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ORIGINAL RESEARCH ARTICLE

Screening and Optimization of Alkaline Protease Productivity from *Bacillus* sp. Agt from Tannery Effluent

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ABSTRACT

Bacillus sp. *AGT* strains was isolated from tannery effluent were optimized with respect to temperature, pH and incubation time, different carbon sources, different nitrogen sources and also it was optimized with respect to different agro industrial waste. Substantial level of protease enzyme activity for *Bacillus* sp. AGT isolate was achieved at 40°C, pH 9.0 during 18 hours incubation in our production medium containing maltose as carbon source and 0.5% gelatine as nitrogen source.

Key words: Alkaline protease, *Bacillus* sp., Microbial enzymes and Enzyme production.

1. INTRODUCTION

Alkaline protease endorsed as a most important enzyme in both scientific and industrial community as it has wide range of analytical and industrial applications. These proteolytic enzymes are the most important group of enzymes produced commercially [1,2]. Microbial alkaline proteases dominate the worldwide enzyme market, accounting for a two-third share of the detergent industry and also find wide application in leather industry. Although many enzymes have their way into biotechnological application still there is drought as far as industrial demand is concerned and to overcome this industrial demand there is a need to emulate a focus towards optimization of protease production from microorganisms.

Microbes which produces alkaline protease needs to be screened and should be optimized to produce substantial amount of protease by adapting favourable conditions like optimal pH, temperature and favourable media should be demonstrated to increase its yield. Alkaline protease from extreme organisms should be produced commercially in high yield at a low-cost method [3]. Currently a large proportion of commercially available alkaline proteases are derived from *Bacillus* strains [4-6]. *Bacillus* strains are recognized as important sources of commercial alkaline protease because of their ability to secrete large amounts of enzymes with high activity [7-14]. Media components and culture conditions were found to have great influence on protease production and are different for each microorganism [15-18]. In this present study, attempts made to enhance alkaline protease production by optimization of the medium and culture conditions from strains of *Bacillus spp* isolated from tannery effluent.

2. MATERIALS AND METHODS

2.1. Isolation and Screening of Bacteria

Bacillus sp. AGT was isolated from the tannery effluent and it was grown in production media with pH 8.0 containing glucose 5%, peptone 7.5%, calcium chloride 0.5%, magnesium sulphate 0.4%, Potassium di hydrogen phosphate 0.5%, and ferrous sulphate 0.01%. All assays were performed by cell-free supernatant of the fermentation broth. The proteolytic activity were performed by using pour plate and spread plate technique in skin milk agar medium. Culture were regenerated every months on a fresh plate from the frozen stock culture

2.2. Determination of Protease Enzyme Production at Various Time Intervals

Production media was prepared and inoculated with 1ml of 24 hrs old culture. Protease enzyme activity was carried out for every one hour interval after 12 hrs till 32 hrs.

2.3. Effect of Different Carbon Source on Protease Enzyme Production

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Production medium containing 0.5% of different carbon source such as Glucose, Galactose, Maltose, Lactose and Ribose were inoculated with 1ml of culture. The production media ware kept for incubation at shaker (240 rpm) for 18 hours at room temperature. After incubation, production media was centrifuged at 10000 rpm for 15 minutes and the supernatant was collected and used for the estimation of protease enzyme activity.

2.4. Effect of Different Nitrogen Source on Protease Enzyme Production

Production medium containing 0.5% different nitrogen source such as Peptone, Beef Extract, Gelatin, Yeast Extract and Casein were inoculated with 1ml of culture. The production media were kept for incubation at shaker (240 rpm) for 18 hours at room temperature. After incubation production media was centrifuged at 10000 rpm for 15 minutes and the supernatant was collected and used for the estimation of protease enzyme activity.

