Thermostable, Raw-Starch-Digesting Amylase from *Bacillus stearothermophilus*

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An endospore-forming thermophilic bacterium, which produced amylase and was identified as *Bacillus stearothermophilus*, was isolated from soil. The amylase had an optimum temperature of 70°C and strongly degraded wheat starch granules (93%) and potato starch granules (80%) at 60°C.

Although raw-starch-granule-digesting amylases from molds (3, 5, 9) and bacteria (7, 8) have recently been reported, there is no published report of thermostable amylase capable of degrading raw starch granules. Isozo and Yutani et al. (10) reported on thermostable amylase from *Bacillus stearothermophilus* but did not refer to its ability to digest raw starch granules. Most of the experiments so far reported on degradation of raw starch granules were performed at ordinary temperatures (30 to 40°C) (3, 5, 7-9). Miyoshi et al. (6), however, suggested that degradation efficiency of raw starch granules by bacterial β-amylase was increased by raising the reaction temperature from 40 to 60°C.

The purpose of this research was to isolate thermophilic microorganisms capable of producing raw-starch-granule-digesting amylases at higher temperatures (50 to 60°C). This paper (i) describes the isolation and identification of a bacterium capable of producing a thermostable and raw-starch-digesting amylase and (ii) gives a preliminary characterization of that amylase.

Soil suspensions in sterilized water were poured and spread onto agar plates containing 0.5% meat extract (Wako Pure Chemical Industry, Ltd.), 1% Polypeptone (Wako), and 2% wheat raw starch (Nakarai Tesque, Ltd.). These plates were incubated at 55°C for 24 h. Among bacterial colonies, 3 colonies showed clear halos on the agar plates. The type culture of *B. stearothermophilus* IFO 12550 was examined in the same way but showed no clear halos around the colony. The three strains were transferred to the liquid culture medium containing 0.5% meat extract, 1% Polypeptone, and 1% soluble starch and shaken at 55°C for 24 h. Each culture filtrate (1 ml) was first mixed with 3 ml of 10 mM phosphate buffer (pH 7.2) which contained 2% wheat raw starch and then further incubated at 55°C for 6 h. The reducing sugars formed in each reaction mixture were measured by the method of Bernfeld (1). One strain of the isolates which showed high ability to digest raw starch was selected for further experiments. This strain, tentatively named strain B1, was gram positive, Voges-Proskauer test negative (at pH 7.2), and facultatively anaerobic and had a rod shape 1.0 to 2.5 μm in diameter. Spore formation was observed at the terminal position at the swollen sporangium. Strain B1 possessed the ability to hydrolyze both starch and gelatin. Strain B1 grew in nutrient broth at 45 to 70°C at neutral pH but could not grow at pH 5.5. Acid formation from D-glucose was positive, but gas formation from glucose was negative. From these results, strain B1 was identified as *B. stearothermophilus* according to *Bergey’s Manual of Systematic Bacteriology* (2).

The procedures to obtain the amylase fraction from the strain B1 culture were as follows. Ammonium sulfate was added to the culture filtrate at up to 70% saturation, and the precipitates obtained were collected and dissolved in 10 mM phosphate buffer (pH 7.2). The supernatant was subjected to Sephadex G-100 gel filtration and DEAE-cellulose ion-exchange column chromatography. The DEAE-cellulose column was equilibrated with 50 mM Tris hydrochloride buffer (pH 9.0), and the elution was performed by increasing the linear gradient of NaCl up to 0.3 M. The amylase activity was measured at 70°C for 5 min in 1% soluble starch solution and expressed as micromoles of maltose formed in 1 min.

Figure 1 shows the influence of temperature on the amylase activity and the thermostability of the enzyme. Optimum temperature of the amylase reaction was 70°C. The remaining activity after 1 h of incubation at 80°C was 50% of the original activity, which is higher than the remaining activity for the α-amylases from *B. stearothermophilus* BS-1 (4, 10). The optimum temperature of the amylase was the same as for amylases from other *B. stearothermophilus* strains (4, 10). Enzyme solution (4 ml; 8.7 IU at 50°C) was mixed with 40 mg of wheat, corn, sweet potato, and potato raw starch granules (Nakarai Tesque). Each reaction mixture was put into a 50-ml flask and incubated at 50 or 60°C at 240 rpm in a Gyrotory water bath shaker (model G-76; New Brunswick Scientific Co., Inc.).

Figure 2 shows the rate of degradation of four types of starch by the amylase at both 50 and 60°C. The final degradation rates of wheat, corn, potato, and sweet potato raw starch granules after 24 h of incubation were 93, 86, 80, and 83% at 60°C and 87, 51, 15, and 33% at 50°C, respectively. The amounts of sugars formed were expressed according to the calibration curve for maltose. The relatively high increase of the degradation rate of potato raw starch granules was observed when the reaction temperature was raised from 50 to 60°C. This phenomenon may be due to more effective enzyme attack on raw starch granules at 60°C.

A differential-interference-constant microscope (Olympus model BHS-N) was used to show further evidence of the degradation of the raw wheat starch granule by the amylase at 50°C (Fig. 3). The sugars formed from the degradation of the soluble starch were analyzed by using a Hitachi 665 A-12 R401 high-pressure liquid chromatograph. Maltose (50.7%),
maltotriose (39.1%), and glucose (2.4%) were formed, indicating that the enzyme may be an α-amylase.

This thermostable α-amylase showed a higher rate of degradation of raw starch granules when compared with α-amylases from other B. stearothermophilus strains. Therefore, if a system to induce hyperproduction of the amylase could be exploited, it would be useful in the industrial saccharification process of raw starch granules.

**LITERATURE CITED**


