ISSN: 2277-9655
Impact Factor: 1.852

IJESRT

INTERNATIONAL JOURNAL OF ENGINEERING SCIENCES & RESEARCH TECHNOLOGY

Pectinase Enzyme Production by using Agrowastes

Priya V[*1], Sashi V[2]

[*1] Ph.D. Scholar, Department of Botany, P.S.G.R.Krishnammal College for Women, Coimbatore, Tamilnadu, India

[2]Associate Professor, Department of Botany, P.S.G.R.Krishnammal College for Women, Coimbatore, Tamilnadu, India

priyasrikumar17@gmail.com

Abstract

In this work an investigation to find Pectinase enzyme with low cost production. These studies deal with to produce Pectinase enzyme using Penicillium sp strain in liquid state fermentation. Agro-industrial residues used as carbon sources were orange peel, mosambi peel, pineapple, wheat bran, papaya and pectin.Best pectinolytic activity, as indicated by the diameter of clear, hydrolyzed zones on the medium plates containing commercial citrus pectin as sole carbon source, was obtained with Penicillium lividum using pineapple closely followed by Aspergillus sps using orange peel.The optimum temperature was found to be 30˚C and pH at 8.0. Peak pectinase activity of 397mg/ml and 346mg/ml protein was respectively obtained by Liquid State Fermentation (LSF) at 48 h for Penicillium lividum and Aspergillus sps. The strains of Penicillium lividum and Aspergillus sps have good prospect for Pectinase production. Pineapple peel and orange peel is a good low-cost fermentation substrate for pectinase production by the investigated fungus.

Keywords: pectinase, Penicillium, Aspergillus, agrowastes.

Introduction

Over the recent years, there has been a tremendous increase in the awareness regarding the effects of population, and public pressure has influenced both industry and government. With the advancement of biotechnology; enzymes have found their way into many new industrial processes (Sonia, *et al.,* 2008). Most of the reactions in living organisms are catalyzed by protein molecules called enzymes. Enzymes can rightly be called the catalytic machinery of living systems. Man has indirectly used enzymes almost since the beginning of human history.

Production of enzymes from agrowastes could be important because they contain large amounts of cellulose, hemicelluloses and pectin, which could serve as inducers for the production of cellulose, xylanase and pectinase respectively (Whitaker, 1990; Nagodawithana, *et al.*,1993; Hours, *et al.,* 1998 and Bocccas, 1994). Microbial pectinase have tremendous potential to offer mankind. Most pectic enzyme preparations are used in the fruit processing industry and pectic enzymes alone account for about one quarter of the world's food enzyme production. Fungal pectinase are among the most important industrial enzymes and are of great significance with wide range application in juice clarification, textile processing, degumming of plant bast fibers, treatment of pectic wastewaters, papermaking and coffee and tea fermentations. Therefore, the biotechnological potential of pectinolytic enzymes from fungi has drawn a great deal of attention from various researchers worldwide (Patil and Dayanad, 2006).

Pectinases have attracted attention globally as biological catalysts in numerous industrial processes and are used in processing agricultural and agro-industrial waste (Jin, 2001; Kashyap, *et al.*,2001; Bai, 2004; Patil and Dayanad, 2006). Several agrowastes mainly citrus peel, apple pomace, coffee pulp, wheat bran(Taragano, *et al.*,1997), sugarcane bagasse(Solis-Pereyra,1993 and 1996), lemon peel (Larios, *et al.*,1989), saw dust, pineapple and mosambi peel (Hours, *et al.,* 1988) have been explored for the microbial production of pectinase. Several authors have reported a wide range of pectin (15-25%) in sunflower head (Federico, *et al.*,1988). This high content of pectin was a stimulant to explore sunflower head as a substrate for the microbial production of pectinase(Whitaker, 1990; Nagodawithana, *et al.*,1993; Hours, *et al.,* 1998; Bocccas, 1994 Patil and Dayanad, 2006). In the present study involves screening of strains of fungi for pectinolytic activity

ISSN: 2277-9655
Impact Factor: 1.852

using different agrowastes as fermentation substrates of Pectinase production by liquid state culture.