2.5. Effect of Various Agro Industrial Waste on Protease Enzyme Production

Production medium containing 1% of orange peel waste, 1% of Rice bran, 1% of Wheat husk, 1% of Sugarcane baggase and 1% of raw potato starch were kept at shaker (240 rpm) for 18 hours at room temperature. After incubation production media was centrifuged at 10000rpm for 15 minutes and supernatant was collected and used for the estimation of protease enzyme activity.

2.6. Effect of pH on Protease Stability and Enzyme Activity

The optimum pH of extracellular protease was determined with casein 2% as a substrate dissolved in citrate phosphate buffer of different pH, from pH 1.0 - pH 10. Reaction mixture was incubated under assay condition and the activity of protease enzyme was measured.

2.7. Effect of Temperature on Protease Stability and Enzyme Activity

The optimum temperature of extracellular protease was determined using 2% casein as a substrate by incubating the samples at appropriate temperature, from 20°C to 100°C. After incubation samples were collected and estimated for protease enzyme activity.

2.8. Enzyme Assay Procedure (Mac Donald & Chand method)

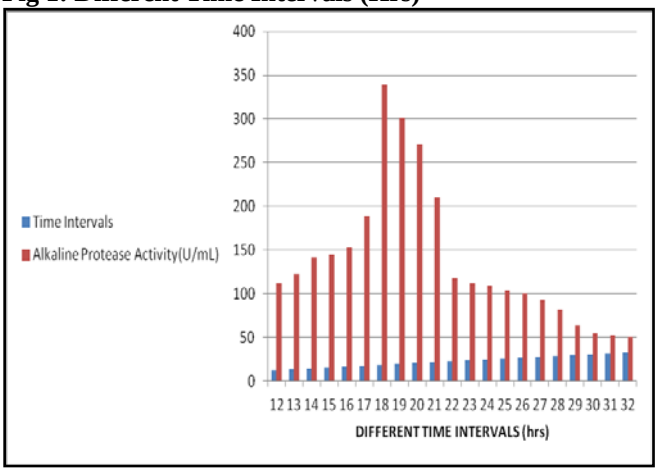
Enzyme assay performed as per Mac Donald & Chand method. The proteolytic activity of the enzyme was determined using 2% casein as a substrate. Crude culture filtrate of about 1.0 ml was boiled in a water bath at 100°C for 20 minutes. Both live and killed samples were incubated at 37°C for 1 hour. After incubation, 1.0 ml cold TCA (Trichloraceticacid 10%) was added and centrifuged at 8000 rpm for 10 minutes, the supernatant was collected and mixed with 2.5 ml of reagent (sodium carbonate and sodium hydroxide) and then 0.7 ml of Folin-Phenol reagent was added and incubated at room temperature for 20 minutes. The samples were read at 660nm using Spectrophotometer.

3. RESULTS AND DISCUSSION

3.1. Determination of Protease Enzyme Production at Various Time Intervals

The 24 hours old culture was inoculated in production medium to determine the enzyme production. The maximum enzyme production was seen in 18 hours 339U/ml (**Fig 1**).

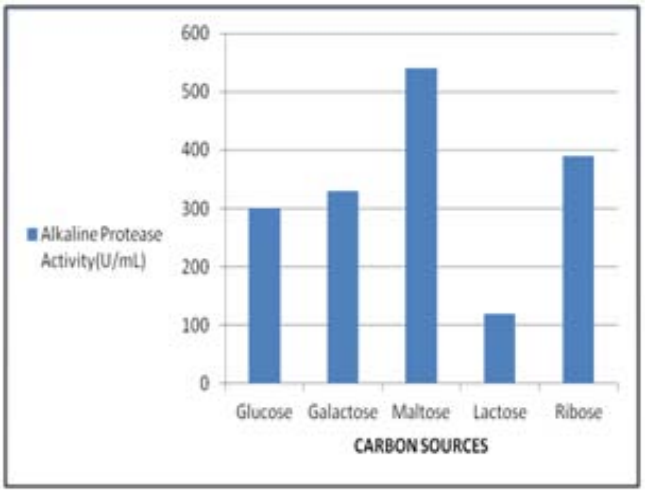
Fig 1: Different Time Intervals (Hrs)



3.2. Effect of Different Carbon Source on Protease Enzyme Production

Various carbon sources added to the production media and incubated for 18 hours. After incubation culture filtrate was collected. Production of enzyme for Glucose 300 U/ml, Galactose 330 U/ml, Maltose 540 U/ml, Lactose 120 U/ml and for Ribose 390 U/ml. The maximum production of protease was observed in Maltose (540 U/ml) (**Fig 2**).

