ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

MICROBIAL PRODUCTION OF ALKALINE PROTEASES USING AGRICULTURAL BY-PRODUCTS

Shubhangi Dixit and Vinod Kumar Nigam*

Department of Bio-Engineering, Birla Institute of Technology, Mesra, Ranchi – 835215 (Jharkhand), India

Manuscript Info

Manuscript History:

Received: 12 April 2014
Final Accepted: 23 April 2014
Published Online: May 2014

Key words:
Agro-wastes, Alkaline proteases, Biomass, Environment pollution, Lignocelluloses

***Corresponding Author**

Vinod Kumar Nigam

Abstract

In recent years, the environmental friendly approaches have attracted lots of attention towards the production of fine chemicals, therefore the use of agricultural waste for production of various enzymes have increased tremendously. Various industries focused on use of agro-residues as sole source of carbon and nitrogen in fermentation medium for large scale production. These residues are easily available in large amount with low cost and minimizing the capital investment in terms of medium constituents. But, uses of agro-residues are having some limitations such as biomass transport, proper handling and efficient pretreatment methods for total delignification of lignocelluloses. The utilization of agro-wastes for production of commercially important compounds does not disturb food-chain as it is based on use of waste to energy concept and decrease dependence on non-renewable resources. Various enzymes including alkaline proteases are being produced from agricultural wastes by solid state and submerged fermentation processes. In the present review, the production of alkaline proteases from various agricultural by-products is highlighted along with the types of microorganisms involved and modes of production process.

INTRODUCTION

The agro-residues with high amount of organic matter consists of 50-60% of total solid wastes which are being used as an alternative source for production of important compounds as these are valuable raw materials; rich in sources like energy and other nutrients (lignocelluloses, proteins, carbohydrates, lipids etc.) which would be lost if they are disposed in the open dumps and landfills. In recent years, due to rapid industrialization there has been trend to use novel technologies, on biological processes for reuse and recycling of agro-wastes. The increased accumulation of biomass increases the rate of deterioration of the environment and if not properly utilized there would be loss of nutrients present in the agro-wastes that can be converted into value added products (Okonko et al. 2009). Bioconversion of agro-residues is associated with certain advantages such as increasing the nutritive values and also contributing to environmental safety (Alofe et al. 1996; Bisaria et al. 1997). The production of bulk chemicals and several value added fine products such as ethanol, single cell protein (SPC), organic acids, amino acids and biologically active secondary metabolites are studied and optimized by reuse of agro-residues by appropriate process technology (Pandey and Soccol 1998).

The release of monomers from the polysaccharides and other polymers are being evaluated by hydrolyzing the agro-wastes in presence of chemicals as well as by different enzymes from microbial sources with certain advantages of enzymatic hydrolysis. Agro-wastes are used as a substrate for production of several enzymes either by solid state or submerged mode of fermentation processes with reduced production cost. Among different agro-residues, wheat bran and rice bran are being used to maximum extent because of presence of considerable amount of carbohydrates which stimulate the cells to express many hydrolytic enzymes (Acebal et al. 1986).

Proteases are commercially important enzymes and find various applications in leather processing, meat processing, dairy, silk industry, detergent, food, pharmaceuticals, paper and pulp, and also in recovery of silver from used X-ray films (Chellappan et al. 2006; Makhija et al. 2006; Singhal et al. 2012). Among all proteases, alkaline proteases are robust in nature and mainly used in detergent additives at very high pH. Alkaline proteases contribute about 40% of total worldwide enzymes sales and it is assumed that this trend has to increase in future. The production cost of alkaline protease enzyme is high because of use of high cost substrates in the production medium. To meet the demand of alkaline proteases in the upcoming decade, industries are in search of cheaper methods of enzyme production processes that could decrease the production cost with increase in the productivity and yield of enzyme (Mukherjee et al. 2008).