Pectinase Enzyme

Pectinase are a complex group of enzymes that that act on the pectic substances and cause its breakdown into galacturonates. Pectinase are produced by microorganisms as well as plants (Sathyanarayana, *et al.*,2009). Pectic substances are glycosidic macromolecules with high molecular weight. Pectic polysaccharides comprise between 30-50% of the cell walls of dicotyledonous plants (Carpita, *et al.*,1993). Pectinase are a super family of enzymes acting on the polymers and mainly composed of pectin hydrolases, lyases and esterase with different specificities (Ladjama, *et al.*,2007).

Materials and Methods

Preparation of Raw Materials

Fresh orange, Mosambi peel and pulp waste was collected from nearby fruit shop. The so called "Starter culture" was prepared as follows: 25g of orange peel waste was mixed with soil in a pot. This can be followed by the other starter culture such as orange, mosambi peel and pulp. Every day the water could be sprinkled. After two months, the soil degraded with fruit waste, which was used as an inoculum "Precursor". This can be used for screening the pectinolytic fungal isolation.

Isolation and Identification of Pectinolytic Microfungi

The fungi were isolated from precursor inoculum using Potato Dextrose Agar(PDA) medium. The soild medium contained (g/l): potato extract - 200ml, dextrose-20g, agar -20g. A supplement of 0.1% ampicillin was added to avoid the contamination. p^H value was adjusted to 5.6 before autoclaving at 121˚C for 15 min. Inoculated plates were incubated at incubated at 30˚C for 5-7 days. Pure cultures were obtained by repeated sub-culturing on PDA plates and maintained at 4˚C on PDA slants. Fifteen isolates were isolated from the precursor and the isolates were examined and identified in the Centre for Advanced studies in Botany, Madras University, Chennai. The microscopic structures of the isolates were studied using a light Microscope with the help of books described in Kenneth and *et al* (1968), Kenneth and *et al* (1965) and Domsch and *et al* (1980).

Maintenance and Growth of Microorganism

PDA slants were incubated at 30˚C for 4-5 days for the growth of spores and were then stored in refrigerator at 4˚C for further use. Spores were first cultivated in an Erlenmeyer flask of 250ml capacity containing 50 ml PDB (Potato Dextrose Broth) at 30˚C for 4-5 days, under stationary conditions for development of fungal spores.

Preparation of Inoculum

50ml of PDB in an Erlenmeyer flask of 250 ml capacity was inoculated with fungal spores maintained on PDA slants and incubated at 30˚C for 4 days, under stationary conditions for development of fungal spores. After 4 days, the content of the flask was decanted off carefully. 50 ml of sterilized 0.9% isotonic NaCl solution (saline solution) was poured on the fungal spores in the flask and shaken vigorously to facilitate the release of spores into the saline solution. This spore suspension was used as inoculums for the further use. After 4 days incubation, the number of spores as counted by Haemocytometer was found to be 5 x 10^{-7} spores/ml (Satvinder singh dhillon, *et al.*, 2004).

Screening of fungal isolates for pectinolytic activity

Fifteen isolates were cultivated on modified Czapek-Dox agar medium. They contained(g/l):$NaNo_3$-3.0g, K_2HPO_4-1.0g, $MgSo_4.H_2O$-0.50g, Kcl-0.50g, $FeSo_4$-0.01g,Sucrose-30g, agar-15.0g with 1.5% as the sole carbon pectin was added. Agar medium was amended with 0.1% of ampicillin to restrict bacterial growth. p^H value was adjusted to 5.6 before autoclaving at 121˚C for 15 min. 2% of fungal spore suspension was centrally inoculated and plates were incubated at incubated at 28±2˚C for 3-5 days. Pectin utilization was detected by flooding the culture plates with freshly prepared Iodine-Potassium iodide solution, Iodine-1.0g,Potassium iodide-5.0g in 330ml distilled water (Hankin, *et al.*,1971).This solution gives color to the medium containing pectin resulting in a translucent halo in the region where pectin is degraded, which indicated the pectinolytic activity. From fifteen isolates one of best organism were identified for pectinase enzyme production based on the zone diameter of the clearing zone.

