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PRODUCTION AND PARTIAL CHARACTERIZATION OF PECTINASES FROM MANGO PEELS BY *Aspergillus tamarii*

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ABSTRACT

Pectinases are a group of enzymes that are able to breakdown or transform pectin. Sources of pectinase comprise a wide variety of bacteria, yeast and filamentous fungi, especially *Aspergillus* sp. In this study pectinases (polygalacturonase and pectin lyase) were produced from mango peels by *Aspergillus tamarii* in solid state fermentation and a fraction of the crude enzyme solution obtained by ultracentrifugation was used for partial characterization assay. The maximum polygalacturonase production was 141.0095 U/g at day 3, 6 and 9 of incubation while the maximum pectin lyase production was 5670.50 U/g obtained at day 6. The optimum temperature and pH for polygalacturonase activity was between 40 – 70°C and 5.0 respectively while that of pectin lyase was 60°C and 7.5 respectively. The polygalacturonase produced was stable between pH 3.6 – 10.0 and at a temperature range of 30 – 70°C while the pectin lyase was stable between pH 7.0 – 8.5 and at 40°C. Na⁺, Mn⁺, Cu²⁺ and Zn²⁺ caused a significant increase in the activity of polygalacturonase whereas Fe²⁺ and Mg²⁺ caused a significant decrease in its activity (P≤0.05). The activity of pectin lyase was significantly increased by Fe²⁺, Mn⁺ and Zn²⁺ but significantly decreased by Cu²⁺, Mg²⁺ and Na⁺ (P≤0.05). Mango peel is a cheap, available and valuable substrate for pectinase production which could be useful for industrial applications especially in the food industry for processing fruit juices.

Keywords: Pectinase, polygalacturonase, pectin lyase, solid state fermentation, mango peels

INTRODUCTION

Pectic substances are group of complex colloidal carbohydrate derivatives which occur in plants and contain a large proportion of anhydro-galacturonic units (Rahman, 1970). They exist abundantly in the middle lamella of plant cells (Willats et al., 2001). There are three types of pectic substances – pectic acids, pectin and protopectin. During ripening of fruits, protopectin is converted to pectic acid and pectin. Pectin is present in all stages of plant development (Parenicova, 2000).

Pectinase is the collective term for a group of enzymes that are able to breakdown or transform pectin. They are produced by plants and microorganisms (Kongruang and Penner, 2003). Pectinases include polygalacturonase (PG or PGase), pectin esterase (PE), pectin lyase (PL) and pectate lyase. The complete degradation of pectin is due to the synergistic action of methylesterase (EC.3.1.11.1), endo – polygalacturonase (EC.3.2.1.15), exopolygalacturonase (EC.3.2.1.67), endo-pectate lyase (EC.4.2.2.2), exopectate lyase (EC.4.2.2.9) and pectate lyase (4.2.2.10) (Rangarajan et al., 2010).

Mango (*Mangifera indica*) contain substantial amount of pectin having a high gelling grade (Bhardwaj and Garg, 2010). In the processing of mango products, mango peels is a major by-product which ends up as a waste product. However, mango peels can be used as a valuable, economic and abundant media source for the commercial production of natural enzymes such as pectinases (Loelillet, 1994). Pectin acts as an inducer for the production of pectinolytic enzymes by microbial systems. The current commercial sources of pectinase comprise a wide variety of bacteria, yeast and filamentous fungi (Yazid et al., 2011). Amande and Adebayo-Tayo (2012) noted that among the filamentous fungi *Aspergillus* spp. are the strains of choice for polygalacturonase production.

Pectinases are widely used in food industries for juice extraction and clarification, coffee and tea fermentation, oil extraction, improvement of chromacity and stability of red wines (Amande and Adebayo-Tayo, 2012). They are also useful in the textile industries, paper and pulp industries and in waste water treatment. About 75% of estimated sales value of industrial enzymes today is contributed by pectinase (Satyanarayana and Panda, 2002) and microbial pectinases account for 25% of the global food enzymes sales (Jayani et al., 2005).

The objective of this study was to evaluate the production of pectinases (polygalacturonase and pectin lyase) in solid state fermentation by *Aspergillus*

tamarii using mango peels as substrate and to partially characterize the enzyme produced.

