PRODUCTION OF FUNGAL PROTEINASE

By

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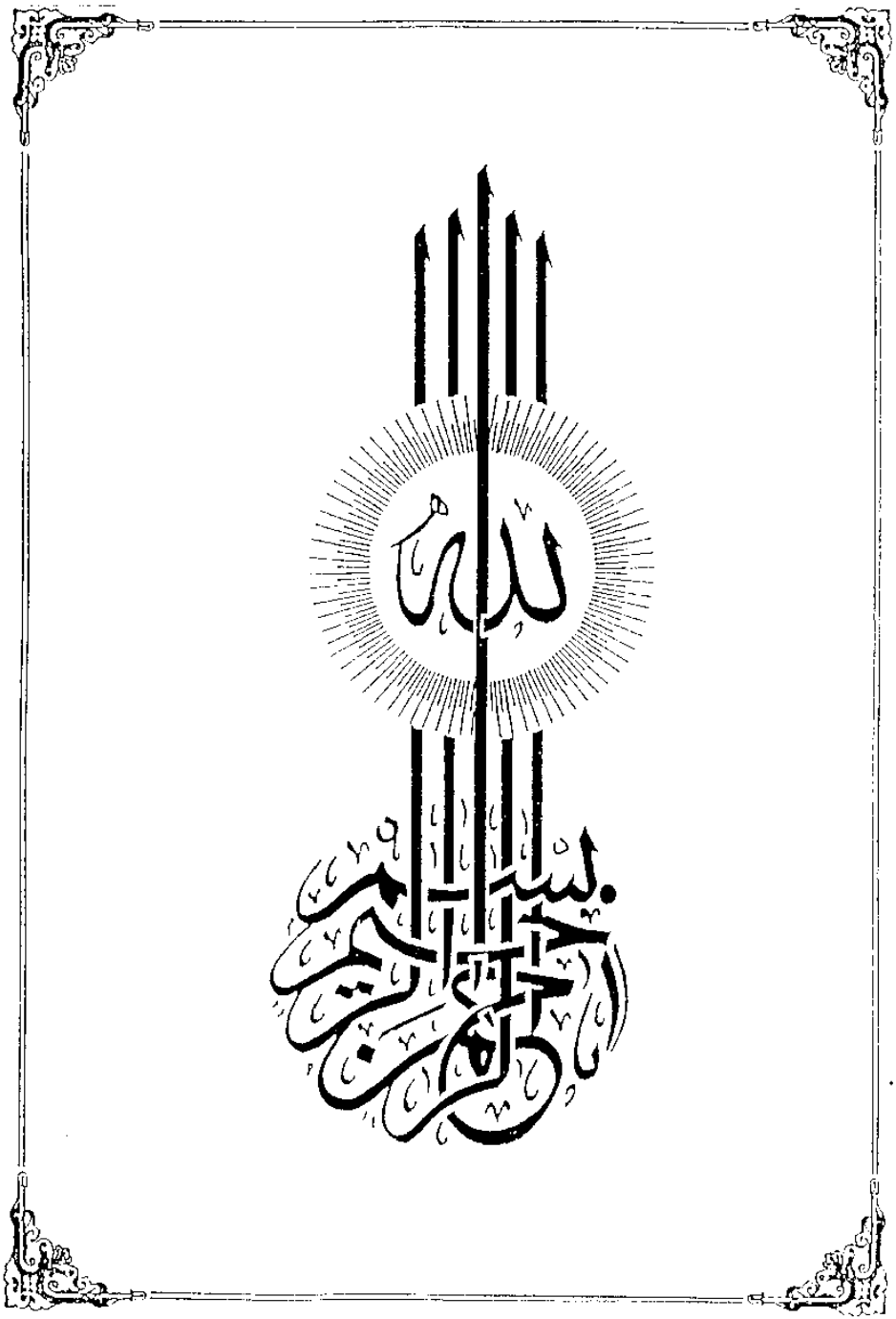

A C K N O W L E D G M E N T

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C O N T E N T S

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INTRODUCTION

The enzyme proteinase is considered of great industrial importance. It is widely used in meat tendering, leather tanning, synthetic detergents, and pharmaceutical preparations including preparation of digestive tablets and treatments of burns and ulcers. The use of microbial proteinases as substitution for rennet enzymes is of increasing importance. As it has been stated by the USDA Statistical Reporting Service (1965 a-b)the rapid increase in cheese production was faced by decrease in rennet enzymes produced from the fourth stomach of the calf. Thus the use of enzymes of microbial origin became important. The Food and Drug Administration (Federal Registeration, 1966 a) has been petitioned for the clearance to use an enzyme produced by the fungus Endothia parasitica. The FDA (Federal Registeration, 1966 b) has also been petitioned for a change in the cheese standards to include additional safe milk clotting enzymes.