Pectinase production with different agro-wastes

Substrate (Agrowaste) preparation

The disposed different agrowastes were collected from nearby fruit shop. They were sorted out manually based on their fine texture and rigidity. The collected peels were minced to pieces and were hot air oven dried at 55˚C until constant weight was achieved. The dried peel was diminuted in a Ball Mill and they were clarified in a sieve shaker.

One of the best organism *Penicillium lividum*. was studied for Pectinase production using different

ISSN: 2277-9655
Impact Factor: 1.852

agrowastes including orange peel, mosambi peel, pineapple, wheat bran, papaya and pectin.

Fermentation studies

The medium for liquid state culture contained per liter of distilled water: Rose Bengal Broth-KH_2PO_4 -1.0g, $MgSo_4$ – 0.5g, Ammonium sulphate – 5.0g, different agrowaste- 5 g, Rose Bengal -0.035g, p^H value was adjusted to 8.5 autoclaving at 121˚C for 15 min. In this medium inoculating 2.0ml of spore suspension containing 5 x 10^{-7} spores/ml. Cultures were incubated at 28±2˚C, with agitations at 150rpm on orbital shaker for 48 hrs. The extract was filtered through Whatman filter paper No.1. and this clear extract used for enzyme assay.

Optimization studies

The operating variables for fermentation were tried to optimize which include p^H, temperature, time of incubation and substrate concentration. Optimal temperature and p^H were obtained by varying the ranges from 20˚C -60˚C and 5 - 9. In addition, enzyme production was monitored from 24 – 120hrs of incubation and substrate concentration was checked from 0.5 – 2.5%.The optimized culture conditions were used for in vitro enzyme production.

Enzyme Assay

Protein assay

The amount of protein in the crude enzyme was estimated using Bovine serum album as standard (Lowry *et al*,1951).

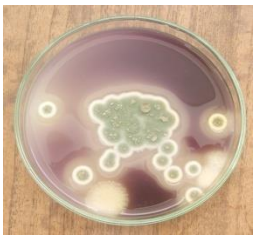
Polygalacturonase assay

Pectinase activity was determined using pectin as substrate. The reaction mixture containing equal amounts of 1% pectin prepared in sodium acetate buffer(0.05M; p^H 5.4)and suitably diluted crude enzyme, was incubated at 50˚C in water bath for 30 min. The reaction was stopped with 1.0ml dinitrosalicyclic acid solution (Miller, 1959), after which the mixture was boiled for 10 min and cooled. The colour was read at 540nm using a spectrophotometer. The amount of reducing sugar released was quantified using glucose as standard. The specific enzyme activity (IU mg^{-1} protein) was calculated as the amount of enzyme required to release one micromole(1µmol) equivalent of galacturonic acid per min per protein (mg) under the assay condition.

Results

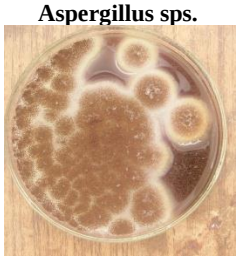
Out of fifteen isolates, four fungi were selected showing clearing zone, based on their ability to grow on the medium containing pectin as the carbon source. The maximum pectinolytic activity was shown in the *Penicillium lividum*. Studies on Pectinase production by *Penicillium* was carried out using orange peel, papaya peel, pineapple peel, mosambi peel, wheat bran and pectin as the carbon source.

Penicillium lividum.(A) Showed 397 IU/ml when pineapple peel used as carbon source and then, *Aspergillus sps(B) showed* 346 IU/ml when orange peel was used as a carbon source, followed by *Aspergillus sps* (*C*) *and Fusarium* sps (D)(Fig 1) showed when wheat bran used as a carbon source.



