International Research Journal of Natural and Applied Sciences

ISSN: (2349-4077)

Impact Factor 5.46 Volume 8, Issue 5, May 2021

Website- www.aarf.asia, **Email** : editor@aarf.asia , editoraarf@gmail.com

AN EMPERICAL STUDY OF ISOLATION AND OPTIMIZATION OF PROTEASE PRODUCING BACTERIA

Dr. Praveen Goswami [1] & Preeti Sharma [2]

1. Professor, Department of Zoology, Poddar International College, Jaipur (Raj)

2. Research Scholar, Department of Zoology, Poddar International College, under University of Rajasthan, Jaipur, Jaipur (Raj)

ABSTRACT

The production of extracellular alkaline protease was studied from the bacterial organism isolated from the soil. Different agro residues as substrate were studied for enzyme production. The highest enzyme production was expressed with Sugarcane baggase, Maltose, Soya bean. Enzymes producing bacterial growth parameters were optimized as pH 3.0 and Temperature 37°c. The high level of alkaline protease was obtained in the medium containing Sugarcane baggase followed by Cheese whey, Na_2SO_4, and Glucose. Among various nitrogen sources, Cheese whey was found to be the best inducer of alkaline protease, while other nitrogen sources repressed enzyme production. Among metal salts Na_2SO_4, was found to increase protease production. The maximum enzyme production (1033 U/I) was observed.

Keywords: *Alkaline protease, Bacillus macerans, Industrial enzyme and submerged fermentation.*

INTRODUCTION

Enzymes are well known biocatalysts that perform a multitude of chemical reactions and are commercially exploited. Protease refers to a group of enzymes whose catalytic function is to hydrolyze (breakdown) proteins. Proteases represent one of the three largest groups of industrial enzymes and account for about 60% of total worldwide enzyme sales (Nurullah Akcan *et al.,* 2011). These enzymes mainly function in a narrow range of pH, temperature, and ionic strength. Thus, the search for new microbial sources is a continual exercise (Kumar, 2008). Many bacteria and fungi excrete alkaline pro-teases. The most important producers are Bacillus strains such as *B. licheniformis, B. amyloliquefaciens, B. firmus, B. megaterium, and B. pumilis;* Alkaline proteases are generally produced using submerged fermentation due to its apparent advantages in consistent enzyme production characteristics with defined medium

and process conditions and advantages in downstream in spite of the cost-intensiveness for medium components (Prakasham *et al.,* 2006). At present, the overall cost of enzyme production is very high and therefore, development of novel processes to increase the yield of proteases with respect to their industrial requirements coupled with lowering down the production cost is highly appreciable from the commercial point of view (Mukherjee *et al.,* 2008). Hence, in the presence investigation the bacterial isolated collected from soil sample and characterized for protease production.

MATERIALS AND METHOD

Bacterial Strain and Culture Conditions: The Soil sample was collected in sterile plastic bag from meat stall at Rajasthan, India. The sample was immediately transferred to the laboratory for further analysis. The microbial colonies of 1gm soil sample were isolated using pour plate techniques using skim milk agar media Proteolytic activity of Microorganism was detected by observing the presence of the clear zones in skim milk agar plate. Identification of organism is done by gram staining, spore staining and biochemical test.

Effect of Various Carbon Sources, Nitrogen sources, Mineral sources: Media screening were done based on the production protease with different carbon source (Potato, Rice bran, Sugarcane baggage, Starch, Fructose, Sucrose, Maltose) and nitrogen source (Soya bean, Yeast extract, Beef extract, Cheese whey, Glycine, Peptone and Trypton) and mineral source (K_2SO_4, $FeSO_4$, Na_2SO_4, $MgSO_4$ and NH_2SO_4) by random screening. Optimum pH, temperature for enzyme activity was determined by conducting the assay at different temperatures 25^0C, 37^0C, 42^0C and pH in between 1 to 5.

Enzyme extraction: The enzyme from the fermented bacterial bran was extracted twice with tap water. The slurry was squeezed through cheesecloth. Extracts were pooled and centrifuged at 4°C for 15 min at 10,000 rpm to separate small particles of different substrates, cells and spores. The brown, clear supernatant was used in enzyme assays.

Assay of protein concentration: Crude Enzyme activity was measured by using 1% casein as a substrate and protein concentration was determined by the Lowry method (Lowry *et al.*, 1951) by using bovine serum albumin as a standard.

