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Biosynthesis of Industrially Relevant Extracellular Pectinase from *Aspergillus terreus* NCFT 4269.10 Using Orange Peels as Substrate

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ABSTRACT

In this study, *Aspergillus terreus* NCFT4269.10 was selected for the biosynthesis of pectinase after screening from fifteen native fungal strains as the performance of *A. terreus* was best in pectin hydrolyzing activity (2.75 cm). Aiming at the cost effective fermentation, several agro-industrial residues such as, Pearl Millet (PM), Finger Millet (FM), Orange Peels (OP), Mustard oil Cake (MoC) and Chikling Vetch Peels (CVP) were evaluated for highest pectinase production using Liquid Static Surface Fermentation (LSSF) and Liquid Shaking Fermentation (LShF). Among these substrates, orange peel was found to be the most suitable substrate for optimal pectinase biosynthesis (LSSF: 633.33 ± 57.73 U mL⁻¹, LShF: 733.33 ± 115.47 U mL⁻¹). Liquid shaking fermentation at 120 rpm for 96 h at 30°C was suitable for pectinase biosynthesis as compared to LSSF. Total protein content was also higher in OP (LSSF: 880.33 ± 12.5 µg mL⁻¹, LShF: 992.7 ± 4.16 µg mL⁻¹) when the culture was grown under static and shaking condition at 30°C for 96 h. Maximum biomass was formed when OP was implemented for fermentation at static condition followed by CVP. But maximum biomass did not always support the enhanced pectinase biosynthesis in most of the cases. Partial purification of pectinase revealed a 3.86 fold purification and 27.63% yield of protein. Fermentation kinetics study revealed that purified enzyme activity yield per gram of substrate was maximum at both LSSF (1866.6 U gds⁻¹) and LShF (2026.7 U gds⁻¹) when biosynthesis of pectinase was carried out using OP. Ratio of enzyme yield and biomass were best with OP as compared to the other substrates both in static and shaking fermentation at crude and purified conditions.

Key words: *Aspergillus terreus*, fermentation kinetics, liquid static surface fermentation, orange peels, pectinase

INTRODUCTION

The chemical transformation processes implemented in many industries have numerous disadvantages owing to its commercial and environmental standpoint. Keeping in view, the enzymes may be most suitable industrial biocatalyst and are increasingly becoming the alternative of chemical catalysts in many industrial sectors (Smith, 1996). The microbial pectinases are the most imperative enzymes in contemporary biotechnology as they have almost entirely substituted chemical hydrolysis of pectin. In contemporary, pectinases have occupied approximately 25% of the enzyme trading (Sidkey *et al.*, 2011).

Pectinases, the multifaceted hydrolases that function as esterases and depolymerases include mostly pectin esterases (EC. 3.1. 1.11), pectin lyases (E.C. 4. 2. 2. 10) and polygalacturonases. Pectin esterases catalyze the hydrolysis of methylated carboxylic ester groups present in pectin into pectic acid and methanol. Similarly, pectin lyases (E.C. 4. 2. 2. 10) hydrolyze α -1, 4-glycosidic bond by process called transelimination releasing galacturonide with a double bond between C-4 and C-5 at the nonreducing end of the sugar backbone. Generally, polygalacturonases also attack α -1, 4-glycosidic linkages in homogalacturonans (Delgado *et al.*, 1993). Now-a-days, they are the high flying industrial enzymes of textile industries, plant fiber processing, degumming, tea, coffee, vegetables oil extraction industries and treatment of industrial wastewater having pectinacious wastes. They are also having potential applications in purification of viruses (Salazar and Jayasinghe, 1999) and in paper producing industries (Ricard and Reid, 2004). Further, enzyme cocktails having pectinases are used for the preparation of animal feeds. To reiterate, these enzymes are also employed in facilitated extraction, filtration and clarification of fruit juices for enhanced biosynthesis (Kashyap *et al.*, 2001), food processing industry, souring of cotton, bleaching of paper and in alcoholic beverage preparation (Beg *et al.*, 2001).