Penicillium sps.

Aspergillus sps.



Aspergillus sps

. Fusarium sps.

Fig 1: Screening of isolates for pectinolytic activity

ISSN: 2277-9655
Impact Factor: 1.852

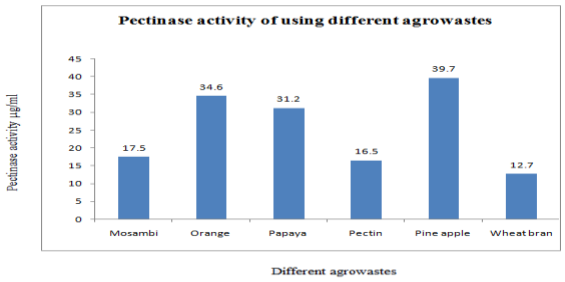


Fig 2: Pectinase activity

Discussion

Fifteen fungal isolates, which exhibited pectinolytic activity were isolated from the fruit waste. The fungal isolates were identified and screened as *Penicillium lividum*, *Aspergillus* spp, and *Fusarium* sps. The best pectinolytic activity based on the plate assay method was given by *Penicillium lividum*, followed by *Aspergillus* sps. The present study, which the strains of *Penicillium lividum* and *Aspergillus* sps to produce pectinase which hydrolyze pectins. The strains of *A. niger* and *P. chrysogenum* isolated by the group were reported to produce cellulases (Chinedu, *et al.*, 2008 and 2007a) and xylanases (Chinedu, *et al.*, 2008b and Okafor, *et al.*,2007a and 2007b). The strains of *A. niger* and *P. chrysogenum* produced the highest pectinase activity with wheat bran as sole carbon source. He concluded that, wheat bran is the most suitable carbon source when compared to other agrowastes (pineapple peel, orange peel, sawdust and sugarcane pulp) for pectinase production by the organisms (Okafor, *et al.*,2010).

The incubation period for pectinase production by the two fungi using wheat bran as sole carbon source was 48 h. Phutela, *et al*., 2005 and Said, *et al*.,1991, reported an incubation period of 48 h for optimal pectinase production for some fungal isolates while Fujio and Eledago, 1993, reported a 72 h incubation time for polygalacturonase production by *Rhizopus oryzae*. This is ideal from an economic viewpoint is based on the enzyme production rate and the low-cost of the substrates. Pectinase production from SSF culture of both organisms was significantly higher than that obtained by SMF. The higher level of pectinase activity by SSF compared to SmF varied with the organisms. For instance, it was more profound with *A. niger* compared to *P. chrysogenum*. Several workers proposed the use of SSF for pectinase production, using different solid agricultural and agro-industrial residues as substrates such as wheat bran and soy bran (Castilho, *et al*.,1999 and Singh, *et al*., 1999). The present result clearly supports the use of LSF for Pectinase production by filamentous fungi.

In conclusion, strians of pectinolytic fungi including *Penicillium lividum*, *Aspergillus* sps, have been isolated from fruit wastes pineapple waste had been identified as a suitable low-cost substrate for pectinase production by the strains of *Penicillium lividum*, *Aspergillus* sps. Higher levels of pectinase activity were obtained by LSF. This is the first report on using pineapple agrowaste for Pectinase enzyme production by liquid state culture fermentation. The use of pineapple waste for pectinase production will not only reduce the production costs of the enzyme but also help decrease pollution-load due to the agro-industrial waste. This is the first report, on to produce pectinase enzyme using pineapple agrowaste by *Penicillium lividum.*

Acknowledgements

I am very much grateful to my Institution to complete my research work.

Conflict of Interest

My research work is very useful to public, production of pectinase enzyme using agrowaste.

References

[1] Bai, Z.H., Zhang, H.X., Qi ,H.Y., Peng, X.W. and Li, B.J. (2004).Pectinase production by Asperillus niger using waste water in solid state fermentation for eliciting plant disease resistance. Bioresource Technology 95(1): 49-52.