RESULTS AND DISCUSSION

Microbial population of soil sample was enumerated in skim milk agar media. After enumeration morphologically different colonies were isolated from streak plate method. The isolated colonies are like of the colonies showed that those colony like circular in shape and smooth in surface (Table 1). In gram staining the organisms were identified as gram positive, rod shape and motile in nature (Table 2). From that single colony was picked up and

biochemical tests were performed for the identification of organism. From the biochemical test the organism was identified as *Bacillus macerans* (Table 3). This organism showed maximum yield in the pH and temperature of 3 and 37°C (Table 4, 5). From the various carbon, Nitrogen and mineral sources sugarcane baggase, soybean meal and Na_2SO_4 were identified as optimal sources (Table 6, 7, 8). From the study media contains Sugarcane baggase 30g/l, Cheese Whey 100ml/l, Na_2SO_4 5 gm/l, Glucose 1gm/l producing 124000 µg/l of enzyme (1033U/l).

Table 1 : Morphology characteristics of organism in soil sample.

S. No.	Dilution Factor	Form	Elevation	Surface	Gelatin Hydrolysis	Color
1	10^{-4}	Irregular	Raised	Concentric	Opaque	White
2	10^{-5}	Circular	Raised	Smooth	Hydrolysis	White
3	10^{-5}	Circular	Raised	Smooth	Hydrolysis	White
4	10^{-7}	Circular	Raised	Smooth	Hydrolysis	White

Table 2 : Gram staining of isolated species.

S. No.	Test	Species Response
1	Gram's staining	Positive
2	Shape	Red shaped
3	Motility	Motile
4	Spore Staining	Endospore

Table 3 : Biochemical characteristics of isolated species

S. No.	Biochemical test	Exhibited result by the organism
1	Anaerobic growth	Negative
2	Indole test	Negative
3	Methyl Red test	Positive
4	Voges-proskauer test	Negative
5	Citrate utilization test	Negative
6	TSI test	Negative
7	Urease test	Negative
8	Nitrate reduction test	Negative
9	Hydrogen sulphide test	Positive
10	Glucose Fructose Sucrose Starch	Positive Positive Positive Negative

Table 4 : Identification of Optimal pH for Fermentation

S. No.	pH	Protease Produced at 2nd day (µg/ml)
1	1	34
2	2	96
3	3	127
4	4	78
5	5	56

Table 5 : Identification of Optimal Temperature for Fermentation

S. No.	Temp. (^{0}C)	Protease Produced at 2nd day (µg/ml)
1	42^{0}c	78
2	37^{0}c	94
3	26^{0}c	37

Table 6 : Identification of Optimal carbon source for Fermentation

S. No.	pH	Protease Produced at 2nd day (µg/ml)
1	Potato	43
2	Rice bran	78
3	Sugarcane baggage	134
4	Starch	26
5	Fructose	58
6	Sucrose	36
7	Maltose	96

Table 7 : Identification of Optimal Nitrogen source for Fermentation

S. No.	Nitrogen source	Protease Produced at 2nd day (µg/ml)
1	Potato	79
2	Rice bran	54
3	Sugarcane baggage	35
4	Starch	145
5	Fructose	34
6	Sucrose	45
7	Maltose	33

Table 8 : Identification of Optimal Mineral source for Fermentation

S. No.	Minerals	Protease Produced at 2nd day (µg/ml)
1	K_2SO_4	78
2	$FeSO_4$	67
3	Na_2SO_4	112
4	$MgSO_4$	76
5	NH_2SO_4	58

Table 9 : Identification of unit activity for the Enzyme supernatant From the experiment 120 (µg/ml) = 1U.

S. No.	Amount of substrate used (ml)	Concentration of protease used (µg/ml)	OD at 600 nm
1	1 % casein	30	0.243
2		60	0.453
3		90	0.566
4		120	0.602
5		150	0.624