Many potential microorganisms like bacteria, fungi and actinomycetes are wide distributed in the nature having the capability to exploit pectin as their sole source of carbon and energy. But, almost all the industrially imperative pectinases are biosynthesized from fungal sources (Singh *et al.*, 1999). *Aspergillus niger* is the most well known fungal species implemented for industrial biosynthesis of pectinases (Naidu and Panda, 1998). Nonetheless, many fungal genera like, *Aspergillus*, *Erwinia*, *Fusarium*, *Kluyveromyces*, *Penicillium*, *Rhizopus* and *Saccharomyces*, have been well recognized for their pectinase biosynthesizing ability (Favela-Torres *et al.*, 2006). However, assortment of the particular fungal species for the biosynthesis of pectinase relies on numerous factors, like, type of fermentation required (solid-state or submerged fermentation), kind of pectinase to be biosynthesized, initial medium pH and temperature profile for the fungus and enzymes and above to all the genotypic trait of the working strain (Favela-Torres *et al.*, 2006).

The features of pectinases though vary with the working strain and diverse group of microorganisms biosynthesize single or a mixture of these pectinolytic enzymes (Castro *et al.*, 1992). Owing to its escalating demand in many industries, searching of pectinases from different novel and innate fungi and its apposite application at cost effective level is a continuous exercise. Hence, this study was aimed at the selection of a suitable strain for the biosynthesis of pectinase using agricultural byproducts as substrates and type of fermentation for maximum enzyme biosynthesis.

MATERIALS AND METHODS

Chemicals and substrates procured for the study: All chemicals implemented in this study were of Analytical Reagent (AR) grade and purchased from Sigma, Hi-Media Limited, SRL Pvt. Ltd. and Merck India Ltd. (Mumbai, India). Commercial citrus pectin was procured from a local supplier of chemicals from Bhubaneswar, Odisha, India. Pearl Millet (PM), Finger Millet (FM), Orange Peels (OP), Mustard oil Cake (MoC) and Chikling Vetch (CV) were obtained from the local market of Cuttack and Bhubaneswar, Odisha, India. The substrates were dried inside a hot air oven at 60°C for 24 h and processed for fermentation.

Screening of fungal isolates for pectinase activity: The seven day old pre-isolated fungi were point inoculated on modified Czapek-Dox agar supplemented with commercial citrus pectin

(1%, w/v) as the sole source of carbon and screened for pectinolytic activity by a modified plate method of Phutela *et al.* (2005). The sterilized and cooled medium (pH 6.5) was supplemented with norfloxacin at 300 mg/100 mL before pouring. The plates were incubated at 30±2°C for five days. The growth of fungi and hydrolysis pattern of pectin were evaluated qualitatively by visual inspection. The clearance zone formed around the colonies was determined using CTAB reagent. Suitable organism was selected and used in subsequent experiments.

Inoculum preparation: *Aspergillus terreus* NCFT 4269.10, a pectinase producing strain was used as the working fungus in the study (Sethi *et al.*, 2013a). The master culture was subcultured and one week old PDA slant culture was processed for inoculum build up using potato dextrose broth. Finally, about 1×10⁷ spores mL⁻¹ was used as the inoculum for fermentation (Sethi *et al.*, 2013b).

Fermentation study: Liquid static and shaking fermentation was carried out using 50 mL of sterilized fermentation medium having PM, FM, OP, MoC, CVP and commercial pectin as the sole source of carbon and substrate for pectinase. Each Erlenmeyer flasks having fermentation medium were inoculated separately with 1×10⁷ cells mL⁻¹ from 7 days old culture and incubated at 30±2°C at static condition. After 96 h, samples were processed for pectinase activity assay. Similarly, one set of the inoculated fermentation flasks were kept in a shaker-cum-incubator at 120 rpm for 96 h at 30±2°C. After completion of fermentation, 50 mL of cultured broth was centrifuged at 10,000 rpm for 10 min at 4°C to obtain a cell-free supernatant. The clear supernatant was filtered through Whatman No. 1 filter paper before pectinase activity assay.

Partial purification of pectinase: Purification of pectinase was performed by ammonium sulphate precipitation. Varying concentration of ammonium sulphate (20-80%, w/v) was added to the crude enzyme gently and mixed by magnetic stirrer at 4°C. The precipitated protein was recovered by cold centrifugation at 10,000 rpm for 15 min and dialyzed against phosphate buffer (pH 6.5). Pectinase activity and total protein content was measured thereafter.