MATERIAL AND METHODS

Microorganism

Aspergillus tamarii used in the study was isolated from contaminated fruits reported earlier by Amande and Adebayo-Tayo (2012). It was maintained on Potatoe Dextrose Agar (PDA) and preserved at 4°C for further experimental work.

Inoculum Preparation

To fully sporulate, *Aspergillus tamarii* was grown on PDA at room temperature (25±2°C) for 7 days. A spore suspension was prepared by adding one disc (using a 7mm-diameter cork borer) to 5ml of sterile distilled water and shaken vigorously. The spore suspension obtained was used as the inoculum.

Substrate

Mango peels were minced into pieces and hot air oven dried at 55°C until a constant weight was achieved (Rangarajan et al., 2010). They were ground into powder (particle size 300µm) and sealed in polyethylene bags for further use.

Solid State Fermentation and Extraction of Enzymes

Mango peels powder was used as the substrate. Solid state fermentation was carried out in 250ml Erlenmeyer flask containing 15g of substrate and 10ml of distilled water. The substrate was autoclaved at 121°C for 15 min. The inoculum (1ml) was added and incubated at room temperature (25±2°C) for 12 days.

The enzyme was harvested on day 3, 6, 9 and 12. Totally 30 ml of sterile distilled water was added to the flask and shaken vigorously to mix. The mixture was filtered with a nylon cloth and centrifuged at 15,000 rpm for 10 minutes. The supernatant was used as the crude enzymes extract and subjected to enzyme assays.

Enzyme Assay

Polygalacturonase and pectin lyase assay was done at the 3rd, 6th, 9th and 12th day of incubation. **Polygalacturonase (PG) activity** was determined by measuring the release of reducing groups from citrus pectin using the 3, 5-dinitrosalicylic acid reagent assay (Miller, 1959). The reaction mixture containing 0.8% citrus pectin in 0.2M acetate buffer, pH 5.0 and 0.2ml of crude enzyme was incubated at 40°C for 10 min (Amande and Adebayo-Tayo, 2012). One unit of enzyme activity (U) was defined as the amount of enzyme which released one μ mole of galacturonic acid per minute. **Pectin lyase (PL) activity** was determined by measuring the increase in absorbance at 235nm (using Lambda 25 UV/Vis Spectrometer) of substrate solution (0.8ml 1% citrus pectin in 0.2M tris-HCl buffer, pH 8.5), hydrolyzed by 0.2ml enzyme solution at 40°C (Martin et al., 2004). One unit of enzymatic activity was defined as the amount of enzyme which released one μ mol of unsaturated uronide per minute based on the molar extinction coefficient (5500) of the unsaturated product (Albersheim, 1966).

Enzyme Characterization

A fraction of the crude enzyme solution obtained after filtration and ultracentrifugation using Hitachi high speed refrigerated centrifuge (Himac CR21GI) at 15,000 rpm for 10 minutes was used for characterization assay.

Optimum pH and Temperature for Enzyme Activity

The enzyme activity was determined at 50°C in different pH using as buffers sodium acetate (pH 3.0 – 5.0), phosphate (pH 5.0 – 7.0), tris-HCl (pH 7.0 – 8.5) and glycine-NaOH (pH 8.5 – 11.0). The optimum temperature was assayed by incubation of the reaction mixture at temperature of 30 -70 °C at optimum pH.

pH and Temperature Stability

Enzyme solution was dispersed 1:1 in 0.2M buffer solutions at pH 3.0 – 5.0 (sodium acetate), pH 5.0 – 7.0 (phosphate), pH 7.0 – 8.5 (tris-HCl) and pH 8.5 – 11.0 (glycine-NaOH) and were maintained at 25°C for 24 hours. An aliquot was used to determine the remaining activity at the optimum pH and temperature of the enzymes.

For temperature stability determination, the enzyme solution was incubated at different temperatures (30 – 70°C) for 1hour at pH 5.0. An aliquot was withdrawn and placed on ice before assaying for residual pectinase activity at optimum pH.

