PRODUCTION OF SOME MICROBIAL ENZYMES

By

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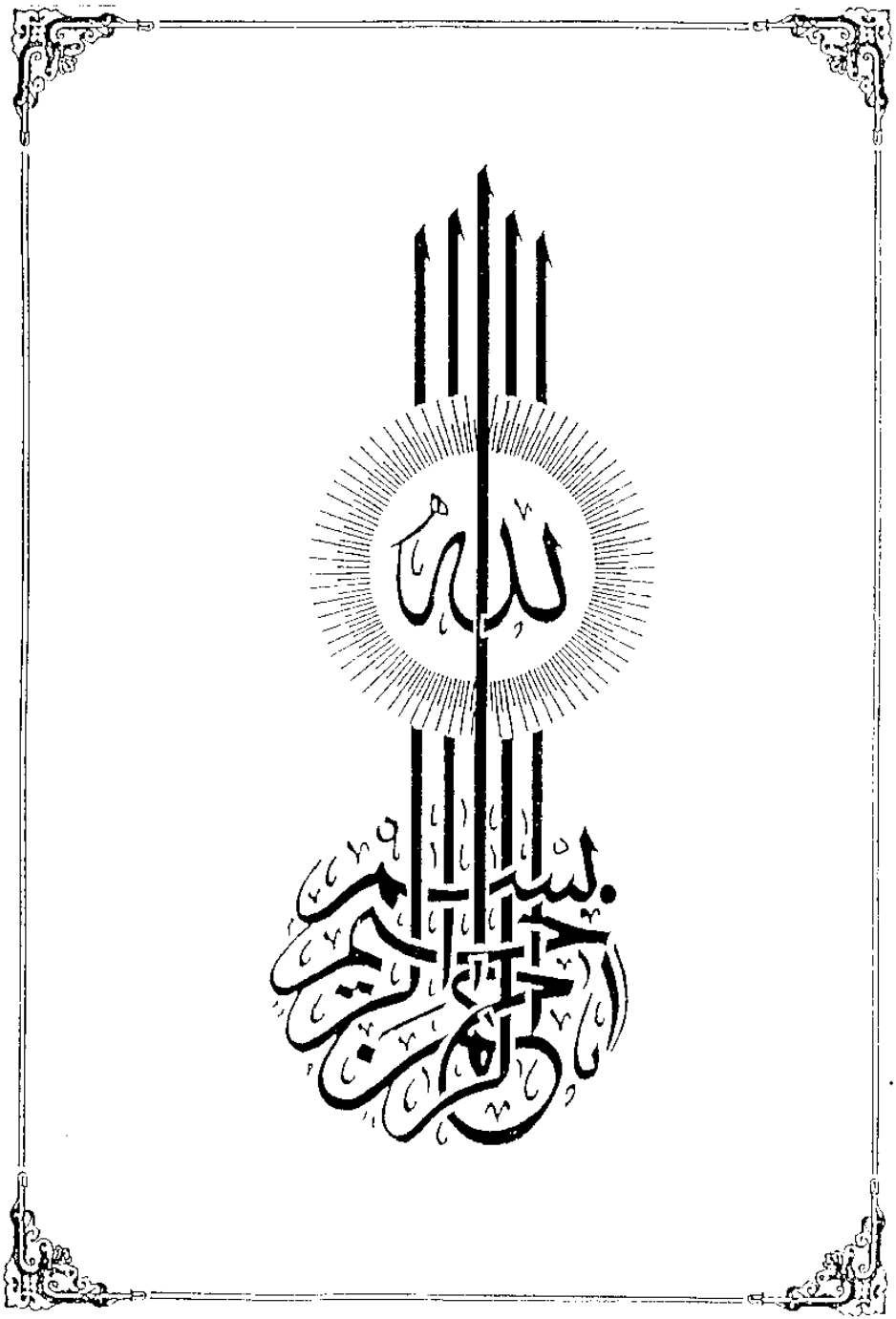

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ABSTRACT

The factors affecting protease production by B. subtilis N' strain were investigated. Results of these studies denoted that modified Luria broth (Zamost et al, 1990) gave the highest enzyme production. 2.0% maltose in the medium was the best carbon source. The optimal levels of yeast extract and tryptone were 0.8 and 2.0% respectively. Enzyme secretion occurs at a wide range of pH between 4-10 with pH 8.0 as optimal. Gradual progressive modifications of the medium resulted in 8 fold increase in enzyme production reaching 303 units/ml of the culture.

The produced enzyme was purified by fractional precipitation by acetone (55% saturation), dialysis by tris phosphate buffer, and gel filtration chromatography. By this

method the specific activity was raised 3.78 fold with 43% recovery of the enzyme.

The characteristics of the purified enzyme were evaluated. The enzyme showed an optimum temperature of 40°C, and even at 80°C about 50% of the original activity was still retained. The enzyme has a considerable thermal stability and at 60°C about 90% of its activity was recorded after 2 hr exposure. $CaCl_2$ considerably increased the thermal stability of the enzyme. The highest enzyme activity was at 0.8 and 0.3% casein and asocasein respectively as substrates.

When enzyme immobilization was tried, it was found that the amount of enzyme retained by the carriers was higher in adsorptive binding than covalent coupling. The enzyme immobilized on trisopor showed the highest specific activity. The enzyme - CPG-10 covalent coupling system was greatly stable. Thus, even after repeated use of this immobilized system in 10 protein digesting cycles, it still retained 100% activity. Both pure commercial subtilisin and the purified enzyme obtained from this work showed similar behavior with respect to retention on carriers and specific activity of the immobilized system.

Key Words: Microbial protease, Microbial proteinase, Alkaline protease, Subtilisin, Immobilized enzymes.

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INTRODUCTION

1. INTRODUCTION

The real progress in microbial enzyme technology started after world war II. This time coincided with the discovery of numerous new enzymes, increase in knowledge of enzyme properties and progress in fermentation technology. Moreover, it had been realized that all enzymes of industrial interest can be produced by microorganisms.

Proteolytic enzymes received special interest because of their large scale production and wide range of application in industry and medicine. Some of these enzymes are produced in large quantities, up to thousands of tons. Among the industrial applications of proteases are : detergents, dairy industries, bating of leather, dehairing and dewooling of skins, removal of turbidity from beverages, dough conditioning, meat tenderization, modification of protein in food industries, reverse hydrolysis in aspertame synthesis (sweeteners preparation), etc. Proteases have also some important medical uses such as removal of dead tissues and dissolution of blood clots, treatment of certain types of hernia, conversion of hog insulin into human insulin and a digestive aid.

The use of immobilized enzymes came to application because of the high cost of enzyme preparation which may affect their economical use in wide range of industrial applications. Consequently it was important to develop systems which allow the recovery and re-use of these