid culture, milk, shochu
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