Analytical method

Total protein content: Protein concentrations were determined according to Bradford (1976) taking Bovine Serum Albumin (BSA) as the standard.

Pectinase assay: The reagents was taken for the estimation of pectinase activity are given in Table 1 (Khairnar *et al.*, 2009).

All reagents were mixed by gentle shaking. Titrated the test and blank with Na₂S₂O₃ (100 mM) until it became light yellow. Then 1 drop of pectin indicator was added and continuously titrated with the reagent (Na₂S₂O₃) until solution becomes colourless.

Table 1: Reagents taken for the estimation of pectinase activity

Reagents	Test sample	Blank
Pectin solution (0.5%)	4.90	5.0
Pectinase	0.10	-
I ₂ /KI (50 mM/200 mM)	5.0	5.0
Na ₂ CO ₃ (1 M)	1.0	1.0
H ₂ SO ₄ (2 M)	2.0	2.0
Pectin indicator (1%)	1-2 drops	1-2 drops

Calculation:

$$\text{Enzyme (U mL}^{-1}\text{)} = (1 \times 100) \times \frac{\text{Sodium thiosulfate for blank (mL)} - \text{Sodium thiosulfate for test (mL)} \times \text{DF}}{5 \text{ mL} \times 0.1 \text{ mL} \times 2 \text{ mL}}$$

Biomass determination: The biomass was determined by measuring the dry weight of sample. The sample was centrifuged at 10,000 rpm for 15 min at 4°C and the fungal biomass was washed with sterilized double distilled water and filtered out using funnel and Whatman No.1 filter paper. The biomass obtained was dried overnight inside the hot air oven at 60°C till the constant weight was obtained. Then, the dry weight of the mycelia was weighed and calculated (Sethi *et al.*, 2013b).

Weight of biomass = Weight of filter paper with fungal biomass - Weight of blank and dry filter paper [Weight of biomass = Weight of organism].

Fermentation kinetics study: Different fermentation yield parameters ($Y_{e/s}$, $Y_{e/x}$ and $Y_{x/s}$) were calculated after the successful fermentation.

RESULTS AND DISCUSSION

Filamentous fungi display much unique individuality that ranks them as superior potential organisms for numerous industrial applications. Remarkably, they have the potentiality for best suited in different fermentation processes, biosynthesis of bulk amount of commercially significant enzymes, cultivation possibilities using cheapest substrates and low-cost-high valued biosynthesis in large bioreactors (De Vries and Visser, 2001; Aro *et al.*, 2005). Among many filamentous fungi, most exploited fungal genera for the biosynthesis of polymer-degrading enzymes are *Trichoderma* and a variety of strains of *Aspergillus* and *Penicillium* (Aro *et al.*, 2005; Cardoso *et al.*, 2008). Furthermore, the biosynthesis of microbial pectinase by fungi is solely dependent on the type of strain and substrate implemented for fermentation, growth conditions like pH, temperature, incubation period, methods of cultivation regimen, cell growth pattern, requirement of nutrients and minimization of cross-contamination during the entire fermentation. This study has emphasized on the fermentation of substrates for biosynthesis of pectinases by a filamentous fungus, *A. terreus* and the strategies for its partial purification for application and enhancement of competence in the food processing industry.

Selection of fungus for pectinase biosynthesis: Selection of potent microorganism and types of fermentation are vital for superlative growth and production of enzyme by fungi. Hence, prior to the biosynthesis of extracellular enzymes in Liquid Static Surface Fermentation (LSSF), it was wise to screen them for identifying the excellent producers of enzymes. Screening on modified Czapek Dox agar media was performed semiquantitatively by measuring the diameter of the hydrolyzing zones formed in the agar after CTAB staining correlating this zone size to the colony size. In this approach, pectin hydrolyzing capabilities of the fungal species were determined. The employment of pectin nutrient agar and CTAB for evaluating pectinase producing potentiality has been reported by Hankin and Anagnostakis (1975). In this study, total fifteen strains were tested for pectin degradation. Among the fifteen strains tested, performance of *Aspergillus terreus* was best in pectin hydrolyzing activity (2.75 cm) followed by *Aspergillus tamari* and *Aspergillus stellatus* (Fig. 1).