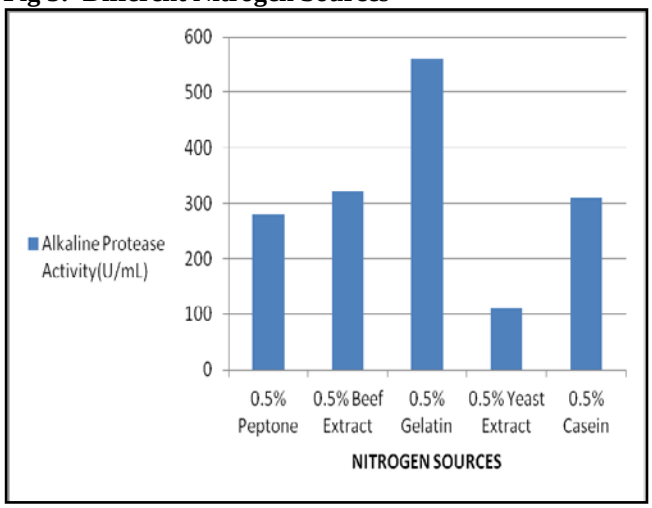
Fig 2: Different Carbon Sources



3.3. Effect of Different Nitrogen Source on Protease Enzyme Production

Various Nitrogen sources added to the production media and incubated for 18 hours. After incubation, the culture filtrate was collected. Enzyme and protein production in 0.5% Peptone 280 U/ml , 0.5% Beef extract 320 U/ml, 0.5% Gelatin 560 U/ml, 0.5% Yeast Extract 110 U/ml and for 0.5% Casein 310 U/ml. The maximum production of enzyme was seen in Gelatin 560 U/ml (**Fig 3**). The carbon and nitrogen sources are important for growth of all the microbes. Improvement in protease production by addition of gelatine is previously reported in case of *Bacillus* sp [3].

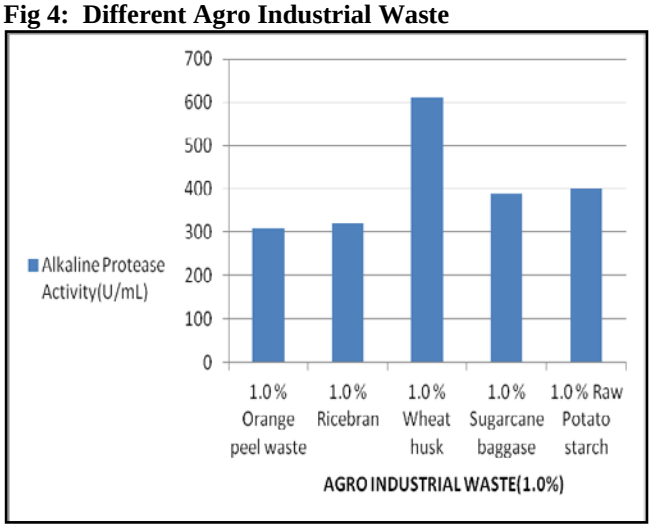
Fig 3: Different Nitrogen Sources



3.4. Effect of Various Agro Industrial Waste on Protease Enzyme Production

Various Agro industrial wastes were added to the production medium. After 18 hours of incubation, production of enzyme in orange peel 310 U/ml, Rice bran 320 U/ml, Wheat husk 610 U/ml, Sugarcane baggase 390 U/ml and for Raw potato starch 400 U/ml. The maximum production of enzyme was seen in wheat husk (610 U/ml) (**Fig 4**).

