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Studies on the Lipase of Thermophilic Fungus *Humicola lanuginosa*

Sir:

The industrial production of the enzyme by thermophilic microorganisms has been reported only in a few case. The interests of those investigators were focussed on α -amylase and protease from *Actinomyces* and *Bacillus*.^{1~4)} Although the industrial production of lipase (EC 3.1.1.3) by microorganisms has aroused a considerable interest of many microbiologists^{5~11)} during the past decade, the production of lipase by thermophilic microorganisms has not been reported. The present investigation

was undertaken as a basic research for the enzyme properties and development of utilization of lipase.

From compost soil samples collected in the vicinity of Tokyo, we have isolated a thermophilic fungus which could produce a remarkable amount of thermostable, alkalistable and extracellular lipase. The taxonomical characteristics of this thermophilic fungus were examined and the fungus was identified as *Humicola lanuginosa* S-38. The thermophilic fungus was cultivated with aeration at 45°C for 80 hr on

FIG. 1. Ultra-centrifuge Patterns of *Humicola* Lipase.

Data: Enzyme concentration=0.75%; 0.05 M Tris-HCl buffer (pH 8.0) contained 0.1 M KCl; Synthetic boundary cell, 60,000 rev./min, 25°C.

a medium consisting of 2.0% soluble starch, 5.0% corn steep liquor, 0.2% K_2HPO_4 , 0.1% $MgSO_4 \cdot 7H_2O$, 0.5% soybean oil, 0.005% deforming agent (Adecanol LG-109) in a 600 liter stainlesssteel fermenter. The cultured broth was filtered to remove cells and the filtrate was precipitated with ammonium sulfate at 60% saturation. The precipitate was collected by centrifugation and desalted by dialyzing against distilled water for 48 hr at 5°C. Then the dialyzed enzyme solution was lyophilized.

Two hundred grams of the crude enzyme powder were suspended in 2 liters of distilled water at room temperature and adjusted to pH 4.0 with 1N HCl. The enzyme solution was stirred gently with a magnetic stirrer for 2 hr. Then the enzyme solution was centrifuged at 8,000 rpm for 20 min. The supernatant was dialyzed against 60 liters of 0.05M Tris-HCl buffer (pH 8.0) at 5°C for 48 hr. After being centrifuged at 8,000 rpm for 10 min, the supernatant was purified 216-fold of specific activity by means of DEAE-Cellulose, DEAE-Sephadex A-50, CM-Sephadex C-50 and Sephadex G-200 column chromatography. The recovery of the activity was 30%. The purified lipase was confirmed to be homogeneous with disc ele-

ctrophoresis and ultracentrifugal analysis (Fig. 1). The average molecular weight of the *Humicola* lipase was given as $27,500 \pm 500$ by Archibald's¹²⁾ and Andrew's¹³⁾ methods. α -Helix content was estimated to be 18.9% according to circular dichroism analysis.²⁾ Sugar and lipid were not detected. The purified lipase was stable in the pH range from 4.0 to 11.0 (Fig. 2). The optimal pH for the hydrolysis of polyvinyl alcohol-emulsified olive oil was 8.0 and the optimal temperature was 60°C. The purified lipase was stable up to 60°C and retained 55% of full activity after heating at 70°C for 20 min. (Fig. 3). When the purified thermostable lipase was stored in a frozen state for more than six months, the loss of the lipase activity was nearly negligible.

Although lipases from many different sources have been described, only a few of these has been investigated in detail. The main reason is that these lipases are very difficult to be purified and hence satisfactory informations on the lipase action can not be obtained. Furthermore, many investigators have been bothered with the fact that these purified lipases are very unstable and lose their activity within a short period. As shown in Fig. 3, the thermostability of *Humicola* lipase was excellent.

FIG. 2. pH Stability of *Humicola* Lipase.

The enzyme was dissolved in various buffer solutions (MacIlvaine buffer, pH 4.0~8.0; ammonium buffer pH 8.0~11.0), and the solutions were incubated at 45°C for 1 hr. Residual activities were measured in a polyvinyl alcohol emulsified olive oil system at 45°C, pH 8.0.

FIG. 3. Heat Stability of *Humicola* Lipase.

The enzyme was dissolved in Tris-HCl buffer of pH 8.0 and incubated at various temperatures for 20 min. Residual activities were measured as described in Fig. 2.

It is obvious that the thermostable property of *Humicola* lipase is useful not only for industrial purposes but also for attempts to obtain more information on the structure and function of lipase.

Details of the full investigation will be published elsewhere in the near future.

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