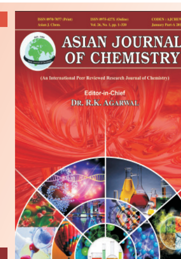
Asian Journal of Chemistry; Vol. 26, No. 7 (2014), 2182-2184

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.16394



Isolation and Production of Proteolytic Enzyme by Bacterial Strains by Using Agrowates as Substrate

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Received: 19 September 2013; *Accepted*: 21 February 2014; *Published online*: 22 March 2014; AJC-14987

Protease production was investigated using *Enterococcus hirae*, *Acinetobacter pittii* and *Pseudomonas aeruginosa* isolated from dairy effluent. Different agro wastes such as mustard oil cake, roasted bengal gram peel, yellow split bean peel, coconut oil cake, sesame oil cake, groundnut oil cake, black gram peels are evaluated to check the possibility of potential utilization as substrates for protease production. *Enterococcus hirae* is found to produce maximum amount of protease (300 µg/mL) in casein than *Acinetobacter pittii* and *Pseudomonas aeruginosa*. The results showed that under optimized conditions among the various oil cakes and peels, protease production is at its peak in black gram peel at 48 h in *Acinetobacter pittii* (310 µg/mL) *Pseudomonas aeruginosa* (510 µg/mL) and *Enterococcus hirae* (610 µg/mL) but predominant production by *Enterococcus hirae*. Among the tested protein substrates, black gram peel served as the most preferable substrate for protease production and *Enterococcus hirae* as potential bacterial strain for protease production.

Keywords: *Enterococcus hirae*, *Acinetobacter pittii*, *Pseudomonas aeruginosa*, Agrowastes, Protease.

INTRODUCTION

Protease or Proteolytic enzymes catalyze the cleavage of peptide bonds in proteins. Protease constitutes one of the most important groups of industrial enzymes accounting for more than 65 % of the total industrial market[1]. The vast diversity of protease in contrast to the specificity of their action has attracted worldwide attention focused on exploiting the physiological and biotechnological applications[2]. Proteases are also envisaged to have extensive applications in the development of environmentally friendly technologies, as well as in several bioremediation processes[3].

Proteases, one among the three largest group of industrial enzymes is widely used in several industries that include detergent, leather processing, meat processing, dairy, preparation of organic fertilizers, as digestive aid, silk industry and also for the recovery of silver from used X-Ray films[4].

Protease production by micro organism is greatly influenced by medium components especially carbon and nitrogen sources and other parameters and other parameters such as temperature, pH, agitation, incubation time and inoculum density[5]. Many processes have been developed for utilization of agro industrial residues as raw materials for the production of bulk protease, which provides alternative substrates and solve the pollution problems. The growth medium that is rich in proteins such as casein, gelatin, tryptone, peptone or skim milk are excellent for acting as inducers for protease production[6].

Organic residues from agriculture are exploited in the few decades. Crop residues are utilized as potential raw materials in bioprocess as excellent substrate for the growth of microorganism supplying the essential nutrients to them[7]. They offer advantages in bioremediation and biological detoxification of hazardous compounds and helpful in production of various value added commercial products like primary and secondary metabolites[8]. A venture is put forth to evaluate the potentiality of agrowastes such as mustard oil cake, roasted bengal gram peel, yellow split bean peel, coconut oil cake, sesame oil cake, groundnut oil cake and black gram peel to serve as substrate for protease production.

EXPERIMENTAL

Enterococcus hirae, *Acinetobacter pittii* and *Pseudomonas aeruginosa* isolated from the dairy effluent was shown to produce significant amount of alkaline protease in the culture medium under optimum conditions.

Preparation of substrates: Different agro wastes such as mustard oil cake, roasted bengal gram peel, yellow split bean peel, coconut oil cake, sesame oil cake, groundnut oil cake and black gram peel were obtained from local market. These materials were washed first with tap water followed by

distilled water. Then blenching operation was carried out by immersing them in hot water (75-80 °C) for 20 min followed by oven drying at 45 °C. Then the dried material was grinded in a mixer grinder and sterilized at 121 °C, 15 lbs pressure for 15 min and stored at 4 °C for further use[9].