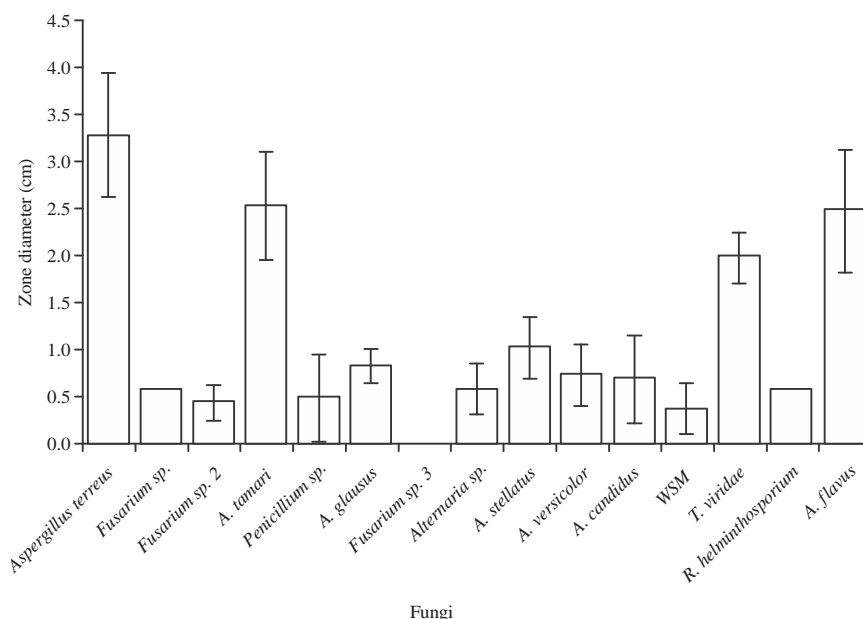


Fig. 1: Screening of fungal isolates for pectinase activity (Mean±SD)

On the basis of the area of clearance one out of fifteen fungal species, *Aspergillus terreus* was selected for subsequent experiments as it exhibited highest pectinolytic activity in pectin agar medium (Fig. 1). Similarly, Hankin and Anagnostakis (1975) first used the solid media to evaluate the pectin degrading ability of seven plants pathogenic, six saprophytic fungi and one leaf composting fungus.

Liquid static surface fermentation versus shaking fermentation: In spite of many fermentation processes, only two types are well accepted for pectinase biosynthesis, such as liquid static surface fermentation and solid-state fermentation. At industrial scale, most of the enzymes are biosynthesized by Liquid Static Shaking Fermentation (LSSF) techniques. In addition, enzymes are usually biosynthesized by LSSF due to its significance in downstream processing despite of its cost intensiveness for medium components (Prakasham *et al.*, 2006). Nonetheless, pectinase is an inducible enzyme and is generally induced in the presence of carbon sources such as pectin, its hydrolytic product or maltose (Morkeberg *et al.*, 1995). Pectinases of bacterial sources have long been employed in industries, but, they are now substituted by pectinase of fungal origin (Ogrydziak, 1993), as the extraction is comparatively easier and swiftly separated from mycelium (Phadatare *et al.*, 1993). Both LSSF and SSF processes are usually employed for biosynthesis of enzymes though various amendments have been suggested. Nevertheless, for cost-effective fermentation, nature, availability and cost of the substrates are of major concern. Thus, process optimization may demand the screening of several agro-industrial residues for its further exploitation in industries. Therefore, abundantly available agro-wastes were selected for screening. Among the various substrates tested, orange peel was found to be the suitable substrate for optimal pectinase biosynthesis (LSSF: 633.33 ± 57.73 U mL⁻¹, LShF: 733.33 ± 115.47 U mL⁻¹). The total protein content was also higher in OP (LSSF: 880.33 ± 12.5 µg mL⁻¹, LShF: 992.7 ± 4.16 µg mL⁻¹) when the culture was grown at static and shaking condition at 30°C for 96 h (Fig. 2). At par protein content was also found when the organism was grown with CV (LSSF: 870.7 ± 17.47 µg mL⁻¹, LShF: 989.3 ± 76.42 µg mL⁻¹) at static and shaking condition (Fig. 2).

