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Study the Effect of Various pH on the Protease Producing Capacity of *Bacillus*

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***Bacillus* was found to produce an extracelluar thermostable protease. The purified enzyme streaked on Nutrient Agar skimmed plates and from which zone producing cultures were screened for maximal activity. The culture was assayed for protease (Azocasein Assay) at different pH i.e. 7,8,9,10 and 11 from which it was found producing 78.5,92.9,101,88.6 and 48.4 units/ml/hr respectively from which culture was found showing maximal units at 9 pH and units found to be decreasing at 10 pH.**

Key words: *Bacillus,* Screening, Protease assay, pH optimization.

Protease catalyses the hydrolysis of proteins to peptides and amino acids.(Brown *et al.*, 1991) The enzymes are important from an industrial perspective and cater to the requirement of nearly 60% of the world enzyme market of these alkaline proteases produced by alkalophiles are of interest from a biotechnological perspective and are investigated not only in scientific areas like protein chemistry and protein engineering but also find application in detergents,food,pharmaceutical and tannery industries (Endo, 1962). Bacteria in the genus Bacilli generally produced large amount of extra cellular protease. These enzymes have similar properties with molecular weight 25000-30000 and optimal pH 9-11 (Kalisz *et al.*, 1988).The ubiquity of *Bacillus* species in nature, the unusual resistance to their endospore to their chemical and physical agents, the developmental cycle of endospore formation the production of antibiotics.In this paper we report on the protease producing capacity of *Bacillus* by the effect of various pH.

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MATERIAL AND METHODS

Micro-organisms

The thermostable bacteria *Bacillus* was obtained from soil samples collected from Barfi Shop.

Media and culture conditions

Soil sample was diluted in normal saline and spreaded on nutrient agar(From Sigma Aldrich) and skim milk (From Sigma Aldrich) plates (Secades 1999, Vaskivyuk, 1981). Then incubated for 24 hrs(hours) at 30°C in Incubator (From Apollo machinery, India). Colony showing zone in the plates was isolated and transferred into liquid Luria Broth media (From Sigma Aldrich) in flasks and again incubated at 30°C in moving shaker

incubator. Samples were drawn after every 2 hrs for Protease analysis.

Samples were centrifuged (Centrifuge of Apollo Machinery, India) at 10000 rpmfor 5 min in which cell mass was separated are used for assay (Foreshteh *et al.* 2003, Polgar, 1989).

Protease assay

The proteolytic activity was assayed by the Azocasein digestion method (Ottensen, 1971, Takani, 1989) In this Tris/Glycine buffer are used in 3 different appendorfs as Enzyme Blank, Substrate Blank and Test with 480µl buffer and 20µl Azocasein, 490µl buffer and 470µl buffer and 20µl Azocasein respectively. All 3 appendorfs were incubated at 55°C using different pH from 7 to 11 in 5 sets separately for 15min. 10µl of crude enzyme was added to Substrate Blank and Test appendorfs of each set. All appendorfs were incubated for 30 min at the respective temperatures. Then enzymatic reaction was stopped by addition of 500µl of 10% Trichloroacetic acid. Further kept test tubes in ice for 15 mins.The reaction mixtures were then centrifuged for 10 mins at 10000 rpm. The supernatant of reaction mixture which was centrifuged above is used for taking absorbance in Spectrophotometer (From Rays scientific instrument) at 420 nm against a Blank. 1 Unit of enzyme activity was defined as the amount of enzyme which hydrolyzed 1mg (mili gram) of Azocasein in 15 mins at 55°C at 9 pH. .Proteolytic activity (units/mili liter/hour) is the increase in absorbance caused by 1.0 ml enzyme/hr under assay conditions.

RESULTS

Production of Protease

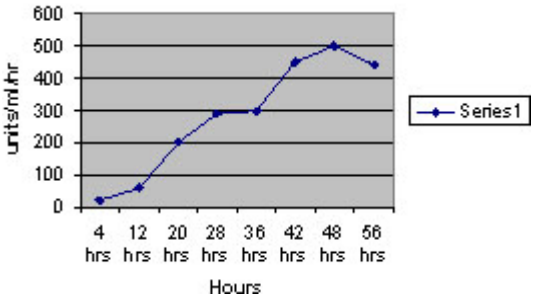
Bacillus was grown in Luria Broth media.Proteolytic activity was detected after every two to three hrs of fermentation at the end of logarithmic growth fails and reached the maximum level i.e. 42-44 hrs after the fermentation starts (Fig. 1).

pH optimization

To identify the effect of various pH on the enzyme producing capacity of *Bacillus* (Table 1), it was cultured in Luria Broth media for 42 hrs and samples were drawn from culture whose Protease assay were performed at 55° C from 7-11 pH. The optimal pH of the enzyme production was found 9 pH with 101 units/ml/hr (Fig. 2) (Rao, 1998, Susana, 2006).

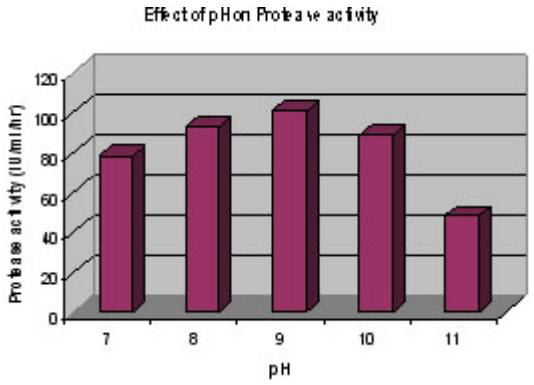
Table 1. Protease activity at various pH

pH	Activity (units/ml/hr)
7	78.5
8	92.9
9	101
10	88.6
11	48.4



ml = mili litre
units = inteernational units
hr = hours

Fig. 1. Growth Curve



pH = hydrogen ion concentration
ml = mili litre
hr = hours

Fig. 2. Effect of pH on Protease activity

DISCUSSION

The Thermostable Protease produced extracellularly by *Bacillus*. The proteolytic activity of enzyme reached maximum at 42hrs at 30°C. The enzyme was most active at 55°C at 9 pH after 42hrs of incubation. It may be economically useful since it can be easily cultivated at 25°-37°C.This Protease may take part in the variation of insecticidal activities in some way and in detergent industries. The use of Protease as active ingredients of detergents is the single largest application of the industrial enzyme.

CONCLUSION

The culture was assayed for Protease (Azocasein assay) at different pH (7, 8,9,10 and 11). The culture was showing maximum units at 9 pH and the units found to decreasing at 10 pH. Hence, pH optimized for highest protease production was 9 pH.

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