In Egypt cheese manufacturers suffer from the variations in the potency of rennet enzyme. Moreover, this enzyme which is marketed as liquid preparation is liable to microbial contaminations and rapid inactivation especially in summer. Leather

and pharmaceutical industries are also dependant on foreign resources of proteinase.

Therefore, it was found valuable to investigate the possibilities of local production of fungal proteinase. A collection of isolates were obtained from soil, deteriorating meat and dry cheese, and their capacities to produce fungal proteinase were qualitatively and quantitatively studied. The most efficient strain was selected. The effect of different media, methods of production (surface and submerged), carbon source, phosphate level and pH on the production were investigated. The most efficient method of enzyme precipitation from the culture was studied. Purification of the enzyme was carried out and potency of the pure and crude enzyme were compared. Furthermore, the effect of pH and temperature on the activity of the pure enzyme were also determined.

REVIEW OF LITERATURE

The most important organisms in fungal proteinase production:

Different species of fungi have been used by various investigators for the production of proteinases.

Yoshida (1956), stated that the black aspergilli, A. awamori, A. niger and A. saiti produce acid proteinases as major components of their protease systems.

Jönsson and Martin (1965), found that five strains of Alternaria tenuissima showed wide variation in the ability to produce extracellular proteinase and the amount of protease produced by a given strain varied greatly with the medium used.

Koase et al., (1966 and 1968) used A. niger var. macrosporus for the production of proteinase. Yuan-Chi Su and Wen-Hsiung Liu (1966), used A. wentii for proteinase formation.

Akiba and Fukimbara (1967) found that Rhizopus(548) was a more stable producer of acid proteinase than Rhizopus 162 or 515 or Rh. javanicus and Aspergillus species tested.

Emad (1967) used A. niger, A. tirricola and A.oryzae

for proteinase production. Somkuti and Babel (1967 and 1969) used Mucor pusillus for the production of this enzyme. Toyama and Miyasato (1967), Dorokhov and Konovalov (1968) and Shakhova and Konovalov (1969) found A. awamori to be active producer of proteinase.

Bilai and Shkurenko (1968) reported that Penicillium cyclopium 5136 and A. sulphureus 5033 were able to produce proteinase.

Day et al. (1968) described a method for culturing Trichophyta granulosum on horse hair digestion for production of proteinase.

Hagemeyer et al. (1968) used Endothia parasitica for proteinase production.

Gernet (1968) used A. flavus for the enzyme production. Kundu et al. (1968) used A. oryza strain E 1212 for the enzyme production. Fujishima and Suzuki (1970) used Aspergillus sp., Penicillium sp. and Mucor sp. for the enzyme production. Marshall (1970) used P. notatum for fungal proteinase production. Rao et al. (1970) tested 12 strains of 3 species of Aspergillus (A. niger, A. flavus-oryzae and A. oryzae), for the ability to degrade animal and plant proteins and found that 4 strains secreted good quantities of protease.

Nasuno and Ohara (1971) obtained mutants of A. sojae which produced large amounts of alkaline proteinase.

Methods and media used for enzyme production :

Maxwell (1950) cultivated 43 species and strains of moulds on steamed wheat bran. A. flavus-oryzae strain 292-4795, yielded culture extracts possessing the highest gelatinase activity. Optimum growth and enzyme production by this strain were obtained on sterilised wheat bran, containing 60 per cent water when spread in layers not exceeding 2 cm. in depth.

Dworschack et al. (1952) described a procedure for surveying mold strains for proteinase production under submerged culture conditions. Four hundred and ninety-one strains of the A. flavus-oryzae group were tested. Eighty strains produced sufficient proteinase activity to warrant further study.

Jönsson and Martin (1964) studied the enzyme production by five strains of A. fumigatus and found that they showed marked variation in their ability to produce protease in submerged culture.

Koase et al. (1966) used a medium containing wheat starch, Corn-steep liquor, defatted soybean meal, $(NH_4)H_2PO_4$,

K_2HPO_4 and $CaCO_3$ for the production of acid proteinase by A. niger var. macrosporus ATCC 16513. Yuan-Chi Su and Wen-Hsiung Liu (1966) stated that for optimal proteinase formation a medium containing defatted soybean powder, rice bran, sucrose and $CaCO_3$ could be used.