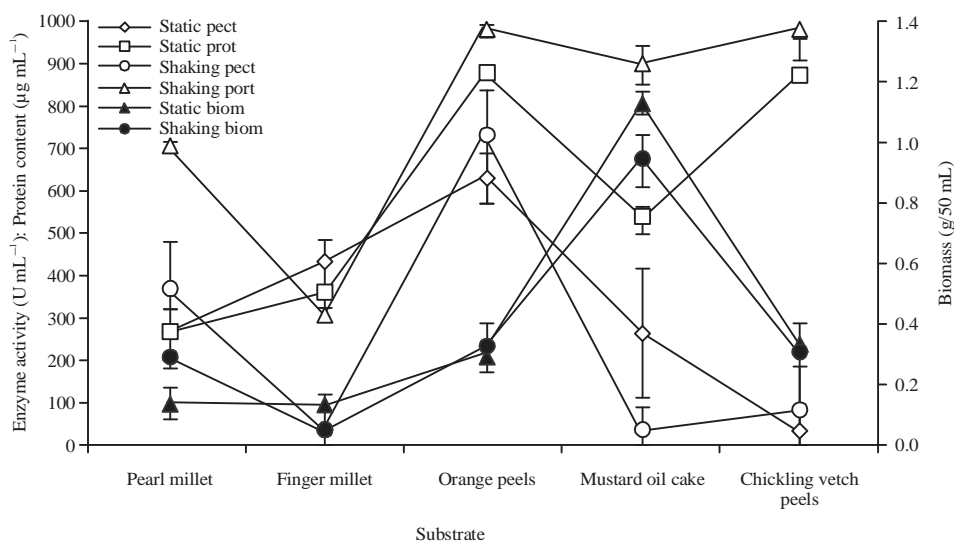


Fig. 2: Pectinase, total protein and biomass production using different substrates at both liquid static and shaking fermentation carried out at 30°C for 96 h using *Aspergillus terreus* NCFT 4269.10

Similarly, Adeleke *et al.* (2012) evaluated the potentials of fungi for the biosynthesis of pectinase and cellulase employing orange peels as the substrate using *Penicillium* sp. Fawole and Odunfa (1992) have reported that *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizopus* have high pectolytic activities. Galiotou-Panayotou *et al.* (1993) have reported biosynthesis of PG using submerged fermentation by *A. niger* in medium supplemented with citrus pectin. Hence, commercial use of orange peel powder for biosynthesis of pectinase can be highly inexpensive, which may trim down the processing cost to a greater extent. Citrus peel was also used for the formulation of fermentation medium by Dhillon *et al.* (2004) for the biosynthesis of pectinase. Employments of such byproducts in industries for the enzyme production in bulk can reduce processing costs as well can bring betterment to the environment and industry.

At the same time, the generation of biomass was also estimated along with the pectinase and total protein content estimation. It was concluded from the finding that the biomass production was dissimilar in response to different substrates. Maximum biomass was formed when OP was used for the fermentation at static condition followed by CVP (Fig. 2). But maximum biomass did not always support for the enhanced pectinase biosynthesis in most of the cases. In case of shaking fermentation, maximum biomass biosynthesis was attained with MoC followed by OP and CVP. Similarly, PM was at par with the biomass produced when shaking fermentation was carried out using OP.

Purification of enzyme: The result of the purified pectinase activity and total protein content is presented in Fig. 3.

After ammonium sulphate precipitation and desalting by dialysis, the enzyme preparation contained 9333 ± 1172 U (LSSF), 10133 ± 1097 U mL⁻¹ (LShF) of enzyme (Fig. 3). After purification by dialysis, a 3.86-fold purification and 27.63% yield was achieved in case of shaking condition.