Batch fermentation: Two grams of each agro waste taken in 250 mL of Erlenmeyer flask and sterilized at 121 °C 15 lbs for 15 min and cooled and inoculated with 1 mL of bacterial strain and incubated at 37 °C and readings were taken at regular interval of time

Protease assay: Protease assay was carried out for the potential protease producing bacterial strains to determine the protease activity using universal protease assay. 1 mL of bacterial sample was taken. Solution with 0.2 M glycine and 0.2 M sodium hydroxide was prepared and 5 mL of this casein solution with glycine -NaOH buffer solution was added and incubated for 10 min at 60 °C. 110 mM trichloroacetic acid (4 mL) was added. 5 mL of 500 mM sodium carbonate solution was added and the test tubes were vortexed in vortex shaker and incubated for 30 min at 37 °C in incubator. The samples were observed under UV spectrophotometer at 660 nm. The enzymatic activity of the bacterial strains was determined with the moles of tyrosine released. One unit of protease activity was defined as the amount of enzyme required to release 1 µg of tyrosine per mL per min under standard assay conditions. Similarly, the protease activity for the 3 bacterial strains was determined after 24, 48, 72 and 96 h of incubation[10].

The sequence data of bacterial strains have been submitted to the GenBank database under accession No. KC991293, KC991294 & KC991295.

RESULTS AND DISCUSSION

In order to identify the best producers of protease, different bacterial strains were examined. 2 gms of casein in 250 mL of Erlenmeyer flask and moistened with distilled water and inoculated with 1 mL of culture under optimum conditions and extracellular protease activity was measured for 24, 48, 76 and 92 h. From the graph, it is evident that *Enteroccocus hirae* dominates *Pseudomonas aeruginosa* and *Acinetobacter pittii* on protease production in casein medium (Fig. 1).

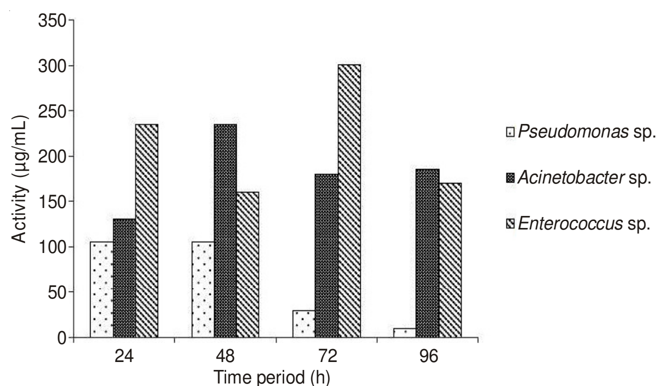

Fig. 1. Screening of isolated microorganism for the protease production in casein medium at different intervals of time

Selection of best substrate: In the present study, we observed that *Enterococcus hirae* to produce protease, which is found better than other two species. Various agro based substrates are used for protease production. The maximum protease production is observed at 48 h in roasted bengal gram peel by *Acinetobacter pittii* (495 µg/mL) (Fig. 2), coconut oil cake at 96 h by *Enterococcus hirae* (260 µg/mL) (Fig. 3). Yellow split bean peel at 48 h by *Enterococcus hirae* (245 µg/mL) (Fig. 4), sesame oil cake at 72 h by *Acinetobacter pittii* (390 µg/mL) (Fig. 5), groundnut oil cake at 96 h by *Pseudomonas aeruginosa*. (340 µg/mL) (Fig. 6). We infer that, among the various oil cakes and peels, protease production is at its peak in black gram peel at 48 h in all three microorganisms but predominant production by *Enterococcus hirae* (610 µg/mL).

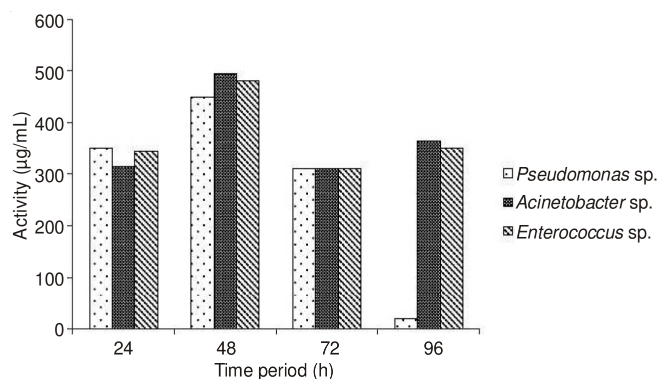

Fig. 2. Comparative analysis on microorganism for the protease production in roasted Bengal gram peel medium at different intervals of time

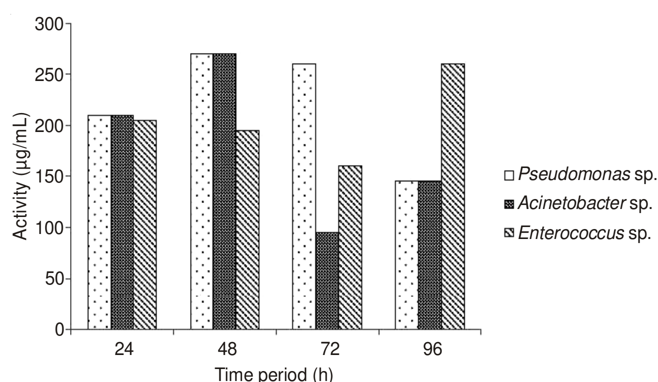

Fig. 3. Comparative analysis on microorganism for the protease production in coconut oil cake medium at different intervals of time