Effect of metallic Salts on Pectinase Activity

The effect of various metal ions on pectinase activity was tested at 10 mM. The salts studied included MnSO₄, FeSO₄, ZnSO₄, MgSO₄.7H₂O, CuSO₄ and NaCl. The crude enzyme solution was dispersed 1:1 in 10 mM of the metal ion solution. Crude enzyme solution dispersed 1:1 in sterile deionized water was used as the control. An aliquot (0.2 ml) was drawn from the mixture and analyzed for pectinase activity.

Statistical Analysis

Results obtained from this study were subjected to analysis of variance using one way ANOVA and differences between means were separated by Duncan Multiple Range Test (Steel and Torrie, 1980; Duncan, 1955).

RESULTS AND DISCUSSION

In the present study, the maximum polygalacturonase (PG) production by *Aspergillus tamarii* was 141.0095 U/g at 3 – 9 days of incubation while the maximum pectin lyase (PL) produced was 5670.50 U/g obtained at day 6 (Table 1). *Aspergillus tamarii* produced pectinases using mango peel as the sole carbon source. Mango peel is a rich source of pectin and a good substrate for pectinase production. This finding is in agreement with an earlier report by Loeliliet (1994). The maximum pectinase (PG and PL) production was observed at day 6 (144 hours) of fermentation. This is in agreement with an earlier finding by Amande and Adebayo-Tayo (2012) that the maximum PG production by *Aspergillus sclerotioniger* using mango peels was obtained at day 6. Martin et al. (2004) also detected the highest PL activity produced by *Moniella* sp. at 144 hours.

The optimum pH for PG activity was 5.0 (Fig. 1) while the optimum pH for PL activity was 7.5 (Fig. 2). Several other researchers reported optimum PG activity at pH 5.0 (Beg et al., 2000; Afifi et al., 2002; Maciel et al., 2011). Tucker and Seymour (2002) maintained that PG had maximal activities at slightly acidic pH. Lisker et al. (1975) and Hamdy (2005) also reported PL with optimum activity at pH 7.5. Lisker et al. (1975) isolated PL from *Rhizoctonia solani* with an optimum pH of 7.5.

The optimum temperature for PG activity was found to be between 40 - 70°C (Fig. 3) while the optimum temperature for PL activity was 60°C (Fig. 4). Blanco

et al. (1999) reported some yeasts (*Tephrosia candida* and *Kluyveromyces fragilis*) produced PG with maximal activities at 60°C. Favela-Torres et al. (2006) and Onilude et al. (2009) made similar observations. Hayrannisa et al. (2010) reported the optimum activity of pectin lyase produced by *Bacillus pumilus* (P9) to be 60°C. Pectin lyase secreted by *Rhizopus oryzae* expressed its maximum activity at 50°C (Hamdy, 2005).

The PG was reasonably stable over the pH range of 3.6 - 10.0 (Fig. 5) and the temperature range of 30 - 70 °C for 1hr (Fig. 7). However, PL was only stable between pH 7.0 – 8.5 (Fig. 6) and at 40°C for 1hr but was inactivated at higher temperatures (Fig. 8). Martin et al. (2004) observed that PG was stable in the acidic to neutral pH range and at 60°C for 1hr whereas PL was stable at acidic pH and at 60°C for 5 hrs. Endo-PG and PL produced by *Aspergillus niger* MTCC 281 were stable at 50°C after 1hr but were inactivated at higher temperatures (Palaniyappan et al., 2009). Silva et al. (2002) showed that at 40°C for 1hr, the activities of PG and PL were 100% and 80% retained. The stability shown by the enzyme is sufficient for application for the processing of fruit juices.

The effect of metallic ion on pectinases activity is shown in Table 2. Na⁺, Mn²⁺, Cu²⁺ and Zn²⁺ caused a significant increase in the activity of PG whereas Fe²⁺ and Mg²⁺ caused a significant decrease in the PG activity (P<0.05). The activity of pectin lyase was significantly increased by Fe²⁺, Mn²⁺ and Zn²⁺ but significantly decreased by Cu²⁺, Mg²⁺ and Na⁺ (P<0.05). Hamdy (2005) observed that ions of Mg, Na and K showed a stimulatory effect and ions of Zn, Co, Mn and Hg showed inhibitory effects on PL secreted by *Rhizopus oryzae*. Spalding and Abdul-Baki (1973) also observed that manganese ions stimulated pectin lyase activity. However, Yandav and Shastri (2005) reported that Ca²⁺, Cu²⁺, Fe³⁺, Mn²⁺, Mo⁴⁺, Ba²⁺, Co²⁺, Ni²⁺, Pb²⁺ and Ag⁺ did not stimulate pectin lyase activity.

