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RESEARCH ARTICLE

SCREENING OF NEW ISOLATES FUNGAL STRAINS FOR POLYGALACTURONASE PRODUCTION IN SUBMERGED FERMENTATION

Tivkaa AMANDE[1]*, Bukola ADEBAYO-TAYO[2]

[1]*Department of Microbiology, Faculty of Science, University of Uyo, Uyo, AkwaIbom State, Nigeria*
[2]*Department of Microbiology, Faculty of Science, University of Ibadan, Ibadan, Oyo State, Nigeria*

Abstract
Thirty–two fungi isolate belonging to the genera *Aspergillus*, *Fusarium* and *Mucor* were isolated from fungal contaminated fruits (Green beans, grape, cucumber, plantain, banana and oranges**).** Nine pectinolytic fungi were selected based on the screening of the isolates on solid agar using pectin as the single carbon source. The selected strains belong species: *Fusarium compactum, Aspergillus flavus, Aspergillus tamarii, Aspergillus sclerotioniger, Aspergillus parasiticus, Aspergillus terreus, Mucor piriformis, Aspergillus piperis* and *Aspergillus niger*. The selected isolate were used for polygalacturonase production in submerged fermentation using different substrates derivated from fruits, pure citrus pectin, mango peels, watermelon peels, plantain peels and banana peels. The polygalacturonase yield ranged from 0.0212 - 5.8850 U/ml in which *Aspergillus tamari* produced the highest level of enzyme by cultivation on medium containing pure citrus pectin. The studies confirm that some selected fungal species isolated from contaminated fruits can be used for polygalacturonase production.

Keywords: *Aspergillus spp., Fusarium spp., Mucor spp., microbiota of fruits, polygalacturonase*

Introduction

Polygalacturonase (PG or PGase, E.C.3.2.1.15) is hydrolytic pectin depolymerase produced by both microorganisms and plant tissues (Kongruang and Penner, 2003). In apple, PG has been shown to be involved in fruit ripening and softening via degradation of apple cortical cell walls (Wu *et al*., 1993).

It exists in two forms; Endo-PG and Exo-PG. Both types of enzymes act only on pectin with a degree of esterification of less than 50-60% (Aehle, 2007). Endo-PG acts randomly at the α-1,4-polygalacturonic backbone and results in a pronounced decrease in viscosity. Exo-PGs act at the non-reducing ends of the chain. Exo-PG releases small fragments from the chain and does not significantly reduce the viscosity.

Pectinase is produced by a large number of microorganisms including bacteria, actinomycetes, yeasts, and filamentous fungi (Kurowski and Dunleavy, 1976, Soares *et al*., 1999, Oliveira *et al*., 2005, Ladjama *et al*., 2007, Kwi *et al*., 1994, Gomes *et al*., 2009, Martin *et al*., 2004 and Teixeira *et al*., 2000). Bacteria produce mainly alkaline and thermostable PGases, whilst fungi are the major producers of acidic PGases (Favela-Torres *et al*., 2006). At present, majority of these commercial preparation of pectinase are obtained from fungi, since, they produce different extracellular enzymes with pectinolytic activity (Aguiler and Huitron, 1987). More than 30 different genera of bacteria, yeasts and moulds have been used for the production of PGases (Favela-Torres *et al*. 2005).

The substrates commonly used for polygalacturonase production are of plant origin. Agro-wastes have been used increasingly for enzyme production because they contain large amounts of cellulose, hemicelluloses and pectin, which could serve as inducers for the production of cellulase, xylanase and pectinase respectively

*Corresponding author: tivkaajoe@yahoo.com

(Patil and Dayanand, 2006). Several agro - industrial wastes and by – products such as orange baggasse, mandarin orange peels, sugar cane bagasse, wheat bran and other food processing waste have been used as substrates for depolymerizing enzyme production (Martin *et al*., 2002, Yandav and Shastri, 2005, Silva *et al*., 2002, Cavalitto *et al*.,1996 and Zheng and Shetty, 2000).

Pectinases are widely used in food industries for fruit juice extraction, coffee and tea fermentation, oil extraction, improvement of chromaticity and stability of red wines etc. Fungal polygalacturonase are used in industrial processes for juice clarification (Gupta and Singh, 2004). More so, pectinases are used in the textile industries, paper and pulp industries and in waste water treatment.

This study was carried out to identify polygalacturonase producers from fungi contaminated fruits and to use the isolates for the production of the enzymes by cultivation in submerged conditions on media based on specific agro- wastes produced in Nigeria.

Materials and Methods

Agro-waste samples collection and processing

Fresh banana, plantain, watermelon, and mangoes were purchased from Bodija Market in Ibadan, Oyo state, South West Nigeria and were conveyed in clean plastic containers to the Postgraduates Laboratory of the Microbiology Department, University of Ibadan. They were washed thoroughly with tap water to remove adhering substances. Using a clean knife, the fruits were peeled into clean trays and they were labeled accordingly. The peels were minced into pieces and hot air oven dried at 55^{o}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.

Isolation and identification of fungi

The test organisms were isolated from green beans, grape, cucumber, plantain, banana and oranges by cultivation on Potatoes Dextrose Agar (PDA). Pure cultures were obtained by subculturing 3 – 4 times on PDA and were maintained on the same media at 4^{o}C for further experimental work. Identification of genus was based on cultural and microscopic characteristics by using Fungi Compendium (Domsch and Anderson, 1980) and others literature data (Singh *et al*., 1991; Samson *et al*., 2004).

