International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.5, No.1, pp 550-553, Jan-Mar 2013

Production Of Alkaline Protease From Bacillus licheniformis (NCIM 2044) And Media Optimisation For Enhanced Enzyme Production

Sathyavrathan P.*, Kavitha M.

Research and Development – Biotechnology Division, SVBiotech, Periyar TBI, Periyar Maniammai University, Vallam, Thanjavur – 613403;India.

Corres.author: p.sathyavrathan@gmail.com,
Mobile no: +91-9944282608

Abstract: Production of alkaline protease from *Bacillus licheniformis* (NCIM 2044) was studied and characterized. Of different cheap source media chosen, maximum production of 172.425 U/ml was obtained for 1% wheat bran containing media. The enzyme assay at various optimum pH and temperatures were also determined.
Keywords: Protease, *Bacillus licheniformis*, cheap source media, NCIM 2044.

INTRODUCTION:

Proteases (E.C.3.4.21.14) are enzymes that break peptide bonds between amino acids of proteins. They are also called proteolytic enzymes [1]. It is very important in digestion as they breakdown the complex proteins to simpler amino acids.

Alkaline proteases are produced by a wide range of micro organisms including bacteria, moulds and yeasts, actinomycetes etc [2]. Proteases are of six groups, serine proteases, threonine proteases, cysteine proteases, aspartate proteases, and metalloproteases [3]. In bacteria, protease enzyme is produced mainly *Bacillus licheniformis, B. horikoshii, B. sphaericus, B. furmis, B. alcalophilus, B. subtilis* [4].

Proteases have a variety of functions and have many important biotechnological applications [5]. It is one of the three largest groups of industrial enzymes and finds application in detergents (to remove the proteins from cloths soiled with blood, milk, sweat, grass etc.) [6, 7]; In silk industry proteases has been used in the process of silk degumming with synthetic detergents which implies the partial non ionic surfactants [8, 9]; In leather industry (for bating, un-hairing, degreasing and soaking which makes clean and green leather tanneries) enzyme based leather dehairing has been considered an environmentally friendly alternative to the conventional chemical process [10, 11]; In food industry, to remove unwanted bitterness in ripening cheese is an example of the proteases in flavor production in foodstuffs [12, 13]. Proteases are also used to recover protein parts of animals and fish which would otherwise go to waste after butchering. Proteases are also used in the treatment of fish skins for industrial uses [14]. In pharmaceutical industry it is used for preventing excessive blood clotting and reducing clotting the tendency for platelets and red blood cells. Proteases are also used in drug manufacturing where the enzyme can be used as an aid for digestion by the pancreas. Also, in bio drugs, proteases help in preventing or treating diseases that escape traditional drug action, such as cancer or other widespread multifactorial diseases [15]. Another major application is in waste water

treatment where the alkaline proteases can solubilize proteins in wastes through a multistep process to recover liquid concentrates which are of high nutritional value for fish and livestock [16].

In this paper we have investigated the alkaline protease production levels in *Bacillus licheniformis* NCIM 2044 in commercial and various cheap source media. The enzyme assay at various pH and temperatures was carried out and the optimum enzyme conditions were determined.

MATERIALS AND METHODS:

1. **Micro Organism:**
   The bacterial strain, *Bacillus licheniformis* NCIM 2044 obtained from National Collection of Industrial Micro-organisms (NCIM), was used in the study.

2. **Chemicals And Reagents:**
   Beef extract powder (HIMEDIA), Casein (HIMEDIA), Trichloro acetic acid (MERCK), protease peptone-A (HIMEDIA), Yeast Extract(HIMEDIA), Di-Sodium hydrogen phosphate (HIMEDIA), Glycine (QUALIGENS), Citric acid (QUALIGENS) of analytical grade were used.
3. **Shake Flask Study:**
   SF_1 (shake flask): Inoculation of a loopful of inoculum from the maintained *Bacillus licheniformis* stock to 3mL Nutrient broth, incubated at 37 °C, 150rpm for 24h.
   SF_2: To 30mL Nutrient media, 10%inoculum was aseptically transferred and maintained at 37 ºC, 150 rpm for 24h.
   SF_3: For a 300mL production media, 10%inoculum from SF2 was aseptically transferred and maintained at 37 ºC, 150 rpm for 24h.
4. **Media Optimization:**
   The inoculum and production medium were defined as follows: Media(1): Wheat Bran- 1%, dextrose-0.5%, soyabean-0.5%, peptone-0.2%; Media(2): Ricebran-0.15%, dextrose-0.5%, di-sodium hydrogen phosphate-0.5%, yeast extract-0.2%; Media (3): Dextrose 0.6g%, di-sodium hydrogen phosphate 0.5%, peptone 0.2%, yeast extract 0.2%. Media (4): Wheat Bran-1%, dextrose-0.2%, soybean meal-0.3%, peptone-0.2%; Media (5): Ricebran-1%, Dextrose-0.2%, di-sodium hydrogen phosphate-0.4%, yeast extract-0.2%.
5. **Enzyme Extraction:**
   24 h grown bacterial cells were centrifuged at 10000 rpm for 15min and the supernatant was filtered and used for enzyme activity assays [17].
6. **Enzyme Assay:**
   Protease activity was determined using a modification of Yang and Huang method [18]. The reaction mixture containing 1.5mL casein in 0.1M carbonate-Bicarbonate buffer (pH 10.5), 0.5mL 5mM calcium chloride, 0.5mL of enzyme solution was incubated for 30 min at 40 ºC. After incubation 2mL of trichloroacetic acid solution was added, incubated at 30 ºC for 45minutes. Then the reaction mixture was centrifuged at 3000rpm for 10 minutes and the supernatant was collected. The absorbance was measured at 280nm.
   Tyrosine was used as a standard (0-100 µg/mL).
   1 Unit of enzyme activity is equal to 1µg of tyrosine released per minute at the assay conditions.

