International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.5, No.4, pp 1541-1544, April-June 2013

Production Of Lipase And Protease From Pseudomonas fragi NRRL-B727 And Optimization Studies Of The Protease Without The Lipase Presence.

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Abstract: Production of protease and lipase was carried out in *Pseudomonas fragi* NRRL-B727 with both the enzyme being secreted concomitantly. Media optimization studies were carried out in cheap source carbon sources to maximize the protease and lipase production. Also, the protease production without the lipase activity was also studied along with the enzyme characterization studies to find optimum pH and temperature.
Keywords: *Pseudomonas fragi* NRRL-B727, protease, lipase.

Introduction:

Enzymes are high valuable bio-products produced widely in various industries for a wide range of applications [1]. Most of the industrial enzyme productions employ micro-organisms which make the production and processing comparatively easier and economically feasible. Some of the industrially important enzymes are amylases, proteases, xylanases, cellulases, oxidases, isomerases, catalases etc [2].

Proteases and lipases are highly demanding enzymes which are used in numerous applications such as food, detergent, pharmaceuticals, leather, digestive aids, effluent treatment etc [3, 4, 5, 6, 7, 8].

Micro-organisms such *as Bacillus, Aspergillus, Pseudomonas, Mucor, Candida, Penicillum Serratia* etc. are known to produce protease and lipase which can be used for wide range of industrial application [9, 10, 11]. Since not many studies have been done, P. fragi was chosen for enzyme productions.

In this study concomitant production of lipase and protease was analyzed using cheap source media components. Individual production and optimization of protease without lipase was also carried out.

Materials and method:

Micro-organism:

The microorganism used in the study was *Pseudomonas fragi* NRRL – B727 obtained from Agricultural Research Service (ARS), USA. The cultures were grown on nutrient agar and incubated at 30°C for 72h.

Production media:

The media used for protease production were: (1) wheat Bran (10g/L), dextrose (2g/L) (MERCK), soya bean (3g/L), and peptone (2g/L) (HIMEDIA). (2) Rice bran (10g/L), yeast extract (2g/L) (HIMEDIA), dextrose (2g/L) (MERCK), di-sodium hydrogen phosphate anhydrous, extra pure (4g/L) (HIMEDIA).

Two different media were used for lipase production: (1) yeast extract (0.3%) (HIMEDIA), peptone (0.1%) (HIMEDIA), olive oil (1%), di-sodium hydrogen phosphate anhydrous, extra pure (0.07%) (HIMEDIA), di-sodium hydrogen phosphate anhydrous, extra pure (0.03%) (REACHEM), ammonium sulphate (0.025%), calcium chloride (0.01%) (RANKEM) and dextrose (2%) (MERCK). (2) yeast extract (0.3%) (HIMEDIA), peptone (0.1%) (HIMEDIA), di-sodium hydrogen phosphate anhydrous, extra pure (0.07%) (HIMEDIA), Tween 20 (1%), di-sodium hydrogen phosphate anhydrous, extra pure (0.03%) (REACHEM), ammonium sulphate (0.025%), calcium chloride (0.01%) (RANKEM) and dextrose (2%) (MERCK).

Enzyme extraction:

The 72h grown culture was centrifuged at 10,000 rpm for 15 min and the supernatant which contains the crude enzyme was stored at 4 ℃ for further studies [12].

Protease assay:

0.5mL of crude enzyme was added with 0.5mL casein (1% w/v), 0.5 mL calcium chloride and incubated at 40°C for 30 min. The reaction was stopped by addition of 2mL trichloro acetic acid and incubated at 30°C for 45 min. Then the reaction mixture was centrifuged at 10000 rpm for 5 -10 min. The supernatant was collected and absorbance was measured at 280 nm using UV spectrophotometer. Tyrosine was used as standard (0-100 µg/mL) [13].

Unit of enzyme activity is defined as µg of tyrosine released per mL in unit time.

Lipase assay:

Lipase activity was determined titrimetrically by olive oil hydrolysis method [14]. 0.5mL sodium chloride (1N), 0.5mL calcium chloride (50mM), 4mL phosphate buffer (maintained pH7) were added and mixed well using a magnetic stirrer and the pH was checked and maintained at pH 7. Then 5mL olive oil was added and an emulsion was prepared by continuous stirring followed by addition of 1mL crude enzyme solution. After 15 min pH was checked and adjusted to pH 7 by addition of 50 mM sodium hydroxide. The volume of sodium hydroxide added was noted.

Enzyme activity= [(NaOH*1000*Amount of NaOH used)/ (Amount of enzyme used)].

