RESEARCH PAPER

Asian Journal of Bio Science, Vol. 4 Issue 2 : 216-220 (October, 2009 to March, 2010)

Effect of growth media pH and incubation temperatures on the production of proteinases from bacillus *sp.* 26

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(Accepted : August, 2009)

Bacillus sps-26 isolated and characterized from dairy effluents was challenged for its ability to produce alkaline proteinase using different temperature and pH as bioparameters with a chemically defined medium containing 5% casein. The maximum enzyme production was achieved at 30.C at pH 10, which was assayed using Genov method. Although moderate enzyme level was obtained at 10.C, it can pave the way for its affordable use in detergent industries.

Key words : *Bacillus sp*-26, Alkaline proteinase, Bioparameters, Tseushida method, Lowry method.

INTRODUCTION

A variety of enzyme products has been developed for use in biological or enzyme detergent to enhance the removal of organic material from textile fibre. The most widely used of these proteinase when function and mode of action is to remove proteins stains such as grease, egg and human sweat by proteolytic degradation to polypeptides that are more soluble and amino acids.

Proteinases are the most important industrial enzymes for about 60% of the total enzyme market and have a number of practical applications in industries such as detergent, tanning, dairy, baking, brewing, leather and textile etc. (Ward *et al.*, 1985). Fungal proteinase is being used for centuries in the Orient for the production of soy sauce. The process involved growing proteolytic strains of A.oryzae on the soybean to produce a heavily sporulated koji (Nahara *et al.*, 1982).

Proteinases are mainly classified into four groups based on their mode of action: Aspartic-proteinase, Serine-proteinase, Metalo-proteinase, Cysteine- proteinase. Alkaline proteinase falls onto either serine-proteinase or metalo-proteinase.Alkaline proteinase are produced by a wide variety of microorganisms such as bacteria, fungi, yeast and actinomycetes.

Bacterial species in particular; *B.subtilis, B.lentus, B.licheniformis* and *B.brevis* are reported to be prolific producers of alkaline proteinase, which constitute a major source of enzymes used in detergents. Different authors reported production, proliferation and characterization of chemo stable alkaline proteinase from *Bacillus* spp. (Takami, 1989; Ferrero *et al.*, 1995; Adinarayana and Elliah, 2002; Prakasham *et al.*, 2005; Enshasy, 2008).

In search of novel extracellular alkaline proteinase active at low and moderate temperatures, isolation and screening of cold active alkaline proteinase have been carried out at RRL, Trivandrum.Extensive screening procedures performed a different stages resulted in the isolation of more than 50 bacterial isolates, which could tolerate alkaline pH. Further screening of those organisms in salt high patent media resulted in the isolation of potent bacterial cultures (identified as *Bacillus* sps-26) that was capable of producing alkaline proteinase in the early growth hours (Sandhia and Prema, 1998).

The influence of various bioparameters on metabolites has been well established. By manipulating the cultural and nutritional parameters of an organism, the enzyme production could be over expressed (Gupta *et al.*, 2002). Following this context, investigation has been carried out to study the effect of growth media, temperature and pH in the production of alkaline proteinase by *Bacillus* sp-26.

MATERIALS AND METHODS

Organism and its maintenance:

Bacillus sps-26 strain used in this study was earlier isolated in RRL, Trivandrum from soil samples. It was maintained on nutrient protein agar medium (pH 10) stored at 4^0C and was periodically subcultured.

Medium used for growth:

The composition of the growth medium (g/100ml) consist of Casein-0.15, Yeast extract-1.0, $MgSO_4$-0.005, KH_2PO_4-0.005, $FeSO_4$-0.001, pH-10.