Fermentation yield kinetics: After successful fermentation, the yield kinetics was calculated. The results obtained are presented in Table 2. It is evident from the table that the enzyme yield

Table 2. Fermentation yield kinetics of crude and purified pectinase produced at both liquid static and shaking fermentation

Substrates	Ye/s (U gds ⁻¹)				Ye/x (U g ⁻¹)				Yx/s (g gds ⁻¹)			
	LSSF	LShF	LSSF - purified	LShF - purified	LSSF	LShF	LSSF - purified	LShF - purified	LSSF	LShF	LSSF - purified	LShF - purified
Pearl millet	53.332	73.332	1760.0	1893.20	1918.410	1262.603	63309.35	32643.68	0.0279	0.0580	0.0279	0.0580
Finger millet	86.660	6.660	1706.6	1786.60	3209.850	666.660	63681.56	178666.70	0.0269	0.0100	0.0269	0.0100
Orange peels	86.660	126.660	1866.6	2026.66	1430.130	1936.780	30803.07	30988.77	0.0606	0.0654	0.0606	0.0654
Mustard oil cake	53.330	6.660	1833.2	1826.60	235.150	35.232	8083.48	9644.48	0.2268	0.1893	0.2268	0.1893
Chickling vetch peels	6.660	16.660	320.0	1673.33	98.318	268.800	4705.88	26989.25	0.0678	0.0621	0.0678	0.0621

LSSF: Liquid static surface fermentation, LShF: Liquid shaking fermentation, both the fermentations were carried out at 30°C for 96 h using *Aspergillus terreus* NCF 4269.10

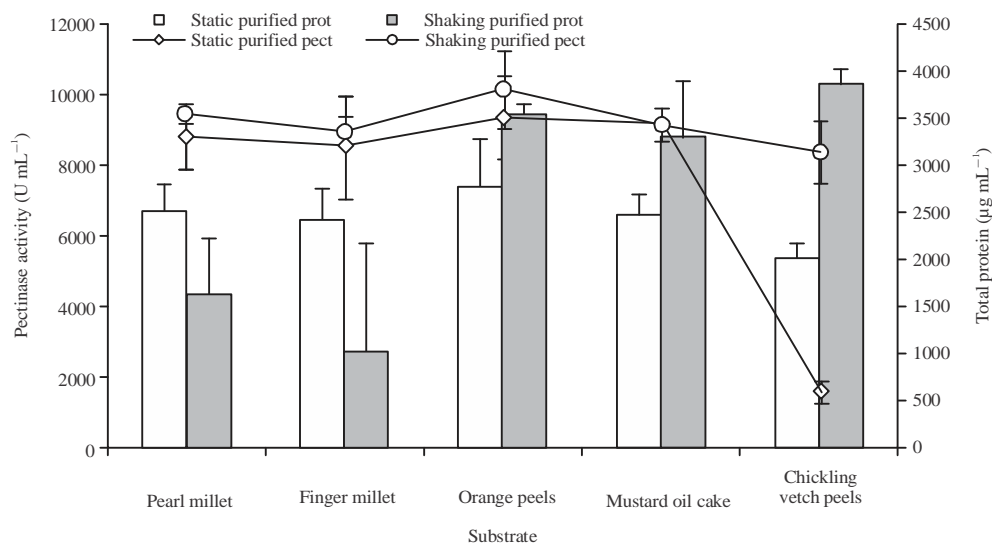


Fig. 3: Pectinase activity and total protein content obtained after partial purification of crude extracts produced using different substrates at both liquid static and shaking fermentation carried out at 30°C for 96 h using *Aspergillus terreus* NCFT 4269.10

per gram of substrate was maximal at both LSSF (1866.6 U gds⁻¹) and LShF (2026.7 U gds⁻¹) when biosynthesis of pectinase was carried out using OP. It can be stated that pectin is a high molecular weight acidic and heterogeneous structural polysaccharide (Janani *et al.*, 2011) which is mostly present in fruits and vegetables, but, the amount of pectin is excellent in citrus fruits like, orange peel (Blanco *et al.*, 1999). Therefore, in present days, citrus fruit peels and apple pomade are the principal resources of commercial pectin. To reiterate, during the extraction of juices, the use of pectinase to soft fruits guarantees the elevated yield of juice by partially destructing the pectin present (Itoandon *et al.*, 2011). Meanwhile, ratio of enzyme yield and biomass were best with OP as compared to the other substrates both in static and shaking fermentation at crude and purified conditions. Further, the yield of biomass per gram of substrate was not uniform as compared to the enzyme yield (Table 2). From this, it can be concluded that the biosynthesis of enzyme is not exclusively growth associated; rather it is growth independent and depends on the nature of the substrates available. Further, establishment of an economic production protocol is conceivably the main limitation in industrialization of novel sources of enzymes (Siddiqui *et al.*, 2013). Therefore, optimization of conditions and analyzing the fermentation kinetics, higher yields of pectinase at low-cost investment may be achieved.