Unit of enzyme activity is defined as 1 micro equivalent of fatty acid hydrolyzed from a triglyceride in one hour at pH7 at 30° C.

Effect of pH and temperature on protease activity:

The effect of the different pH and temperature was studied for protease with pH values varying from 4 to 8 and temperature 20 to 70°C. Acetate buffer for pH 4, 5; Citrate buffer for pH 6; Phosphate buffer for pH 7; Tris Hcl buffer for pH 8 were used.

Result:

The maximum protease activity value from *Pseudomonas fragi* NRRL-B727 was 626.32 µg/mL/min at pH 8, temperature 50°C and maximum lipase activity was 2486 Units.

Discussions:

The lipase production was carried out at pH7 and temp 30°C for which 2486 Units was obtained. But the level of lipase was less compared to other lipase producing micro-organisms. For both the media, the enzyme activity was not obtained. Mencher *et al* had discussed that they had obtained an enzyme activity of 2890 units for this production [15]. Lu and Liska had reported a maximum activity of 1.545 units at temperature 54°C and pH between 7.5 and 8.9 [16]. Reports regarding rice bran and wheat bran media for lipase production were not available.

The protease activity of 276.83 µg/mL/min was obtained for pseudomonas fragi grown in nutrient broth. Sterling *et al* had reported an enzyme activity of 0.59U/mL for protease grown in commercial media [17]. Jayathi and Rintu had obtained an activity of 3.81 U/mg from Pseudomonas species in commercial media [18]. As higher amount of proteases were obtained, further production in cheap source media was carried out.

In wheat bran based media 626.32 µg/mL/min was obtained at pH8 and temperature 50°C (Fig 1) whereas in rice bran containing media the highest enzyme activity of 509.57 µg/mL/min at pH5, temperature 50°C (Fig 2). Kalaiarasi and Sunitha had mentioned for Pseudomonas fluorescens, an enzyme activity of 0.389 U/mL when wheat bran was used where as enzyme activity less than 0.05 U/mL was obtained for rice bran carbon source [19].

The proteases had higher activity at temperature 50°C, pH8 (for media 1) whereas lowest activity was measured at temperature 70°C, pH6 (for media 2) (Table 1). Koka and Weimer had reported the dependence of protease activity from *P.fluorescens* at various pH and temperature where pH5 and temperature 35°C had maximum enzyme activity [20].

Table 1: Optimum values of Enzyme activity parameters for Protease production:

Conditions	Optimum value
Media (1) [Wheat bran]	
(i) pH	8
(ii) Temperature	50 °C

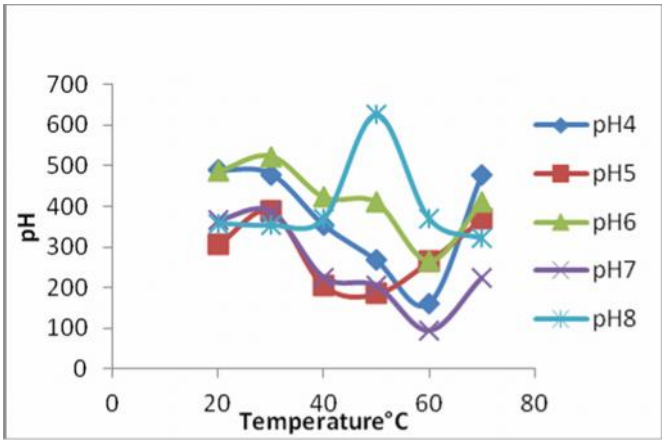

Fig 1: Enzyme activity for media (1) at various pH and temperatures

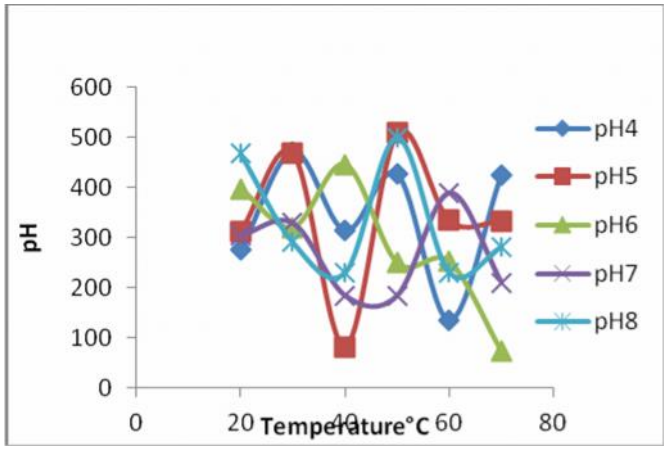

Fig 2: Enzyme activity for media (2) at various pH and temperatures

Conclusions:

Pseudomonas fragi NRRL-B727 had proved to be a promising organism for concomitant production of protease and lipase and can be used for industrial scale productions using cheap source media components for economical enzyme production and wide range of application.