Role of agricultural residues/ biomass for enzyme production:
Every year large amount of biomass accumulated in nature which creates environmental problems and if not utilized properly the loss of valuable products occurs (Sarath et al. 2008). Agro-residues are rich source of lignocellulosic materials and it consists of cellulose and hemicelluloses which converted into commercially important bio-compounds such as organic acids, biofuel, and enzymes etc. (Koutinas et al. 2007). Various agricultural by-products such as agave tequilana waste, rice bran, shrimp shell waste, sugarcane bagasse, wheat bran, wheat straw etc. are used in production of several enzymes such as carboxymethyl cellulose, cellulose, inulinase, lipase, protease and so on (Table 1). The utilization of agro-wastes as substrates has several advantages as these are inexpensive, non-toxic in nature, easily available and non-renewable resource. The use of agro-residues in production of valuable products is more advantageous than conventional process. The process of biological treatment of agricultural residues is also more effective and economic to larger extent in waste management.

Microbial sources and role of various factors influencing production of alkaline proteases:
Commercial productions of proteases are carried out by several microbes (bacteria, fungi, yeast etc.) as animal and plant sources are unable to meet the current demand of proteases. Proteases production from microbial sources contributed 40% of total worldwide sources. Table 2 illustrates types of microbial sources, modes of protease production and the respective substrates for maximum production of proteases. In microbes, *Bacillus* sp. is widely used for protease production due to its major application in detergent and leather industries. Proteases production is carried out either by solid state fermentation (SSF) or by submerged fermentation (SmF). The major factor in production process is choice of substrate. The important consideration is that the substrate should be inexpensive and inducing in nature by which the production of enzyme could increase and subsequently the cost could decrease. Solid state fermentation are preferred over submerged fermentation as SSF provides several advantages such as products are highly concentrated after the process, use of inexpensive substrates, use semi-sterilized conditions, simple down streaming processing and reduced pollution etc. Effect of particle size of agro-residues and moisture contents (humidity) play major roles in production of alkaline proteases. Maximum production of enzyme was noted with small particle sizes as it increases the surface area of substrates. The moisture content in case of SSF of protease production should be in the range of 30-80% (optimum), above which the yield of enzyme decreased due to mass transfer limitation of nutrients and oxygen. In one of the study, up to 1.5 times increase in activity was observed by increasing the humidity from 50% to 75% (Foda et al. 2013).

Conclusion:
Agricultural residues are produced in large quantity every year and hence it is the need of hours to find some alternative for recycling of the biomass into the valuable compounds by utilizing the nutrients and energy present in the agro-residues. Certain microbes produce valuable bio-molecules from these agricultural by-products in high yield as environmental safe and cost efficient process. Agricultural by-products are mostly used as solid supports which provide carbon, nitrogen and mineral sources and their use as a nutrient source avoid expensive constituents present in the fermentation medium. Though several studies have been carried out for the production of proteases using these agricultural wastes but a large scale unit has not been operational. Also, the microbial diversity from extreme environment with proteolytic properties has to be optimized for higher yield using wheat bran, rice bran, bagasse and other agro-residues. Certain other properties of alkaline proteases from biomass like applicability in leather and detergent industries and stability at various operating conditions need to be evaluated so that a complete process protocol could be developed for large scale production

.

Acknowledgement:

Authors wish to acknowledge the financial assistance received from University Grants Commission, New Delhi, India, [(Ref. No.: 41-504/2012(SR)] for carrying out this work and Centre of Excellence (COE), Department of Bio-Engineering under TEQIP-Phase II (Ref No NPIU/TEQIP II/ FIN/31/158; dated 16th April 2013) for instruments and infrastructure support.

Table 1: Production of various enzymes using different agro-residues.