Table 1 Production of polygalacturonase and pectin lyase by *Aspergillus tamarii* using mango peels as substrate in solid state fermentation

Incubation time	Polygalacturonase (U/g)	Pectin Lyase (U/g)
Day 3	141.0093 ^a	3261.55 ^b
Day 6	141.0095 ^a	5670.50 ^a
Day 9	141.0095 ^a	1343.55 ^c
Day 12	10.00329 ^b	944.55 ^d

Values with the same letter in a column are not significantly different (P<0.05)

Table 2 Effect of metallic ions on pectinase activity

Chemical compound	Polygalacturonase activity (U/g)	Pectin lyase activity (U/g)
Control	93.4636 ^c	1842.55 ^d
NaCl	107.7459 ^a	1455.55 ^b
MnSO ₄	105.2850 ^c	5135.55 ^b
FeSO ₄	73.3366 ^f	6250.50 ^a
ZnSO ₄	100.5719 ^d	2956.55 ^c
MgSO ₄ .7H ₂ O	67.6919 ^e	1546.55 ^f
CuSO ₄	105.5977 ^b	1782.55 ^c

Values with the same letter in a column are not significantly different (P<0.05)

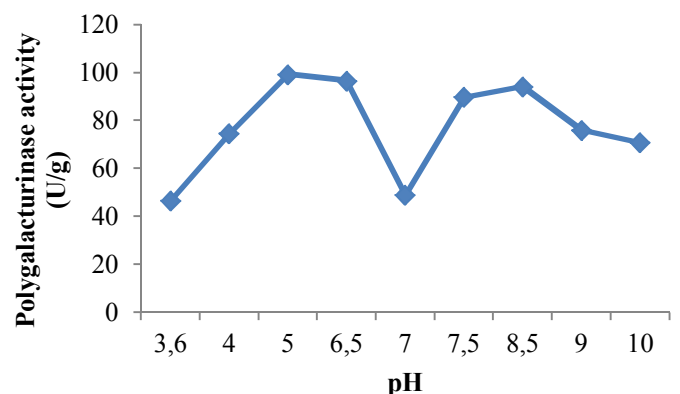


Figure 1 Effect of pH on polygalacturonase activity

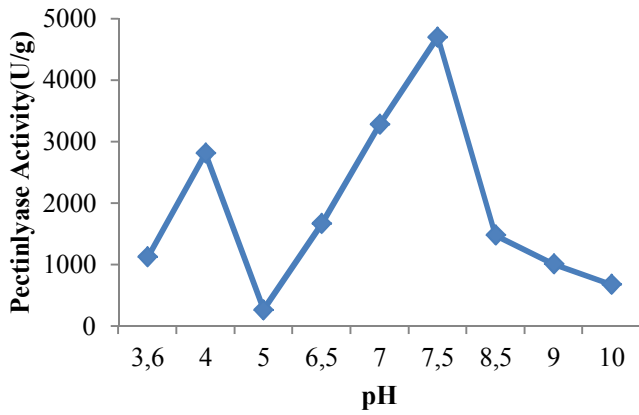


Figure 2 Effect of pH on pectin lyase activity

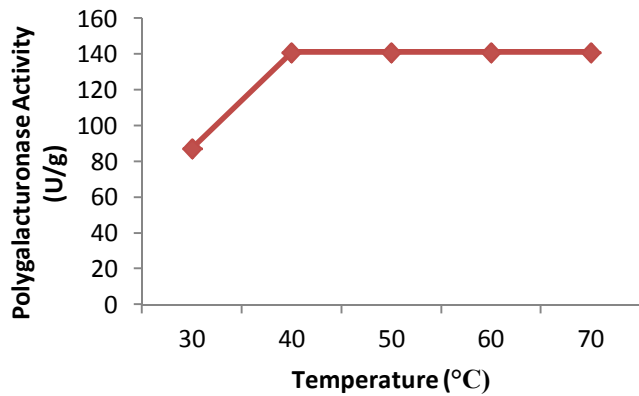


Figure 3 Effect of temperature on polygalacturonase activity

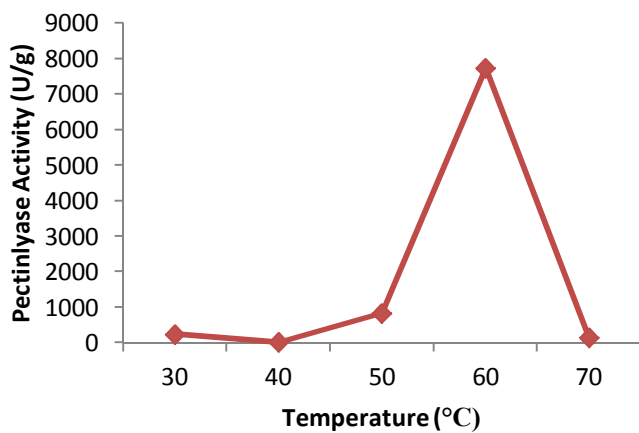


Figure 4 Effect of temperature of pectin lyase activity

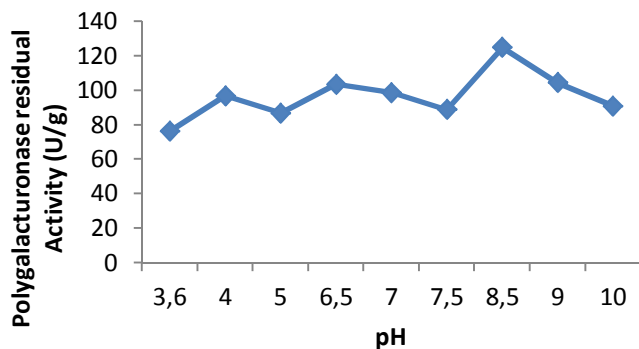


Figure 5 pH stability of polygalacturonase produced by *Aspergillus tamarii* in solid state fermentation

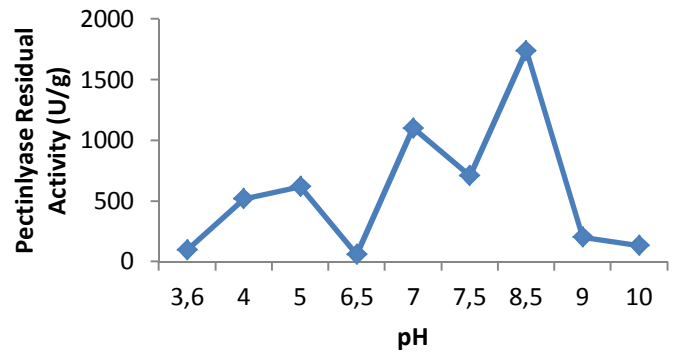


Figure 6 pH stability of pectin lyase produced by *Aspergillus tamarii* in solid state fermentation

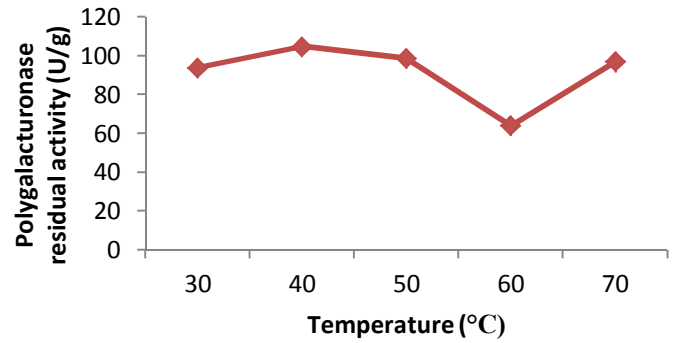


Figure 7 Temperature stability of polygalacturonase produced by *Aspergillus tamarii* in solid state fermentation