Fig. 1 : Identification of Optimal pH for Fermentation

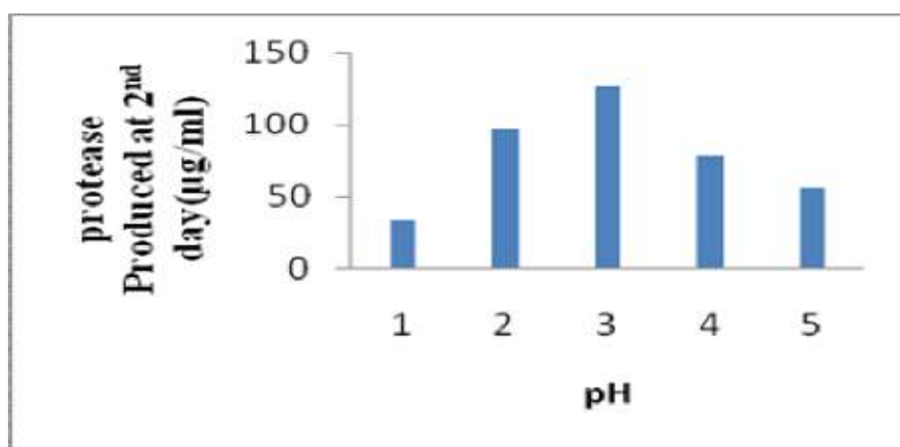


Fig 2 : Identification of Optimal Temperature for Fermentation

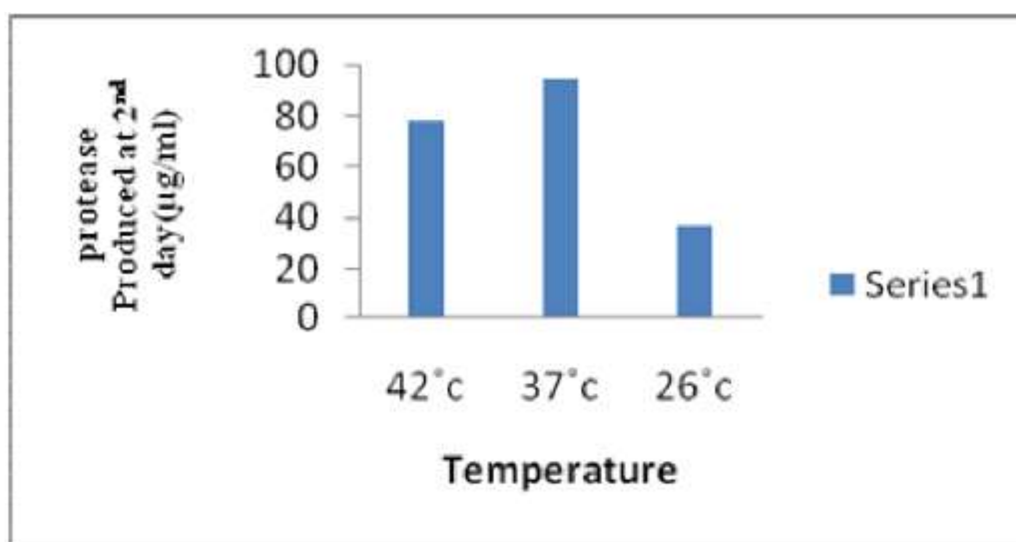


Fig 3 : Identification of Optimal carbon source for Fermentation

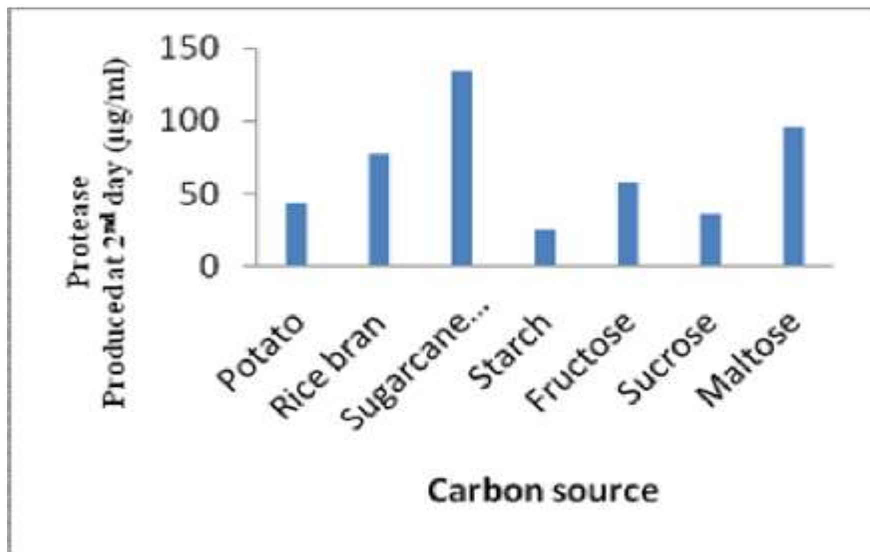


Fig 4 : Identification of Optimal Nitrogen source for Fermentation

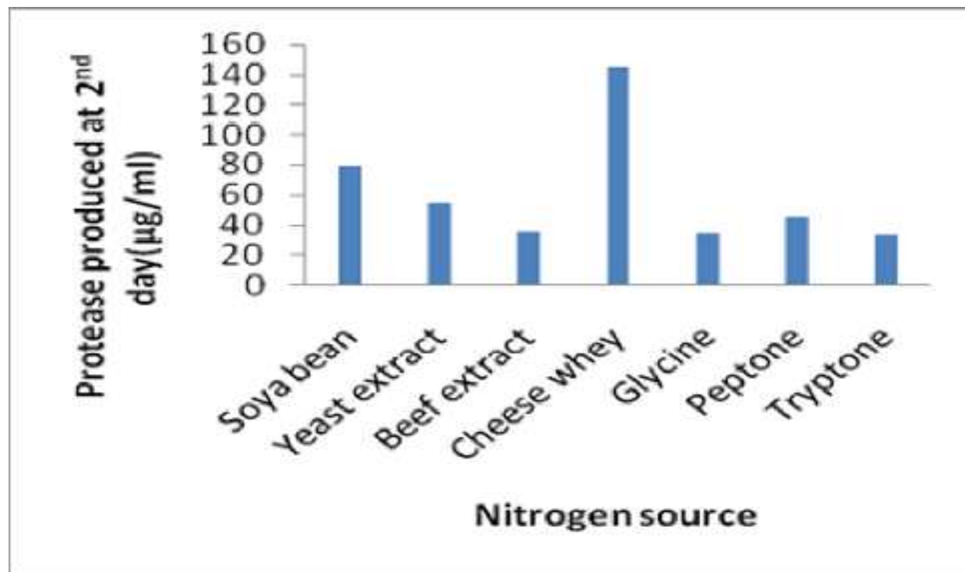
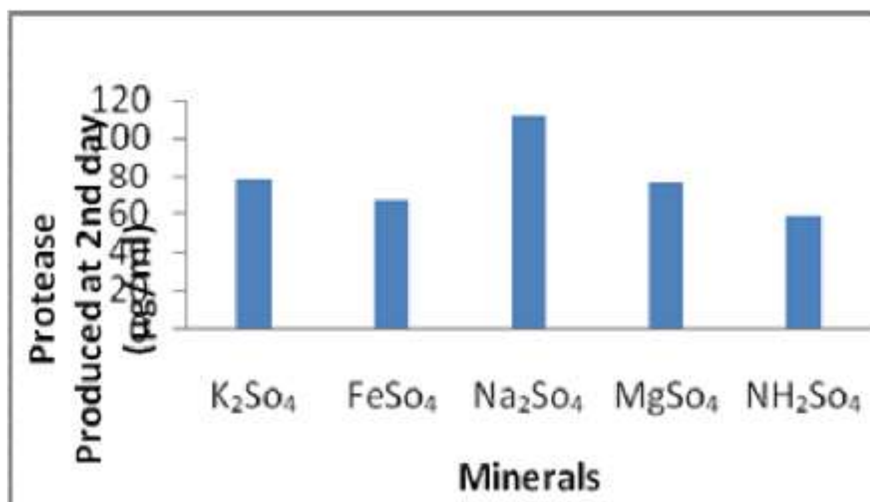


Fig 5 : Identification of Optimal Mineral source for Fermentation



© Association of Academic Researchers and Faculties (AARF)
A Monthly Double-Blind Peer Reviewed Refereed Open Access International e-Journal - Included in the International Serial Directories.

CONCLUSION

The production of extracellular alkaline protease was studied from the bacterial organism isolated from the soil. Different agro residues as substrate were studied for enzyme production. The highest enzyme production was expressed with Sugarcane baggase, Maltose, Soya bean. Enzymes producing bacterial growth parameters were optimized as pH 3.0 and Temperature 37°c. The high level of alkaline protease was obtained in the medium containing Sugarcane baggase followed by Cheese whey, Na_2SO_4, and Glucose. Among various nitrogen sources, Cheese whey was found to be the best inducer of alkaline protease, while other nitrogen sources repressed enzyme production. Among metal salts Na_2SO_4, was found to increase protease production. The maximum enzyme production (1033 U/I) was observed.