Acknowledgements:

We sincerely thank our company M/s. SVBIOTECH and Periyar TBI, Thanjavur for great co-operation and technical support.

References:

1. Grisham, Charles M., Reginald H., Garrett, Biochemistry, Philadelphia: Saunders College Pub., 1999, 426-427.
2. Padmapriya V. and Anand N., Evaluation of some industrially important enzymes in filamentous cyanobacteria, ARPN Journal of Agricultural and Biological Science, 2010, 5 (5), 86-97.
3. Mala B. Rao, Aparna M. Tanksale, Mohini S. Ghatge and Vasanti V. Deshpande, Molecular and biotechnological aspects of microbial proteases, Microbiology and Molecular Biology Reviews, 1998, 62 (3), 597 -635.
4. Ganesh Kumar C., Hiroshi Takagi, Microbial Alkaline proteases: From a bioindustrial view point, Biotechnology Advances, 1999, 17, 561-594.
5. Sumantha A., Larroche C. and Pandey A., Microbiology and Industrial biotechnology of food-grade proteases: A perspective, Food Technology and Biotechnology, 2006, 44 (2), 211-220.
6. Houde A., Kademi A., Leblanc D., Lipases and their industrial applications: An overview, Applied Biochemistry and Biotechnology, 2004, 118 (1-3), 155-170.
7. Aravindan R., Anbumathi P. and Viruthagiri P., Lipase applications in food industry, Indian Journal of Biotechnology, 2007, 6, 141-158.
8. Hasan F., Ali Shah A. and Hameed A., Industrial applications of microbial lipases, Enzyme and Microbial Technology, 2006, 39 (2), 235-251.
9. Mahanta N., Gupta A. and Khare SK., Production of protease and lipase by solvent tolerant Pseudomonas aeruginosa PseA in solid state fermentation using Jatropa curcas seed cake as substrate, Bioresource Technology, 2008, 99 (6), 1729-1735.
10. Sangeetha R., Geetha A. and Arulpandi I., Concomitant production of protease and lipase by Bacillus licheniformis VSG1: Production, purification and characterization, Brazilian Journal of Microbilogy, 2010, 41, 179-185.
11. Henriette C., Zinebi S., Aumaitre MF., Petitdemange E. and Petitdemange H., Protease and lipase production by a strain of Serratia marcescens, Journal of Industrial microbiology, 1993, 12, 129-135.
12. Mehta VJ., Thumar JT. And Singh SP., Production of alkaline protease from an alkaliphilic actinomycete, Bioresource Technology, 2006, 97, 1650-1654.
13. Yang SS. And Huang C., Proteases production by amylolytic fungi in solid state fermentation, Journal of Chinese Agriculture and Chemical Society, 1999, 32 (6), 589-601.
14. Borkar PS., Bodade RG., Rao SR., Khobragade CN., Purification and characterization of extracellular lipase from a new strain-Pseudomonas aeruginosa SRT9, Brazilian Journal of Microbiology, 2009, 40, 358-366.
15. Mencher JR. and Alford JA., Purification and characterization of the lipase of Pseudomonas fragi, Journal of General Microbiology, 1967, 48, 317-328.
16. Lu JY. And Liska BJ., Lipase fron Pseudomonas fragi, Applied Microbiology, 1969, 18 (1), 108-113.
17. Sterling ST., Naidu YM. and James JP., Ultrastructural localization of an extracellular protease in Pseudomonas fragi by using Peroxidase-Antiperoxidase reaction, Applied and Environmental Microbiology, 1985, 50 (4), 1038-1042.
18. Jayanti RD. and Rintu B., Isolation and characterization of a newly isolated Pseudomonas mutant for protease production, Brazilian Archives of Biology and Technology, 2006, 49 (1), 37-47.
19. Kalaiarasi K. and Sunitha PU., Optimization of alkaline protease production from Pseudomonas fluorescens isolated from meat waste contaminated soil, African Journal of Biotechnology, 2009, 8 (24), 7035-7041.
20. Koka R. and Weimer BC., Isolation and characterization of a protease from Pseudomonas fluorescens RO98, Journal of Applied Microbiology, 2000, 89, 280-288.

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