CONCLUSION

Pectinases have gained significant recognition in biotechnological industries especially in food, beverage and textile sectors. Exploitation of such agro-based substrates like citrus peels and other wastes rich in pectin can produce high-valued products. Further, liquid shaking fermentation is more suitable for biosynthesis of pectinase as compared to the liquid static surface fermentation. For these reasons, biosynthesis of stable, highly active and high-valued enzymes requires a detailed study. Regardless of biotechnological improvements, more detailed studies on enzyme characterization and optimization of fermentation conditions are still essential for suitable applications in industries.

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REFERENCES

- Adeleke, A.J., S.A. Odunfa, A. Olanbiwonninu and M.C. Owoseni, 2012. Production of cellulase and pectinase from orange peels by fungi. *Nat. Sci.*, 10: 107-112.
- Aro, N., T. Pakula and M. Penttila, 2005. Transcriptional regulation of plant cell wall degradation by filamentous fungi. *FEMS. Microbiol. Rev.*, 29: 719-739.
- Beg, Q.K., M. Kapoor, R.P. Tiwari and G.S. Hoondal, 2001. Bleach-boosting of *Eucalyptus* kraft pulp using combination of xylanase and pectinase from *Streptomyces* sp. QG-11-3. *Res. Bull. Panjab Univ.*, 57: 71-78.
- Blanco, P., C. Sieiro and T.G. Villa, 1999. Production of pectic enzymes in yeasts. *FEMS Microbiol. Lett.*, 175: 1-9.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72: 248-254.
- Cardoso, P.G., J.B. Ribeiro, J.A. Teixeira, M.V. de Queiroz and E.F. de Araujo, 2008. Overexpression of the *plg1* gene encoding pectin lyase in *Penicillium griseoroseum*. *J. Ind. Microbiol. Biotechnol.*, 35: 159-166.
- Castro, P.M.L., P.M. Hayter, A.P. Ison and A.T. Bull, 1992. Application of a statistical design to the optimization of culture medium for recombinant interferon-gamma production by Chinese hamster ovary cells. *Applied Microbiol. Biotechnol.*, 38: 84-90.
- De Vries, R.P. and J. Visser, 2001. *Aspergillus* enzymes involved in degradation of plant cell wall polysaccharides. *Microbiol. Mol. Biol. Rev.*, 65: 497-522.
- Delgado, L., B.A. Trejo, C. Huitron and G. Aguilar, 1993. Pectin lyase from *Aspergillus* sp. CH-Y-1043. *Applied Microbiol. Biotechnol.*, 39: 515-519.
- Dhillon, S.S., R.K. Gill, S.S. Gill and M. Singh, 2004. Studies on the utilization of citrus peel for pectinase production using fungus *Aspergillus niger*. *Int. J. Environ.*, 61: 199-210.
- Favela-Torres, E., T. Volke-Sepulveda and G. Viniegra-Gonzalez, 2006. Production of hydrolytic depolymerising pectinases. *Food. Technol. Biotechnol.*, 44: 221-227.
- Fawole, O.B. and S.A. Odunfa, 1992. Pectolytic moulds in Nigeria. *Lett. Applied Microbiol.*, 15: 266-268.
- Galiotou-Panayotou, M., P. Rodis and M. Kapantai, 1993. Enhanced polygalacturonase production by *Aspergillus niger* NRRL-364 grown on supplemented citrus pectin. *Lett. Applied Microbiol.*, 17: 145-148.
- Hankin, L. and S.L. Anagnostakis, 1975. The use of solid media for detection of enzyme production by fungi. *Mycologia*, 67: 597-607.
- Itoandon, E.E., S.O.A. Olatope, F.A. Orji, O. Morenike, K.A. Shittu and L.O. Adebajo, 2011. Studies on production and activities of amylase, cellulase and pectinase enzymes of *Bacillus* and *Aspergillus* species from stale fast foods. *Nig. J. Agric. Food Environ.*, 7: 81-91.
- Janani, L.K., G. Kumar and K.V. Bhaskara Rao, 2011. Screening of pectinase producing microorganisms from agricultural waste dump soil. *Asian J. Biochem. Pharm. Res.*, 2: 329-337.
- Kashyap, D.R., P.K. Vohra, S. Chopra and R. Tewari, 2001. Applications of pectinases in the commercial sector: A review. *Bioresour. Technol.*, 77: 215-227.