S. No.	Microorganisms	Agro-residues	Production Process	Activity	Enzymes	References
1.	*Bacillus* sp. JB-99	Rice bran	Solid State fermentation	3644 U/g DBB	Xylanase	Virupakshi et al. (2005)
2.	*Aspergillus* CH-A-2010	Agave tequilana waste	Submerged fermentation	1.48 U/ ml	Inulinase	Huitron et al. (2008)
3.	*Aspergillus niger* CH-A-2016	Agave tequilana waste	Submerged fermentation	1.52 U/ ml	Xylanase	Huitron et al. (2008)
4.	*Trichoderma harzianum*	Wheat straw	Solid State fermentation	480±4.22 µM/ml /min	Carboxy methyl Cellulase	Iqbal et al. (2010)
5.	*Aspergillus oryzae*	Coffee by products	Solid State fermentation	12236 U /gds	Protease	Murthy and Naidu (2010)
6.	*Bacillus subtilis* 2724 NCIM	Convalia ensiformis beans	Solid State fermentation	680 µg/ ml	Alkaline Protease	Srinivas et al. (2010)
7.	*Volvariella volvacea*	Saw dust	Submerged fermentation	122 µmol /min/ml	Cellulase	Akinyele et al. (2011)
8.	*Aspergillus oryzae*	Suagarcane bagasse and Wheat bran	Solid State fermentation	330 µg/ ml/min	Gluco-amylase	Parbat and Singhal (2011)
9.	*Pseudomonas aeruginosa* A2	Shrimp shell waste	Submerged fermentation	1230 U/ ml	Alkaline Protease	Ghorbel-Bellaaj et al. (2012)
10.	*Bacillus cereus*	Ground nut shell	Solid State fermentation	76.75 U/ Gds	Alkaline Protease	Rathakrishnan and Nagarajan (2012)
11.	*Bacillus stearothermophilus* MTCC 37	Wheat bran	Submerged fermentation	22.04 U/ ml	Lipase	Sabat et al. (2012)
12.	*Candida rugosa* NCIM 3462	Sesame oil cake	Solid State fermentation	22.40 U/ g	Lipase	Rajendra and Thanyavelu (2013)

Table 2: Microorganisms and modes of proteases production.

S. No.	Microorganisms	Modes of production	Substrate	References
Bacterial sources				
1.	*Bacillus* sp. strain GX6638	Submerged fermentation	Casein, sAAPFpNA, CBZ-Ala-p-nitrophenylester, CBZ-Gly-p-nitrophenylester	Durham et al. (1987)
2.	*Conidiobolus coronatus*	Submerged fermentation	Casein	Phadatare et al. (1993)
3.	*Streptomyces rimosus*	Submerged fermentation and Solid State fermentation	Sweet potato residues, Peanut meal residue	Yang and Wang (1999)
4.	*Aspergillus* sp.	Solid State fermentation	Coconut oil cake and Wheat bran	Sumantha et al. (2005)
5.	*Penicillum fellutanum*	Submerged fermentation	Casein	Manivannan and Katiresan (2007)
6.	*Bacillus firmus* 7728	Submerged fermentation	Casein	Rao and Narasu (2007)
7.	*Bacillus circulans*	Submerged fermentation	Wheat bran, Rice bran, Cotton deoiled meal	Jaswal et al. (2008)
8.	*Bacillus subtilis*	Solid State fermentation	Potato peel, I.cylindrica grass	Mukherjee et al. (2008)
9.	*Streptomyces* sp. CN902	Solid State fermentation	Wheat bran and Chopped date stone	Lazim et al. (2009)
10.	*Serratia marcescens* sp.7	Solid State fermentation	Ground nut shell and Wheat bran	Joseph and Palaniyandi (2011)
Fungal sources				
11.	*Aspergillus oryzae* AWT 20	Submerged fermentation	Casein	Sharma et al. (2006)
12.	*Aspergillus tamari*	Submerged fermentation	Bovine serum albumin, Casein, Gelatin, Hemoglobin	Sharma and De (2011)
13.	*Aspergillus flavus* AS2	Submerged fermentation	Casein	Rani et al. (2012)
Yeast sources				
14.	*Saccharomyces lipolytica*	Submerged fermentation	Casein	Ogrydziak and Mortimer (1977)
15.	*Candida olea*	Submerged fermentation	Bovine serum albumin	Nelson and Young (1987)
16.	*Aureobasidium pullulans*	Submerged fermentation	Casein	Chi et al. (2007)
17.	*Yarrowia lipolytica*	Solid State fermentation	Fish flour and Polyurethane foam	Hernandez-Martinez et al. (2011)
Halophilic sources				
18.	*Bacillus* sp. tk1 and tk2	Solid State fermentation	Moon dhal husk, Green gram husk, Sugarcane bagasse, Rice bran, Wheat bran, Cotton seed	Kuberan et al. (2010)
19.	*Bacillus* sp. HS-4	Submerged fermentation	Casein and Gelatin	Sehar and Hameed (2011)