Akiba and Fukimbara (1967) stated that the most effective culture medium for acid proteinase production contained wheat bran, starch, rice bran, defatted soybean meal, $NaNO_3$ and KH_2PO_4. Brad (1967) used a wet mixture of wheat bran and corn meal as a suitable assay medium for proteinase production by Aspergillus niger, A. terricola and A. oryza. Somkuti and Babel (1967) used wheat bran medium under submerged conditions for the production of protease synthazed by Mucor pusillus.

Misumma and Nakadai (1967) used a medium consisting of soybean and wheat containing 50% H_2O for proteinase production. Toyama and Miyasato (1967) found that in the production of proteinase by A. awamori strain 29-2 was obtained after between 30 and 60 hrs. on wheat-bran culture.

Wang (1967) found that when Mucor hiemalis NRRL 3103 was grown in soybean medium, only a small fraction of the proteinase produced by the organism appeared in the culture filtrate, whereas the bulk of the enzyme was bound to the

Zielinska and Kaczkowski (1971) found that A. niger AS/VII produced the highest proteolytic activity when growing in a nutrient medium containing as a protein source, water extract of wheat bran and soybean flour. Korshunov and Kazakov (1972) found that elevated enzyme yield (proteinase by A. terricola 3-374) was obtained in a medium containing wheat-bran or maise or soybean flour, in addition to mineral components. Optimal concentration of C, N. and P. in the growth medium for synthesis of proteinase were found. The synthesis increased 2-3-fold in a medium containing soybean flour, KH_2PO_4 , $K_2HPO_4.3H_2O$, KNO_3 , $MgSO_4.7H_2O$. Tap water at pH 6.8 was used.

Kumdu et al. (1972) found that under submerged condition, fungi belonging to Aspergillus group secrete protease or collagenase or both depending on the medium. Masuno and Ohara (1972) found that an alkaline proteinase was extracted from A. candidus, which was grown in wheat-bran solid culture, with 1% NaCl.

Sekine (1972a) isolated two neutral proteinase I and II from wheat-bran cultures of A. oryzae.

Popova and Fedotova (1972) studied proteinase biosynthesis by various mutants of A. terricola at 28-29°C for 72-96 hr. in shake culture containing casein, the media had a pH 3.5, 7.3 or 9.5.

Yokotsuka and Hashimoto (1972) cultivated P. duponti in a solid medium at 40°C for 4 days for the production of a new acid stable and thermostable protease.

Effect of pH on enzyme production :

Yuan-Chi Su and Wen-Haiung Liu (1966) found that optimal initial pH for protease formation by A. wentii was 5.0.

Somkuti and Babel (1967) studied protease synthesis by Mucor pusillus in a wheat-bran medium under submerged conditions and found that the initial pH of the medium had a substantial effect on enzyme synthesis; adjustment of the enzyme production medium to pH 5.0 prior sterilisation was desirable.

Toyama and Miyasato (1967) found that the maximum production of protease on wheat bran culture by A. awamori, was at an optimum pH of 2.7.

Jonsson (1968) found that during protease production by species of Entomophthora, pH values during growth rose from 4.5-7.5 in the initial medium to 7.2-7.9, and did not affect the final yields.

Dorokhov and Konovalov (1968) used a medium at pH 5.0-5.5 for the production of the enzyme by fungi such as Aspergillus and Penicillium.

Gernot (1966) found that for the enzyme production by A. fluvus the pH could vary between 2 and 7; however, best yields were obtained between 3.5 and 4.5, in which case 55% of the final proteolytic activity was reached after 24 hrs., and 100% after 3 days.

Marshall (1970) found that the enzyme is produced by incubating Penicillium potatum in nutrient medium at pH 4.8. Thus, spores of P. potatum were used to inoculate wort broth at pH 4.5, in surface cultures in Roux bottles.

Zielinska (1971) found that the pH range 2.5-3.0 gave the best results for production of A. niger acid protease

Zielinska and Kaczkowski (1971) found that the synthesis of extracellular acid protease from A.niger AS/VII occurs both in the logarithmic and stationary phases of growth when pH decreases to < 4.0.

Kundu et al. (1972) found that the optimal pH for production protease by Aspergillus groups is 7.0. Popova and Fedotova (1972) produced the proteinase biosynthesis by various mutants of A. terricola at 28-29°C for 72-76 hr in shake cultures containing casein at pH 3.5, 7.3 or 9.5.