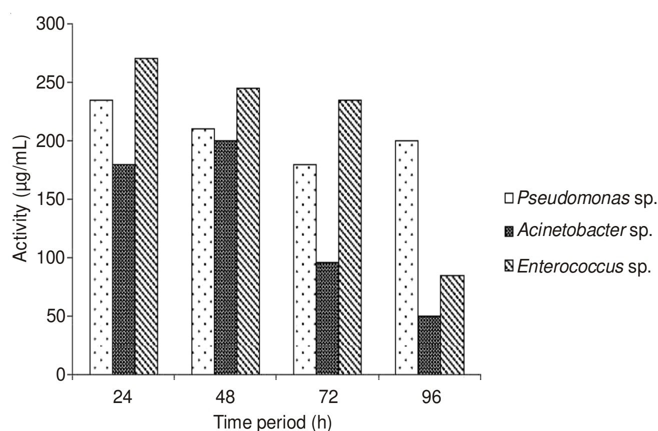

Fig. 4. Comparative studies on microorganism for the protease production in yellow split bean peel medium at different intervals of time

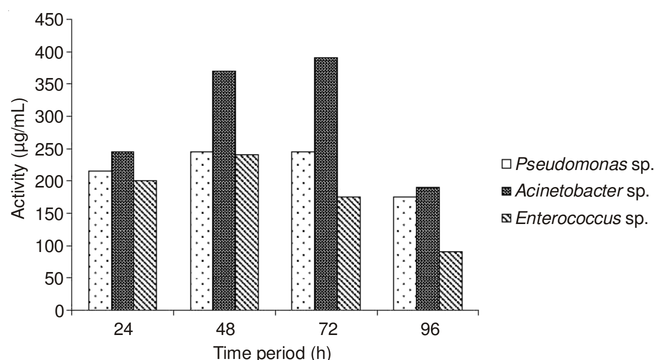

Fig. 5. Comparative analysis on microorganism for the protease production in sesame oil cake medium at different intervals of time

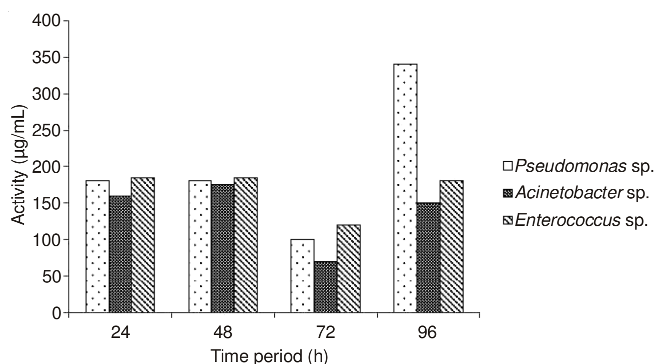

Fig. 6. Comparative analysis on microorganism for the protease production in groundnut oil cake medium at different intervals of time

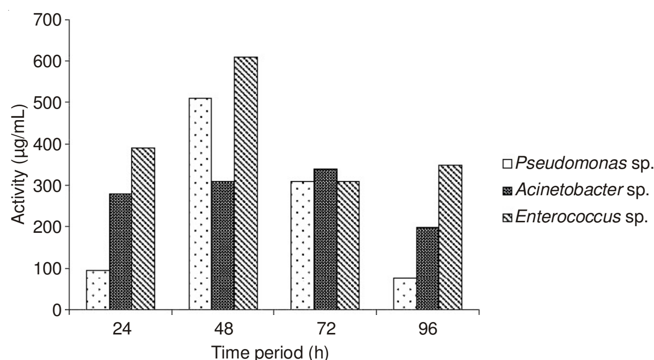

Fig. 7. Comparative analysis on microorganism for the protease production in black gram peel medium at different intervals of time

Conclusion

Our study shows that *Enterococcus hirae* intensively produces extracellular proteolytic enzymes of an industrial value. The enzyme production with these sources would be an economically attractive source. *Enterococcus hirae* produces high level of extracellular protease when black gram peel is used as substrate and influence over other two bacterial strains. Black gram peel as substrate shows higher production of protease than that of other agro wastes. These enzymes could be exploited commercially for their use in industrial detergent formulation, dehairing in tannery industry and for various purposes.

ACKNOWLEDGEMENTS

The authors grateful to Prof. Dr. R. Rangarajan, Chancellor, Vel Tech Dr. RR Dr. SR Technical University for his valuable support in completing this project.

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