REFERENCES

- Kumar, PPK., Mathivanan, V., Karunakaran, M., Renganathan, S and R.S. Sreenivasan. 2008. Studies on the effects of pH and incubation period on protease production by Bacillus spp. Using groundnut cake and wheat bran. *Indian Journal of Science Technology,* 1(4): 1-4.
- Lowry, OH., Rosebrough, NJ., Farr, Al and RJ. Randall. 1951. Protein measurement with the folinphenol reagents. *Journal of Biological Chemistry,* 48: 17-25.
- Mukherjee, AK., Adhikari, H and SK. Rai. 2008. Production of alkaline protease by a thermophilic *Bacillus subtilis* under solid-state fermentation (SSF) condition using *lmperata cylindrica* grass and potato peel as low-cost medium: Characterization and application of enzyme in detergent formulation. *Biochemical Engineering Journal*, 39: 353-361.
- Nurullah Akcan and Hatt Uyar. 2011. Production of extracellular alkaline protease from *Bacillus subtilis* RSKK96 with solid state fermentation. *Eurasia J Biosci*, 5: 64-72.
- Prakasham, RS., Rao, CS and PN. Sarma. 2006. Green gram husk-an inexpensive substrate for alkaline Protease production by *Bacillus sp.* in solid-state fermentation. *Bioresource Technology*, 97: 1449-1454.