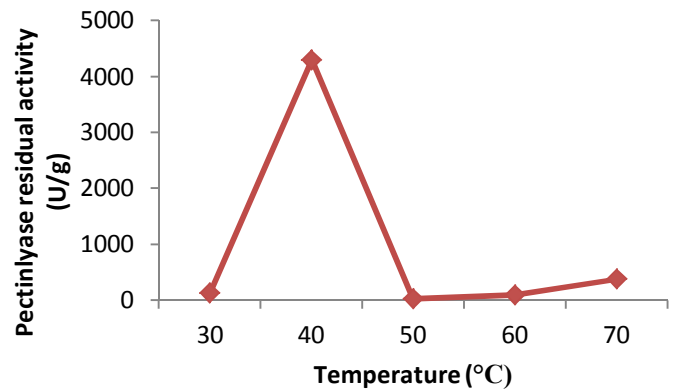


Figure 8 Temperature stability of pectin lyase produced by *Aspergillus tamarii* in solid state fermentation

CONCLUSION

Mango peel is a cheap, available and valuable substrate for pectinase production by *Aspergillus tamarii* in solid state fermentation at an optimum incubation time of 144 hrs (day 6). The optimum temperature and pH for polygalacturonase activity is between 40 – 70°C and 5.0 respectively while that of pectin lyase is 60°C and 7.5 respectively. The PG is stable between pH 3.6 – 10.0 and at a temperature range of 30 – 70°C while the PL is stable between pH 7.0 – 8.5 and at 40°C. The polygalacturonase and pectin lyase produced by *Aspergillus tamarii* could be found useful for industrial applications especially in the food industry for fruit processing.

REFERENCES

AFIFI, A.F. , FAWZI, E.M., FOAAD, M.A. 2002. Purification and characterization of Pectin lyase produced by *Curvularia inaequalis* NRRL 13884 on orange peels waste solid state culture. *Annals of Microbiology*, 52, 287-297.
 ALBERSHEIM, P. 1966. Pectin lyase from fungi. *Methods in Enzymology*, 8, 628-631.
 AMANDE, T., ADEBAYO-TAYO, B. 2012. Screening of new isolates fungal strains for polygalacturonase production in submerged fermentation. *Innovative Romanian Food Biotechnology*, 11, 15 – 22.