[2] Boccas, F., Roussos, S., Gutierrez, M., Serrano, L. and Viniegra, G.G. (1994). Production of pectinases from coffee pulp in solid-state fermentation system: Selection of wild fungal isolate of high potency by a simple three steps screening technique. J.Food Sci. Technol.31:22-26.

[3] Castilho, L.R., Alves, T.L.M. and Medronho, R.A. (1999). Recovery of pectinolytic enzymes produced by solid-state culture of Aspergillus niger. Process Biochem., 34: 181–186.

[4] Carpita, N.C. and Gibeaut, D.M.(1993).Structural models of primary cell walls in flowering plants: Consistency of molecular structure with the physical properties of the walls during growth.Plant Journal.3. 1-30.

[5] Chinedu, S.N., Okochi, V.I., Smith, H.A., Okafor, U.A., Onyegeme- Okerenta, B.M. and Omidiji, O. (2007 a). Effect of carbon sources on cellulase (EC 3. 2. 1. 4) production by wild-type Penicillium

chrysogenum PCL501. Afr. J. Biochem. Res., 1(1): 6-10.

[6] Chinedu, S.N., Nwinyi, O.C. and Okochi, V.I. (2008). A Growth and cellulose activity of wild-type Aspergillus niger ANL301 in different carbon sources. Can. J. Pure Appl. Sci., 2(2): 357-362.

[7] Chinedu, S.N., Okafor, U.A., Emezue, T.N., Okochi, V.I. (2008b). Xylanase production of Aspergillus niger and Penicillium chrysogenum from ammonia pretreated cellulosic waste.

[8] Res. J. Microbiol., 3(4): 246-253.

[9] Domsch, K.H., and Gams, W. (1980). Compendium of Soil Fungi. Vol.1.

[10] Federico, F., Gianfranco, M., Maurizio, S. and Maurizio, P.(1988). Pectic enzyme production by Cryptococcus albidus var. albidus on olive vegetation waters enriched with sunflower calathide meal. Biol. Wastes 25: 291–301.

[11] Fujio, Y. and Eledago, F.B. (1993). Polygalacturonase production by Rhizopus species. J. Gen. Appl. Microbiol., 39: 409-418.

[12] Hankin, L., Zucker, M. and Sands D.C.(1971). Improved soild medium for the detection and enumeration of pectolytic bacteria. Applied Microbiology 22: 205-209.

[13] Hours, R.A., Voget, C.E. and Ertola, R.J. (1988). Apple pomace as raw material for the pectinases production in solid-state culture, Biol. Wastes.24 :147-157.

[14] Jin C. and Masako M, (2001).Evaluation of enzyme treatment of linen fabrics. Fiber 57(9): 270-273.

[15] Kashyap, D.R., Vohra, P.K., Chopra, S., Tewari, R. (2001). Applications of pectinases in the commercial sector: A review. Bioresource technology. 77(3): 215-227.

[16] Kenneth, B. Raper., Charles Thom. and Dorothy, I. Fennell. (1968).A Mannual of Penicillia.

[17] 20. Kenneth, B. Raper., and Dorothy I. Fennell. (1965). The Genus of Aspergillus.

[18] Larios, G., Garcia, J.M. and Huitron, C. (1989). Endo-polygalacturonase production from untreated lemon peel by Aspergillus sp. CH-Y-1043. Biotechnol. Lett. 11: 729–734.

[19] Ladjama, A., Taibi, Z. and Meddour,A.(2007). Production of pectinolytic enzymes using streptromyces strains isolated from palm grove soil in biskra area (Algeria). African crop science conference proceedings. 8 : 1155-1158.

[20] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randal, R.J.(1951). Protein measurement with the Folin Phenol reagent. J. Biol.Chem.,193: 267-275.

[21] Miller, G.L.(1959).Use of Dinitrosalicylic acid reagent for determination of reducing sugar. Anal.Chem.,31: 426 -428.