20.	*Salinivibrio* sp. strain MS-7	Submerged fermentation	Casein	Shahbazi and Karbalaei (2012)
21.	*Bacillus* sp.	Submerged fermentation	Casein	Nigam et al. (2013 a & b)

References:

1. Okonko, I. O., Ogunnusi, T. A., Aloysius, F. E., Adejoye, O. D. and Adewale, O. A. (2009). Utilization of food wastes for sustainable development. Elec. J. of Env. Agri. Food Chem. 8: 263- 286.

2. Alofe, F.V., Odeyemi, O. and Oke, O. L. (1996). Three edible wild mushrooms from Nigeria: Their proximate and mineral composition. Plant Foods Human Nutr. 49: 63-73.

3. Bisaria, R., Madan, M. and Vasudevan, P. (1997). Utilization of Agro-residues as animal feed through bioconversion. Biores. Technol. 59: 5-8.

4. Pandey, A. and Soccol, C. R. (1998). Bioconversion of biomass: A case study of lignocellulosic bioconversions in solid state fermentation. Braz. Arch. Biol. Technol. 41: 379-390.

5. Aceba, C., Castillon, M.P., Estrada, P., Mata, I. and Costa, E. (1986). Enhanced cellulase production from *Trichoderma reesii* QM 9414 m physically treated wheat straw. J. Appl. Microbiol. Biotechnol. 23: 218-223.

6. Chellappan, S., Jasmin, C., Basheer, S. M., Elyas, K. K., Bhat, S. G. and Chandrasekaran, M. (2006). Production, purification and partial characterization of a novel protease from marine *Engyodontium album* BTMFS10 under solid state fermentation. Proc. Biochem. 41: 956-961.

7. Makhija, P., Nigam, V. K., Mohan, M. K., Ghosh, P. and Sasamal, D. (2006). Characterization of extracelluалar alkaline proteases produced by halophilic bacteria. Proc. Nat. Acad. Sci. India 76(B) IV: 362-367.

8. Singhal, P., Nigam, V. K., Vidyarthi, A. S. (2012). Studies on production, characterization and application of microbial alkaline proteases. Int. J. Adv. Biotechnol. 3: 653-669.

9. Mukherjee, A. K., Adhikari, H. and Rai, S. K. (2008). Production of alkaline protease by a thermophilic *Bacillus subtilis* under solid-state fermentation (SSF) condition using imperata cylindrica grass and potato peel as low-cost medium: Characterization and application of enzyme in detergent formulation. Biochemi. Eng. J. 39: 353-361.

10. Sarath, G., Mitchel, R. B., Satler, S. E., Funnell, D., Pedersen, J. F., Graybosch, R. A., and Vogel K. P. (2008). Opportunities and road blocks in utilizing oranges and small grains for liquid fuels. J. Ind. Microbiol. Biotechnol. 35: 343–354.

11. Koutinas, A. A., Arifeen, N., Wang, R. and Webb, C. (2007). Cereal based biorefinery development: integrated enzyme production for cereal fluor hydrolysis. Biotechnol. Bioeng. 97: 61-72.

12. Virupakshi, S., Babu, K. G., Gaikwad, S. R. and Naik, G. R. (2005). Production of a xylanolytic enzyme by a thermoalkaliphic *Bacillus* sp. JB-99 in solid-state fermentation. Proc. Biotechnol. 40: 431-435.