- Khairnar, Y., V.K. Krishna, A. Boraste, N. Gupta and S. Trivedi *et al.*, 2009. Study of pectinase production in submerged fermentation using different strains of *Aspergillus niger*. *Int. J. Microbiol. Res.*, 1: 13-17.
- Morkeberg, R., M. Carlsen and J. Nielsen, 1995. Induction and repression of α -amylase production in batch and continuous cultures of *Aspergillus oryzae*. *Microbiology*, 141: 2449-2454.
- Naidu, G.S.N. and T. Panda, 1998. Production of pectolytic enzymes-a review. *Bioprocess Eng.*, 19: 355-361.
- Ogrydziak, D.M., 1993. Yeast extracellular proteases. *Crit. Rev. Biotechnol.*, 13: 1-55.
- Phadatare, S.U., V.V. Deshpande and M.C. Srinivasan, 1993. High activity alkaline protease from *Conidiobolus coronatus* (NCL 86.8.20): Enzyme production and compatibility with commercial detergents. *Enzyme Microb. Technol.*, 15: 72-76.
- Phutela, U., V. Dhuna, S. Sandhu and B.S. Chadha, 2005. Pectinase and polygalacturonase production by a thermophilic *Aspergillus fumigatus* isolated from decomposing orange peels. *Braz. J. Microbiol.*, 36: 63-69.
- Prakasham, R.S., C.S. Rao and P.N. Sarma, 2006. Green gram husk-an inexpensive substrate for alkaline protease production by *Bacillus* sp. in solid-state fermentation. *Bioresour. Technol.*, 97: 1449-1454.
- Ricard, M. and I.D. Reid, 2004. Purified pectinase lowers cationic demand in peroxide-bleached mechanical pulp. *Enzyme Microbial Technol.*, 34: 499-504.
- Salazar, L. and U. Jayasinghe, 1999. Fundamentals of purification of plant viruses. *Techniques in Plant, Virology, CIP., Training Manual, Virus Purification*, (International Potato Centre, Peru, pp: 1-10.
- Sethi, B.K., J.R. Rout, R. Das, P.K. Nanda and S.L. Sahoo, 2013a. Lipase production by *Aspergillus terreus* using mustard seed oil cake as a carbon source. *Ann. Microbiol.*, 63: 241-252.
- Sethi, B.K., P.K. Nanda and S.L. Sahoo, 2013b. Isolation, identification and conservation of potent hydrolase producer from different soils of Odisha, India. *Int. J. Pharma. Bio Sci.*, 4: 89-100.
- Siddiqui, M.A., V. Pande and M. Arif, 2013. Polygalacturonase production from *Rhizomucor pusillus* isolated from fruit markets of Uttar Pradesh. *Afr. J. Microbiol. Res.*, 7: 252-259.
- Sidkey, N.M., M.A. Abo-Shadi, R. Balahmar, R. Sabry and G. Badrany, 2011. Purification and characterization of α -amylase from a newly isolated *Aspergillus flavus* F2Mbb. *Int. Res. J. Microbiol.*, 2: 96-103.
- Singh, S.A., M. Ramakrishna and A.G.A. Rao, 1999. Optimization of downstream processing parameters for the recovery of pectinase from the fermented broth of *Aspergillus carbonarius*. *Process Biochem.*, 35: 411-417.
- Smith, J.E., 1996. *Biotechnology*. 3rd Edn., Cambridge Press, Cambridge.