[22] Nagodawithana, T., Reed, G. and Taylor,V.(1993).Carbohydrates. In: Enzymes in Food Processes, (Eds.), Academic Press, London, UK. pp.121-158.

[23] Okafor U.A, Emezue T.N, Okochi V.I, Onyegeme-Okerenta B.M, Nwodo- Chinedu S (2007a). Xylanase production by Penicillium chrysogenum PCL501 fermented on cellulosic wastes. Afr. J. Biochem. Res., 1(4): 48-53

[24] Okafor, U.A., Okochi, V.I., Onyegeme-Okerenta, M.B. and . Chinedu, S.N. (2007b). Xylanase production by Aspergillus niger ANL301using agro-wastes. Afr. J. Biotechnol., 6(14): 1710-1714.

[25] Okafor, U.A., Okochi, V.I., Shalom Nwodo Chineu., Ebuechi, O.A.T. and Onygeme, B.M. Okerenta.(2010). Pectinolytic activity of wild type filamentous fungi fermented on agrowaste. African Journal of Microbiology Research 4(24): 2729 -2734.

[26] Phutela, U., Dhuna V., Sandhu, S. and Chadha, B.S. (2005). Pectinase and polygalacturonase production by a thermophilic Aspergillus fumigates isolated from decomposting orange peels. Braz. J. Microbiol., 36: 63- 69.

[27] Said. S., Fonseca, M.J.V., Siersera, V. (1991). Pectinase production by Pencillium frequentans. World J. Microbiol. Biotechnol., 7: 607-608.

[28] Sarvamangala R.Patil and A.Dayanad. Bioresource Technology. (2006). Production of pectinase from deseeded sunflower head by Aspergillus niger in submerged and solid-state conditions. Bioresource Technology. 97: 2054-2058.

[29] Sarvamangala, R.P. and Dayanand, A.(2006). Exploration of regional agrowastes for the production of pectinase by Aspergillus niger. Food Technology and Biotechology. 44(2): 289-292.

[30] Sathyananrayana N.Gummadi and etal (2009).Industrially important carbohydrate degrading enzymes from yeasts: Pectinases,

ISSN: 2277-9655
Impact Factor: 1.852

Chitinases and β-1,3 –Glucanases. Yeast Biotechnology: Diversity and Applications. 673-691

[31]Satvinder singh dhillon, Rajwant Kaur Gill, Sikander Singh Gill and Malkiat Singh. (2004).International journal of Environmental studies. 61(2): 199-210.

[32]Singh, S.A., Plattner, H. and Diekmann, H. (1999). Exopolygalacturonate lyase from a thermophilic Bacillus sp. Enzym. Microb. Technol., 25: 420– 425.

[33]Solis-Pereyra, S., Favela-Torres, E., Viniegra-Gonzalez, G. and GutierrezRojas, M.(1993). Effect of different carbon sources on the synthesis of pectinase by Aspergillus niger in submerged and solid state fermentation. Appl. Microbiol. Biotechnol. 39 : 36–41.

[34]Solis-Pereyra, S., Favela-Torres, E., Gutierrez, Rojas, M., Roussos, S., Saucedo Castaneda, G., Gunasekaran, P. and Viniegra-Gonzalez, G.(1996). Production of pectinases by Aspergillus niger in solid-state fermentation at high initial glucose concentrations. World J. Microbiol. Biotechnol. 12: 257–260.

[35]Sonia Ahlawat.R.P.Mandhan, Saurabh Sudha Dhiman, Rakesh Kumar and Jitender Sharma. (2008). Potential application of alkaline pectinase from Bacilus subtilis SS in pulp and paper industry. Appl.Biochem Biotechnol. 149: 287-293

[36]Taragano, V., Sanchez, V.E. and Pilosof, A.M.R. (1997). Combined effect of water activity depression and glucose addition on pectinase and protease production by Aspergillus niger. Biotechnol. Lett. 19 (3) : 233–236.

[37]Whitaker, J.R. (1990). New and future uses of enzymes in food processing. Food Biotechnol. 4 : 669-697.