13. Huitron, C., Perez, R. , Sanchez, A. E., Lappe, P. and Zavaleta, L. R. (2008). Agricultural waste from the tequila industry as substrate for the production of commercially important enzymes. J. Env. Bio. 29: 37-41.

14. Iqbal, H. M .N., Asgher, M., Ahmed, I. and Hussain, S. (2010). Media optimization for hyper-production of Carboxymethyl Cellulase using proximally analyzed agro-industrial residue with *Trichoderma harzianum* under SSF. Int. J. Agro. Vet. Med. Sci. 4: 47-55.

15. Murthy, P. S. and Naidu, M. M. (2010). Protease production by *Aspergillus oryzae* in solid state fermentation utilizing coffee by products. World Appl. Sci. J. 8: 199-205.

16. Srinivas, Ch., Bhushanam, V., Premsagar, T., Nagamani, Ch., and Rajasekhar, M. (2010). Studies on the production of protease from agro residues Convalia ensiformis beans by solid state fermentation (SSF) using *Bacillus subtalis* 2724 NCIM. J. Chem. Pharm. Res. 2: 10-15.

17. Akinyele, B. J., Olaniyi, O. O. and Arotupin, D. J. (2011). Bioconversion of selected agricultural wastes and associated enzymes by *Volvariella volvacea*: An edible mushroom. Res. J. Microbiol. 6: 63-70.

18. Parbat, R. and Singhal, B. (2011). Production of Glucoamylase by *Aspergillus oryzae* under solid state fermentation using agro industrial products. Int. J. Microbiol. Res. 2:204-207.

19. Ghorbel-Bellaaj, O., Jridi, M., Khaled, H. B., Jellouli, K. and Nasri, M. (2012). Bioconversion of shrimp shell waste for the production of antioxidant and chitosan used as fruit juice clarifier. Int. J. Food Sci. Tech. 47: 1835-1841.

20. Rathakrishnan, P. and Nagarajan, P. (2012). Optimizing factors affecting protease production by a *Bacillus cereus* using ground nut shell under solid substrate fermentation. Int. J. of Sci. Inv. Tod. 1: 114-129.

