Asian J. of Bio Sci. (2007) Vol. 2 No. 2 : (177-179)

A Review :

Enzymatic properties of bacterial protease

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(Accepted : August, 2007)

Enzymes are macromolecules, with highly specialized catalytic functions produced by all living organisms. Enzymes are responsible for many essential biochemical reactions in microorganisms, plants, animals and human beings. Their existence was associated with the history of ancient Greece where they were using enzymes from microorganisms in various processes. Modern genetic engineering techniques have made it possible to produce relatively rare enzyme with many of industrial and medical applications in large quantities. Although enzymes derived from a range of organism are being used commercially, certain properties of these proteins can be further improved. For example, it may be advantageous to increase the heat stability or alter the pH optimum of an enzyme thus improve the efficiency of a defined process. The present article reviews some environmental effects on bacterial strains which are producing proteases.

Key words : Bacterial protease, Environmental conditions, Enzymatic properties.

INTRODUCTION

Many of the goals of industrial protein engineering are to design and construct novel enzymes by modifying the properties of existing enzymes. For example, it may be desirable to alter the kinetic properties K_{cat} and K_m, the substrate specificity, the pH optimum, the temperature stability, the stability in the presence of chemical reagent and the isoelectric point of an enzyme (Singh, 1999).The stability of an enzyme can be defined as its ability to retain its activity under various conditions (Missal, 1993). The enzymes are inherently unstable.

These enzymes are important in a number of diverse and crucial biological processes; they are involved in the regulation of metabolism and gene expression, enzyme modification, pathogenicity, and the hydrolysis of large proteins to smaller molecules for transport and metabolism. The extracellular proteases are of commercial value and find multiple applications in various industrial sectors.Proteinases are found in several microrganisms such as viruses, protozoa, bacteria, yeast and fungi.

Recent studies document the production of hydrolytic enzymes from thermophilic bacteria (Antranikian *et al.*, 1990). So far, however, few thermophilic *Bacillus sp.* that produce proteases have been isolated, the earliest isolate being *Bacillus stearothermophilus* (Salleh *et al.*, 1977) which is found to be stable at 60°C. Enhancement of protease activity excreted from *Bacillus stearothermophilus* had also been possible using economical, chemical additives in the protease reaction involved in water activated sludge (Kim *et al.*, 2002).Protease production by *B. licheniformis* S-40 was reduced to half its maximum level when glucose was present as the carbon source (Sen and Satyanarayana, 1993). Battiaglino *et al.* (1991) reported that glucose repressed protease synthesis while Gomaa *et al. (1990)* reported glucose to be the best carbon source for protease production by *B. subtilis*. Effect of incubation period on protease production.

It is well known that bacterial proteases are extracellular enzymes the synthesis of which depends on the environment conditions. As inductors, in addition to the substrate, the metabolism end products can be effective too. Madan *et al.* (2000) investigated the enzyme production of *Bacillus polymyxa* in modified Reese's medium under stationary and submerged conditions at 50 ^{0}C after different time intervals.

Effect of incubation time on protease activity :

An increase in enzyme production with increase in incubation time after attaining certain peak value there is gradual decrease in the enzymatic activity. Kaur *et al.* (1998) reported that incubation of isolated *Bacillus polymyxa* at 70 ^{0}C for 10 min in modified Reese medium gave about 3 times more protease enzyme activity compared to that in the seed medium. Gajju *et al.* (1996) characterized protease enzyme of thermophilic *Bacillus coagulans* PB-77 at different time intervals (10-50 min)and found that increase in incubation time denaturates the enzyme. Madan *et al.* (2000) investigated the enzyme production of *Bacillus polymyxa* in modified Reese's

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medium under stationary and submerged conditions at 50 ^{0}C after different time intervals.

Effect of pH on enzyme activity :

Majority of organisms producing alkaline protease show growth and enzyme production better under alkaline conditions. Decline in activity was observed with increase in pH (11.0). Kumar *et al.*(2002) observed maximum protease production in case of *Bacillus sp* strain S-4 and *Pseudomonas* strain S-22 at pH 7 and 9.0 respectively and hence indicated that S-4 produced neutral protease and S-22 produced alkaline protease. Peek *et al.* (1992) observed that the protease from a *Thermus sp.* strain Rt41A exhibited stability for at least for 4 hours over a pH 5-10. Chaia *et al.* (2000) reported the optimum pH was between 7-11 for the proteolytic activity of *Brevundimonas diminuta*.

Effect of pH on stability of protease :

Protease from *Bacillus sp*. is reported to be stable over a wide alkaline pH range (9.0-9.5) and up to 50 ^{0}C (Madan *et al.*, 2000).Olajuyigbe and Ajele (2005) investigated the proteolytic activity of three *Bacillus* species over the entire range of pH (pH 5-10). However, maximum protease production was observed at pH 8.0 while at pH 10, the protease production was about 60%.

Effect of temperature on protease activity :

Enzymes are temperature specific, as each enzyme is most active at a specific temperature. Kumar *et al.* (2002) reported highest protease activity at 30 °C from *Bacillus sp*. S-4 *and Pseudomonas* S-22, there was continuous decline in enzyme activity with increase in incubation temperature. Naidu and Devi (2005) reported maximum production of protease enzyme at 50 °C after 96 h in *Bacillus sp.* K-30. Sen and Satyanarayana (1993) observed that *Bacillus licheniformis* S-40 had 45 ^{0}C as optimum temperature for the production of protease. Chaia *et al.* (2000) reported the optimum temperature for the proteolytic activity of *Brevundimonas diminuta* between 40 °C and 50 °C.

Effect of temperature on stability of protease activity:

Extremely thermostable serine proteases are produced by the hyperthermophilic *Archaeum desulfurococcus* strain (Hanazawa *et al.*, 1996), and thermostable metalloproteases are reported from a gram-negative thermophilic bacterium (Pawinee and Wipapat, 1996). Sharma *et al.* (1996) reported maximum protease enzyme activity of *Bacillus laterosporus* and *Flavobacterium* sp. at 60 ^{0}C. Olajuyigbe and Ajele (2005) showed that three *Bacillus* species under study are producer of extracellular protease at high temperature.

Effect of carbon and nitrogen sources on enzyme activity :

The production of alkaline protease was high in starch soya meal medium with wheat bran/starch/glucose/ dextrin as a carbon source. Inorganic nitrogen sources supported better protease production than the organic sources (Sinha and Satyanaryana, 1991). In the presence of inorganic nitrogen and asparagine, very low levels of enzyme were secreted (Sen and Satyanarayana, 1993).

Effect of metal ions and inhibitors on enzyme activity:

The enzyme activity of the thermostable alkaline protease from *Bacillus coagulans* PB-77 was greatly enhanced in the presence of 2mM and 5mM concentration of Fe^{2+}, Fe^{3+} ions, whereas Hg^{2+} severely inhibited enzyme activity (Gajju *et al*., 1996).EDTA inhibited about 60% of the enzyme activity which indicates the partial requirement of metal ions (North, 1982). Some of the metal ions such as Ca^{2+}, Mg^{2+} and Mn^{2+}) increased and stabilized the protease activity of the enzyme; this is possible because of the activation by the metal ions (Adinarayana *et al.,* 2003).

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