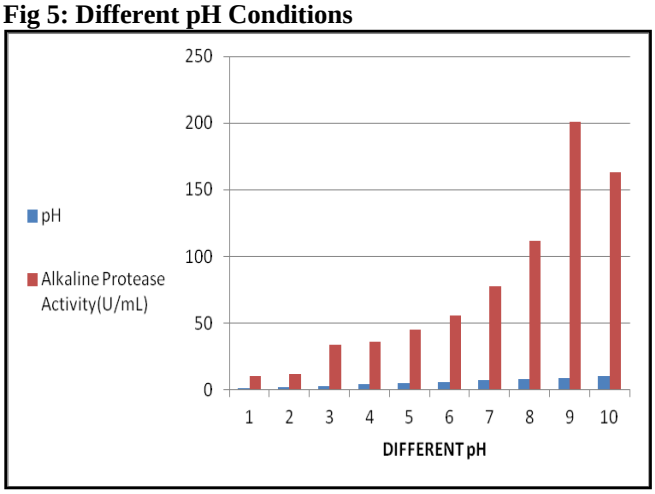
Fig 4: Different Agro Industrial Waste



3.5. Effect of pH on Protease Stability and Enzyme Activity

After incubation under assay condition the activity of enzyme was measured, from pH 1-5 less enzyme production was observed, and in pH 6 (56 U/ml), pH 7 (78 U/ml), pH 8 (112 U/ml), pH 9 (201 U/ml), pH 10 (163 U/ml). The maximum stability of enzyme was observed in pH 9 (201 U/ml) (**Fig 5**). Earlier reports are comparable to our report as greater protease production was reported by *Bacillus* sp. in the alkaline range of 7-12[19-21].

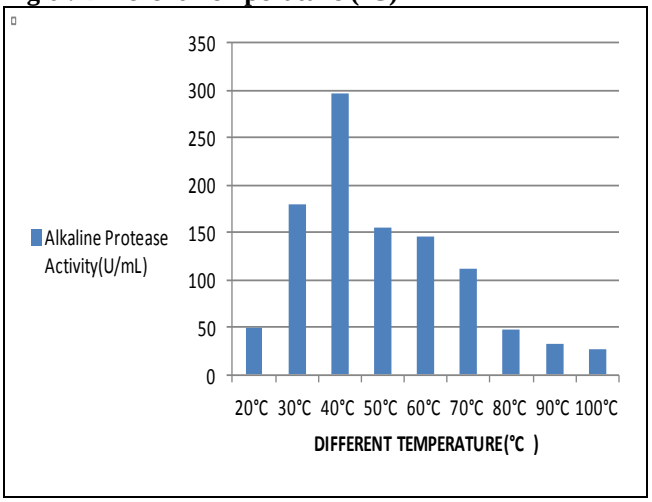
Fig 5: Different pH Conditions



3.6. Effect of Temperature on Protease Stability and Enzyme Activity

After incubating at different temperature, the enzyme activity was measured,20°C (50 U/ml), 30°C (180 U/ml), 40°C (296 U/ml), 50°C (155 U/ml), 60°C (146 U/ml), 70°C (112 U/ml), 80°C (47 U/ml), 90°C (32 U/ml) and for 100°C (27 U/ml). The maximum stability of enzyme was observed in temperature 40°C (296 U/ml) (**Fig 6**). An optimum temperature of 45°C which is very close to our report has been reported earlier [21].

Fig 6 : Different Temperature (°C)



4. CONCLUSION

Thus, it may be concluded that our *Bacillus* sp AGT isolate could be optimized for the maximum production of alkaline protease, at 40°C, pH 9.0

during 18 hours of incubation in our production medium containing maltose as carbon source and 0.5% gelatine as nitrogen source. Further study could reveal its possible exploitation for commercialization.

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