21. Sabat, S., Murthy, V. K., Pavithra, M., PalaMayur and Chandavar, A. (2012). Study of enhanced Lipase production using agro-waste product by *Bacillus stearothermophilus* MTCC 37. Int. J. Eng. Res. Appl. 2: 266-274.
22. Rajendra, A. and Thanyavelu, V. (2013). Utilizing agricultural wastes as substrates for Lipase production by *Candida rugosa* NCIM 3462 in solid state fermentation: Response surface optimization of fermentation parameters. Waste Biom. Valoriz. 4: 347-357.
23. Durham, D. R., Stewart, D. B. and Stellwag, E. J. (1987). Novel alkaline and heat stable serine proteases from alkalophilic *Bacillus* sp. strain GX6638. J. Bacteriol. 169: 2762-2768.
24. Phadatare, S. U., Deshapande, W. and Srinivasam, M. C. (1993). High activity alkaline protease from *Conidiobolus coronatus* (NCL 86.8.20): Enzyme production and compatibility with commercial detergents. Enz. Microbial Technol. 15: 72-76.
25. Yang, S. S. and Wang, J. Y. (1999). Protease and amylase production of *Streptomyces rimosus* in submerged and solid-state cultivations. Bot. Bull. Acad. Sin. 40: 259-265.
26. Sumantha, A., Sandhya, C., Szakacs, G., Soccol, C. R. and Pandey, A. (2005). Production and partial purification of a neutral metalloprotease by a fungal mixed substrate fermentation. Food Technol. Biotechnol. 43: 313-319.
27. Manivannan, S. and Katiresan, K. (2007). Alkaline protease production by *Penicillium fellutanum* isolated from mangrove sediment. Int. J. Biol. Chem. 1: 98-103.
28. Rao, K. and Narasu, M. L. (2007). Alkaline protease from *Bacillus firmus* 7728. Afri. J. Biotechnol. 8: 7035-7041.
29. Jaswal, R. K., Kocher, G. S. and Virk M. S. (2008). Production of alkaline protease by *Bacillus circulans* using agricultural residues: A statistical approach. Ind. J. Biotechnol. 7: 356-360.
30. Lazim, H., Mankai, H., Slama, N., Barkallah, I. and Limam, F. (2009). Production and optimization of thermophilic alkaline protease in solid-state fermentation by *Streptomyces* sp. CN902. J. Ind. Microbiol. Biotechnol. 36: 531-537.
31. Joseph, B. and Palaniyandi, S. (2011). Determination of alkaline protease production in *Serratia Marcescens* sp7 using agro wastes as substrate medium, optimization of production parameters and purification of the enzyme. World Acad. of Sci. Eng. Technol. 1: 247-251.
32. Sharma, J., Singh, A., Kumar, R. and Mittal, A. (2006).Partial purification of an alkaline protease from a new strain of *Aspergillus oryzae* AWT20 and its enhanced stabilization in entrapped Ca-Alginate beads. The Internet J. Microbiol. 2: 1-14.
33. Sharma, N. and De, K. (2011). Production ,purification and crystallization of an alkaline protease from *Aspergillus tamari* [EF661565.1]. Agri. Biol. J. N. Am. 2: 1135-1142.
34. Rani, M. R., Prasad, N. N. and Sambasivarao (2012). Optimization of cultural conditions for the production of alkaline protease from a mutant *Aspergillus flavus* AS2. Asian J. Exp. Biol. Sci. 3: 565-576.
35. Ogrydziak, D. M. and Mortimer, R. K. (1977). Genetics of extracellular protease production in *Saccharomyces lipolytica*. Gen. 87: 621-632.
36. Nelson, G. and Young, T. W. (1987). Extracellular acid and alkaline protease from *Candida olea*. J. Gen. Micobiol. 133: 1461-1469.
37. Chi, Z., Ma., C., Wang, P. and Li H. F. (2007). Optmization of medium and cultivation conditions for alkaline protease production by the marine yeast *Aureobasidium pullulans*. Biores. Technol. 98: 534-538.
38. Hernandez-Martinez, R., Sancho-Solano, A., Loera-Corral, O., Rojo-Dominguez, A., Regalado-Gonzalez, C., Huerta-Ochoa, S. and Prado-Barragan, L.A. (2011). Purification and characterization of a thermostable alkaline protease produced by *Yarrowia lipolytica*. Rev. Mex. Ing. Quim. 10: 333-341.
39. Kuberan, T., Sangaralingam and Thirumalaiarasu, V. (2010). Isolation and optimization of protease producing bacteria from Halophilic soil. J. Biosci. Res. 1: 163-174.
40. Sehar, S. and Hameed, A. (2011). Extracellualr alkaline protease by a newly isolated halophilic *Bacillus* sp. Global J. of Biotechnol. Biochem. 6: 142-148.
41. Shahbazi, M. and Karbalaei-Heidari, H. R. (2012). A novel low molecular weight extracellular protese from a moderately halophilic bacterium *Salinivibrio* sp. strain MS-7 production and biochemical properties. Mol. Bio. Res. Comm. 1: 45-56.
42. Nigam, V. K., Vimal, A., Vidyarthi, A. S. and Mohan, M. K. (2013a). Studies on applicability of alkaline protease from halophilic bacteria in detergent formulations. Ind. J. App. Res. 3: 47-50.
43. Nigam, V. K., Singhal, P., Vidyarthi, A. S., Mohan, M. K. and Ghosh, P. (2013b). Studies on keratinolytic activity of alkaline proteases from halophilic bacteria. Int. J. Pharma. Biosci. 4: 389-399.

44. Foda, M. S., Ali, S. M., Yousef, A. M., Kahil, T., Sahta, H. M. and Roshdy, A.M. (2013). Production physiology of alkaline protease by *Bacillus thuringesis* spp. under solid state fermentation conditions. J. Appl. Sci. Res. 9: 1975-1984.