- BEG, Q.K. , BHUSHAN, B. , KAPOOR, M. , HOONDAL, G.S. 2000. Production and characterization of thermostable xylanase and pectinase from *Sreptomyces* sp. QG-11-3. *Journal of Industrial Microbiology and Biotechnology*, 24, 396–402.
- BHARDWAJ, V., GARG, N. 2010. Exploitation of microorganism for isolation and screening of pectinase from the environment. *Globelic* (8th International conference held at University of Malaya, Kuala Lumpur, Malaysia, 1 – 3 November, 2010), 1 – 16.
- BLANCO, P. , SIEIRO, C. , VILLA, T.G. 1999. Production of pectic enzymes in yeasts, *FEMS Microbiology Letters*, 175, 1–9.
- DUNCAN, D. B. 1955. Multiple Range and Multiple F-tests. *Biometrics*, 11, 1 – 42.
- FAVELA-TORRES, E., VOLKE-SEPULVEDA, T., VINIEGRA- GONZALEZ, G. 2006. Production of Hydrolytic Depolymerizing Pectinase. *Food Technology and Biotechnology*, 44(2), 221-227.
- HAMDY, H.S. 2005. Purification and Characterization of the pectinlyase from *Rhizopus oryzae* grown on orange peels. *Annals of Microbiology*, 55(3), 205-211.
- HAYRUNNISA, N., ESEN, T., AHMET, A., MEDINE, G., NAZAN, D. 2010. Production of novel pectinlyase from *Bacillus pumilus* (P9), Purification and characterization and Fruit juice Application. *Romanian Biotechnological Letters*, 15(2) .
- JAYANI, R.S., SAXENA, S., GUPTA, R. 2005. Microbial pectinolytic enzymes: a review. *Process Biochemistry*, 40, 2931 – 2944.
- KONGRUANG, S., PENNER, M.H. 2003. Detecting enzymatic activity: A case study of polygalacturonase. *Current protocols in food and analytical chemistry*. John Wiley and sons ,Inc.C.1.2.1-C.1.2.14.
- LISKER, N., KATAN, J., HENIS Y. 1975. Sequential production of polygalacturonase, cellulose and pectin lyase by *Rhizoctonia solani*. *Canadian Journal of Microbiology*, 22, 1298 – 1304.
- LOELILLET, D. 1994. The European mango market: A promising tropical fruit. *Fruit*, 49, 332 – 334.
- MACIEL, M. H.C., HECULANO, P.N., PORTO, T.S., TEIXEIRA, M.F.S., MOREIRA, K.A., SOUZA-MOTTA, C.M. 2011. Production and Partial Characterization of pectinases from forage palm by *Aspergillus niger* URM4645. *African Journal of Biotechnology*, 10(13), 2469 – 2475.
- MARTIN, N. , SOUZA, S.R.D. , SILVA, R.D., GOMES, E. 2004. Pectinase Production by Fungal strains in solid state fermentation using Agro-Industrial Byproducts. *Brazilian Archives of Biology and Technology*, 47(5), 813-819.
- MILLER, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Analytical Chemistry*, (31), 426-428.
- ONILUDE, A. A., ODENIYI, O.A., AYODELE, M.A. 2009. Production characteristics of cellulose/polygalacturonase by *Bacillus coagulans* strain from a fermenting palm – fruit industrial residue. *African Journal of Microbiology Research*, 3(8), 407-417.
- PALANIYAPPAN, M., VIJAYAGOPAL, V., VISWANATHAN, R., VIRUTHAGIRI, T. 2009. Screening of natural substrates and optimization of operating variables on the production of pectinase by submerged fermentation using *Aspergillus niger* MTCC 281. *African Journal of Biotechnology*, 8 (4), 682 – 686.
- PARENICOVA L. 2000. Pectinase of *Aspergillus niger*: A molecular and biochemical characterization. PhD. Thesis .Department of Industrial Microbiology. Wogeningen University. Vii + 200.
- RAHMAN, F. 1970. Studies on pectinesterase. PhD Thesis.Institute of Chemistry.University of Punjab. Xi + 260.
- RANGARAJAN, V., RAJASEKHARAN, M., SRIGANESH, K., VAITHEESWARAN, O. 2010. Pectinase production from orange peel extract and dried orange peel solid as substrate using *Aspergillus niger*. *International Journal of Biotechnology and Biochemistry*, 6 (3), 445-453.
- SATYANARAYANA, N.G., PANDA, T. 2002. Purification and biochemical properties of microbial pectinases- a review. *Process Biochemistry*, 38, 987 – 996.
- SILVA, D., MARTINS, E. S., SILVA, R. , GOMES, E. 2002.Pectinase production from *Penicillium viridicatum* RFC3 by solid state fermentation using agricultural residues and agro-industrial by-product. *Brazilian Journal of Microbiology*, 33, 318-324.
- SPALDING, D.H., ABDUL-BAKI, A.A. 1973. In vitro and in vivo production of pectin lyase by *Penicillium expansum*. *Phytopathology*, 63, 231-235.
- STEEL, R.G.D., TORRIE, J.H. 1980. Principles and Procedures of Statistics 2nd edn. McGraw – Hill Book Co., New York, USA. 633p.
- TUCKER, G.A., SEYMOUR, G.B. 2002. Modification and degradation of proteins. *Pectins and Their Manipulation*. Seymour G.B. , Knox J.P (eds.). Blackwell Publishing, Oxford, 150-173.
- WILLATS, W.G.T., MCCARTNEY, L. , KNOX, J.P. 2001. In-situ analysis of pectic polysaccharides in seed mucilage and at the root surface of *Arabidopsis thaliana*. *Planta*, 213, 37-44
- YANNAV, S., SHASTRI, N.V. 2005. Partial purification and characterization of a pectin lyase produced by *Penicillium oxalicum* solid state fermentation. *Indian Journal of Biotechnology*, 4, 501-505.
- YAZID, A.M.M., MEHRNOUSH, A. , SARKER, M. Z. I. , MUSTAFA, S. 2011. Direct purification of pectinase from mango (*Mangifera indica* Cv *Chokanan*) peel using PEG/Salt-Based aqueous two phase system. *Molecule*, 16, 8419 – 8427.