RESULTS AND DISCUSSION:

Maximum protease activity of 141.75 U/ml was obtained for media (4) at temperature of 40 ºC and pH 10.5.

Protease enzyme experimental data obtained production in 5 different media highest enzyme activity of 141.75 U/ml (Table-1) was obtained.

Table-1: Enzyme activity obtained with various cheap source media

Media	Enzyme activity (U/ml)
1	117.675
2	97.8
3	110.25
4	141.75
5	83.25

Srividya *et al* had reported an enzyme activity of 50 U/ml for wheat bran substrate [19]. For rice bran, by *R. microsporus* NRRL 3671, 129 U/gds had been obtained [20] whereas for dextrose 15 U/ml was obtained [21]. Various other substrates like groundnut cake [22], starch [23], corn [24], etc. were also used and the highest protease activity reported was 615 U/ml for groundnut cake media.

The effect of pH and temperature also influences on the enzyme activity [25]. In our experiment, maximum protease activity of 172.425 U/ml was obtained at pH 9 and temperature 60 ºC (Figure-1).

Chaudhuri *et* al had also obtained a maximum protease activity at pH 9 [25, 26]. At lower pH, the enzyme activity was less since alkaline proteases are highly stable only at higher pH ranges. Masumeh Anvari *et* al had reported that at pH 10 and 11, 70 – 80 % of enzyme activity was retained [27].

The influences of temperatures were also reported to alter the enzyme activity values. Most alkaline proteases had temperature optima at 60 ºC. At lower temperatures (below 50 ºC), the enzyme activity was also low [28, 29].

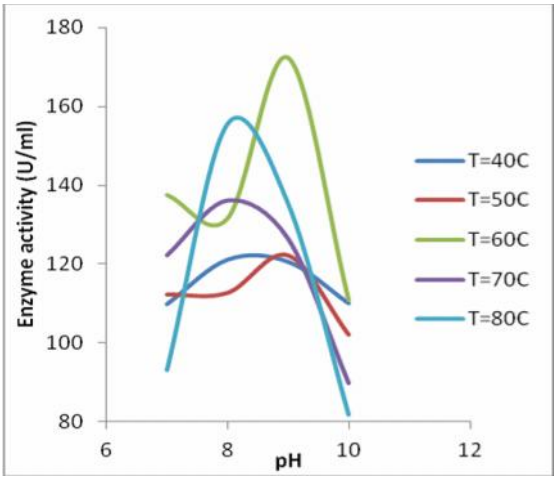

Figure-1: Enzyme activity at various Temperature and pH

CONCLUSION:

Thus the protease produced from *Bacillus licheniformis* (NCIM 2044) was found to be promising for higher enzyme yields. The enzyme had wide adaptability and applicability for various industrial applications. The enzyme production studies has to be studied on large scale levels and further purification steps have to be chosen according to the application requirements.

ACKNOWLEDGEMENTS:

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

REFERENCES:

1. Swapna Vadlamani, Sreenivasa Rao Parcha, Studies on industrially important alkaline protease production from locally isolated superior microbial strain from soil microorganisms, International Journal of Biotechnology Applications, 2011, 3 (3), 102-105.
2. Nurullah Akcan and Fikret Uyar, Production of extracellular alkaline protease from Bacillus subtilis RSKK96 with solid state fermentation, Eurasia Journal of Biosciences, 2011, 5, 64-72.
3. 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.
4. Adinarayana K, Ellaiah P. Investigations on alkaline protease production with B subtilis PE-11 immobilised in calcium alginate gel beads. Process Biochemistry, 2004, 39, 1331-1339.
5. 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.
6. Kumar D., Savitri, Thakur N., Verma R. and Bhalla T. C., Microbial Proteases and Application as Laundry Detergent Additive. Research Journal of Microbiology, 2008, 3, 661-672.
7. Masse F. W. J. L., Van Tilburg R., The benefit of detergent enzymes under changing washing conditions, Jornal of American Oil Chemical Society, 1983, 60, 1672–75.
8. Singh L., Devi Y. and Devi S., Enzymological characterization of pineapple extract for potential application in oak tasar (Antheraea proylei J.) silk cocoon cooking and reeling, Electronic Journal of Biotechnology, 2003, 615, 12.
9. Rinsey Johnny V.A. and Karpagam Chinnammal S., Degumming Of Silk Using Protease Enzyme from Bacillus Species, International Journal of Science and Nature, 2012, 3 (1), 51-59.
10. Arunachalam C. and Saritha K., Protease enzyme: an eco-friendly alternative for leather industry, Indian Journal of Science and Technology, 2009, 2 (12), 29-32.
11. Dayanandana A., Kanagarajb J., Lesley Sounderraja, Govindarajua R., Suseela Rajkumar G., Application of an alkaline protease in leather processing: an ecofriendly approach, Journal of Cleaner Production, 2003, 11 (5), 533–536.

