Short Communication

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MILK COAGULATING ENZYME (RENNET) FROM *ASPERGILLUS ORYZAE*

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The need for microbial rennet has arisen because most of the calves are now raised to become mature animals due to heavier demand of milk and meat and the stomach of these mature animals does not contain rennet. Due to the similar status of microbial and calf rennet, large scale replacement of the later is anticipated in future. Among the few bacteria whose enzymes are used for making cheese, *Bacillus cereus* [1], *B. mesentericus* [2], *B. polymxa* and *B. subtilis* are worth mentioning. Fungi such as *Penicillium citrinum* [3], *Absidia remosa* [4], *Mucor lamprosporus* and *Mucor mucedo* have also been investigated for rennet production but not with significant success [5]. The only microorganisms whose enzymes have so far been used commercially to produce rennet are *Endothia parasitica, Mucor miehi* and *Mucor pusillus* [6]. In the present study the milk curdling enzyme from *Aspergillus oryzae* has been examined.

MATERIALS AND METHODS

Organisms. Aspergillus oryzae was used for the study. Stock culture was maintained on Dextrose Agar medium.

(A) *Preparation of enzyme extract.* A simple medium of the following composition was used:

Peptone, 50 g; $NaNO_3$; 50 g; KH_2 PO_4; 2.0 g; KCl; 50 g; $MgSO_4$, $7H_2$; and glucose, 10 g/ld

Glucose was also substituted by fructose. The pH of the medium was adjusted to 5.6 before inoculation. The medium was distributed in conical flasks and sterilized for 15 min. at 15 lb pressure. After inoculating the sterilized media, the flasks were placed on a rotary shaker with 120 rpm at 30° for three days. Toluene (20 ml) was used in each flask as a preservative and the enzyme was extracted with tap water for twenty hours at room temperature. On filtration, the filtrate was used for the enzyme assay. Substitution of glucose by fructose showed a slight increase in the enzyme activity.

(B) *Assay for the rennet.* The milk used for the study was of Milko Pak Limited, Lahore whose pH value was adjusted to 6.0 with lactic acid (E. Merck). Calcium chloride was added to the milk (50 mg/100 ml) and incubated at 35° for half an hour with 2 ml of enzyme extract for each 10 ml of the milk in a dish. The clotting time in hours was observed and recorded.

RESULTS AND DISCUSSION

Tables 1, 2 and 3 show the results of the present study.

Effects of time pH and temperature on the milk coagulating activity. A. oryzae was cultivated by using glucose and fructose as the carbon source. Effects of incubation time, pH and temperature on milk clotting activity were studied. It was noted that the rennet produced after 96 hr. of incubation was the most efficient to

Table 1. Effect of incubation times and carbon source on the milk coagulatting activity

Incubation time	24	48	72	96	120
Time of clotting					
with glucose (hr.)	5	5	4.40	4	5.10
With fructose (hr.)	4.30	4.20	4.00	3.30	4.30

The enzyme activity was maximum after 96 hours of incubation.

Table 2. Effect of pH on the clotting activity of the enzyme extract

pH	5.5	5.8	6.0	6.5
Clotting time				
with glucose (hr.)	4	4	4	5
With fructose (hr.)	3.30	3.30	3.30	4

Table 3. Interaction between the temperature and the clotting time

Temperature (°C)	30	35	40	50
Clotting time				
with glucose (hr.)	5	4	6	6
With fructose (hr.)	5	3.30	5.25	6

coagulate the milk as compared to those obtained after 24, 48, 72 and 120 hr. incubation.

$CaCl_2$ (50 mg/ml) was also used to improve the milk coagulating activity. It has already been observed that the rennets from *Mucor pusillus* seem to be more sensitive to calcium ion concentration than the calf rennet with coagulating activity having linear relationship with $CaCl_2$ content. The clotting activity of *A. oryzae* rennet is reported to be independent of pH variation between pH 5.5 and pH 6.0 which is also in accordance to the observation made earlier on *Mucor miehi* [8].

Most of the bacteria used for rennet production have been successful due to multitude of factors [9, 10]. Spontaneous microbiol mutation may cause variability in the rennet of the same batch and even of the same bacteria from different manufacturers [11]. In the present studies *A. oryzae* showed encouraging results for the production of microbial rennets but its extract appeared to contain some other types of contaminating enzymes, e.g. esterases and lipases. These later enzymes produced objectionable odours and flavours. Certain measures were adopted to avoid such flavours but no significant success appeared forthcoming in this respect. Microbial rennets contain not only several proteases with different concentrations of milk clotting and proteolytic activities but also other enzymes such as lipases and cellulases. Some proteolytic activity considered to be beneficial on one side may cause problems of bitter taste and softening of texture [12]. Schliech *et al.* have succeeded in removing objectionable odours and flavours in certain strains like *Mucor miehi* and *Mucor pusillus* to a significant extent [13]. The study of milk clotting strains of genus *Streptomyees* has also been failure due to the same problem as observed in the present study, i.e. the greater proteolytic activity of its enzyme extracts.

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