A Rennin-like Enzyme from *Penicillium expansum*

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In a previous paper, Abdel-Fattah et al.\textsuperscript{1)} reported on the production of milk-clotting and proteolytic enzymes by fungi. The latter authors found that *Penicillium expansum* was the potent organism for producing the most active milk-clotting rennin-like enzyme. We now report on the purification and the properties of this fungal enzyme.

The cultivation medium used consisted of corn steep liquor (2\%) and lactose (1\%). The cultures were made in 250-ml flat-bottomed flasks, each containing 50 ml of sterile medium and the inoculated flasks were incubated at 30°C for 7 days. The culture filtrate (0.19 unit/mg protein) was precipitated with one volume of ice-cold acetone and the precipitate was dried under reduced pressure at room temperature. Fractional precipitation of this enzyme preparation was performed with ice-cold acetone as previously described by Abdel-Fattah and El-Hawwary.\textsuperscript{2)} Paper electrophoresis of the enzyme preparations was performed with acetate buffer solutions of different concentrations and at different pH values, using Elphor apparatus. A potential of 300 V (0.2 mA/strip) was applied at room temperature. The protein content of the enzyme sample was done by the method of Lowry et al.\textsuperscript{3)}

The milk-clotting activity was determined according to the method of Berridge,\textsuperscript{4)} using reconstituted skim milk and buffered enzyme solution (0.02 M acetate buffer, pH 3.42). One unit of the enzyme activity was considered to be that which clotted 10 ml of milk in 10 min at 40°C.

Fractionation of the partially purified enzyme preparation with acetone afforded 3 fractions possessing different milk-clotting activities (Table I). The enzyme fraction precipitated between 20~30\% was the most active towards clotting of milk and possessed about 2/3 of the total activity. At lower and higher concentrations of acetone, the fractions obtained showed relatively very weak activity. On the other hand, paper electrophoresis of each of the partially purified enzyme preparation and its 3 fractions indicated that each enzyme sample moved as a single protein component and all showed the same electrophoretic mobility. These results indicated that a mere single precipitation of the culture filtrate with one volume acetone afforded an enzyme preparation of good purity and contained one milk-clotting rennin-like component.

The properties of the fungal milk-clotting rennin-like enzyme were investigated using the fraction precipitated at 20~30\% acetone. Although proportional amounts of calcium chloride were added to milk, yet a substrate concentration higher than 8 g/100 ml was found to be a limiting factor for milk-clotting activity. The fungal rennin-like enzyme fraction showed optimum activity at pH 6.0 and optimal temperature of 50°C. This temperature differed from that found for *Penicillium citrinum* (62°C),\textsuperscript{5)} indicating differences between rennin enzymes of the same genus. Generally, the rennin-like enzyme was more stable at pH 3.42 and gradually lost its activity with increase of pH value.

The effect of some agents on the activity of the rennin-like enzyme fraction indicated that calcium chloride and barium chloride enhanced clotting of milk. On the other hand, sodium chloride inhibited the enzyme action. Complete or partial inhibition of enzyme action was brought about by potassium ferricyanide, iodoacetic acid and p-chloromercuribenzoic acid. On the other hand, reduced glutathione enhanced the clotting of milk by the enzyme. These results do not, however, provide conclusive evidence for the presence of reactive sulfhydryl groups in the active sites of the fungal enzyme.

### Table I. Milk-clotting Activity of the Enzyme Fractions Obtained by Acetone Fractionation of the Partially Purified *Penicillium expansum* Enzyme Preparation

<table>
<thead>
<tr>
<th>Enzyme fractions</th>
<th>Concentration of acetone (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Milk-clotting activity (unit/mg protein)</td>
<td>1.82</td>
</tr>
<tr>
<td>Proportion of milk-clotting activity (%)</td>
<td>25.56</td>
</tr>
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rennin-like enzyme.

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REFERENCES