12. Gupta R., Beg Q. K., Lorenz P., Bacterial alkaline proteases: molecular approaches and industrial applications, Applied Microbiology and Biotechnology, 2002, 59 (1), 15-32.
13. Takagi H., Kondou M., Hisatsuka T., Nakamori S., Tsai Y. C., Yamasaki M., Effects of an alkaline elastase from an alkalophilic Bacillus strain on the tenderization of beef meat, Jouranl of Agriculture and Food Chemistry, 1992, 40, 2364–68.
14. Ganesh Kumar C. and Hiroshi Takagi, Microbial alkaline proteases: From a bioindustrial viewpoint, Biotechnology Advances, 1999, 17, 561–594.
15. Barthomeuf C., Pourrat H., Pourrat A., Collagenolytic activity of a new semi-alkaline protease from Aspergillus niger, Journal of Fermentation and Bioengineering, 1992, 73, 233–36.
16. Shoemaker S., The use of enzymes for waste management in the food industry, Biotechnology in Food Processing, Noyes Publications (Park Ridge, NJ), 1986, 259–67.
17. Mehta V. J., Thumar J. T. and Singh S. P., Production of alkaline protease from an alkaliphilic actinomycete, Bioresource Technology, 2006, 97, 1650-1654.
18. Yang S. S. and Huang C., Proteases production by amylolytic fungi in solid state fermentation, Journal of Chinese Agriculture and Chemical Society, 1999, 32 (6), 589-601.
19. SRIVIDYA Y. S., MALA M., Influence of process parameters on the production of detergent compatible alkaline protease by a newly isolated Bacillus sp., Turkish Journal of Biology, 2011, 35, 177-182.
20. Sumantha, Alagarsamy, Rice bran as a substrate for proteolytic enzyme production, Brazilian archives of Biology and Technology, 2006, 49 (5), 843-851.
21. Fikret Uyar, Ilknur Porsuk, Göksel Kizil, Ebru Ince Yilmaz, Optimal conditions for production of extracellular protease from newly isolated Bacillus cereus strain CA15, EurAsian Journal of BioSciences, 2011, 5, 1-9.
22. Praveen Kumar, 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 and Technology, 2008, 1 (4).
23. Abbas Akhavan Sepahy and Leila Jabalameli, Effect of Culture Conditions on the Production of an Extracellular Protease by Bacillus sp. Isolated from Soil Sample of Lavizan Jungle Park, Enzyme Research, 2011; 2011: 219628.
24. Nadeem M., Effect of Medium Composition on Commercially Important Alkaline Protease Production by Bacillus licheniformis N-2, Food Technology and Biotechnology, 2008, 46 (4), 388–394.
25. Biswanath Bhunia, Debjani Dutta, Surabhi Chaudhuri, Extracellular alkaline protease from Bacillus licheniformis NCIM-2042: Improving enzyme activity assay and characterization, Engineering in Life Sciences, 2011, 11 (2), 207–215.
26. Nikerel I. E., Ateş Ö. and Öner E. T., Effect of bioprocess conditions on growth and alkaline protease production by halotolerant Bacillus licheniformis BA17, Applied Biochemistry and Microbiology, 2008, 44 (5), 487-492.
27. Masumeh Anvari and Gholam Khayati, Production and Characterization of Alkaline Protease from Bacillus licheniformis sp. Isolated from Iranian Northern Soils with Ram Horn Hydrolysate, Trends in Applied Sciences Research, 2011, 6, 1206-1213.
28. Kumar C.G., Purification and characterization of a thermostable alkaline protease from alkalophilic Bacillus pumilus, Letters Applied Microbiology, 2002, 34, 13-17.
29. Jyothi Bezawada S. Yan Rojan P. John R. D. Tyagi and R. Y. Surampalli, Recovery of Bacillus licheniformis Alkaline Protease from Supernatant of Fermented Wastewater Sludge Using Ultrafiltration and Its Characterization, Biotechnology Research International, 2011, Article ID 238549, 2011.

*****