Screening of strains with pectinolytic potential

The isolates were screened for their pectinase producing ability by cultivation medium containing 1% citrus pectin, 0.14% $(NH_4)_2$ SO_4 ; 0.20% K_2PHO_4; 0.02% $MgSO_4.7H_2O$; 0.10% nutrient solution(5mg/l $FeSO_4.7H_2O$; 1.6mg/l $MnSO_4.H_2O$; 1.4mg/l $ZnSO_4.7H_2O$; 2.0mg/l $CoCl_2$), 3% agar, pH 5.0 (Martin *et al*., 2004).

Using a sterile laboratory cork borer (7mm diameter), one disc of fungal hyphae from leading edge of actively growing colonies was cut on Petri plate. With a sterile inoculating needle, the hypha was then transferred to the center of the Petri dish containing the solidified basal medium. The plates were incubated at room temperature (25±2^{o}C) for 6days. Selection was done on the basis of the extent of growth from the center of the medium by measuring the diameter of colonies at different incubation time, 2, 3, 4, 5 and 6 days respectively.

Submerged fermentation

The basal liquid medium contained 0.14% $(NH_4)_2SO_4$, 0.6% K_2HPO_4, 0.20% KH_2PO_4, and 0.01% $MgSO_4.7H_2O$, pH 5.0 (Soares *et. al*. 1999) was supplemented with 1% of the following carbon sources, pure pectin, plantain peel, banana peels, mango peel and water melon peel powder, added separately. A volume of 25ml of the basal medium was added into 125ml Erlenmeyer's flasks and labeled appropriately. The flasks were autoclaved at 121^{o}C for 15minutes. Each flask was inoculated with a suspension containing 10^6 cells/ml of the selected pectinolytic isolates. The flasks were then incubated for 12days at room temperature (25±2^{o}C) in a rotary shaker (150rpm).

Enzyme Assay

The filtrates obtained after biomass separation after incubation was stored at 4^{o}C for enzyme assay. Polygalacturonase assay and protein estimation was done on day 3, 6, 9 and 12 respectively.

Polygalacturonase (PG) activity was determined by measuring the release of reducing groups from citrus pectin using the 3,5-dinitrosalicyclic acid reagent assay (Miller,1959). The reaction mixture containing 0.8ml 1% citric pectin in 0.2M acetate buffer, pH 5.0 and 0.2ml of crude enzyme, was incubated at 40^{o}C for 10 minutes using a modified method of Soares *et al.*, (1999). One unit of enzyme activity (U) was defined as the amount of enzyme which released one µmole of galacturonic acid per min.

Total protein content estimation

The estimation of total protein was done by Biuret method using 5mg albumin/ml as the protein standard. A volume of 3ml of Biuret reagent was added to 2ml of test protein solution in a sterile test tube and the mixture was properly mixed. The tubes were then warmed at 37^{0}C for 10 min with shaking in a water bath. The tubes were cooled and absorbance was read at 540 nm using Lambda 25 UV/Vis Spectrophotometer. An etalon curve was used for protein concentration estimation, in mg/ml.

Statistical analysis

Results obtained in 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

A total of 32 fungal strains belonging to the genera *Aspergillus*, *Fusarium* and *Mucor* were isolated from surface of some contaminated fruits, green beans, grape, cucumber, plantain, banana and oranges. The isolates strains were classified as *Fusarium compactum*, *Aspergillus flavus*, *Aspergillus tamarii*, *Aspergillus sclerotioniger*, *Aspergillus parasiticus*, *Aspergillus terreus*, *Mucor piriformis*, *Aspergillus piperis* and *Aspergillus niger*.

Table 1. *Identified pectinolytic strains isolates and their frequency of occurrence*

Fungal strain code	Classification	Percentage frequency of occurrence
BNa	*Fusarium compactum*	40
CUMa	*Mucor piriformis*	50
GB	*Aspergillus flavus*	25
GRP2	*Aspergillus terreus*	22.2
GB1a	*Aspergillus niger*	37.5
GRP1	*Aspergillus sclerotioniger*	33.3
PLTN1	*Aspergillus parasiticus*	25
ORGi	*Aspergillus piperis*	25
GRP2c	*Aspergillus tamarii*	44.4

Key of isolation sources: BN = Banana, CUM = Cucumber, GB = Green Beans, GRP = Grape, PLTN = Plantain, ORG = Orange

Table 2. *Screening of the fungal strains isolates for pectinase production on stationary cultivation on slant agar medium*

Fungal strain code	Colonial growth, during cultivation, cm				
	Day2	Day3	Day4	Day5	Day6
BNa	1	1.5	3	3.5	4
CUMa	1.5	1.8	2.5	2.8	3
GB	1.8	2	3	3.2	4
GRP2	0.3	0.3	0.4	0.4	0.5
GB1a	0.6	1.2	2	2.5	3
GRP1	0.6	1.5	3	3.5	3.8
PLTN1	1.6	2	2.5	3	3.5
ORGi	0.4	0.7	1.2	1.5	2
GRP 2c	1.5	2.2	2.8	3.5	4

The fungi isolate and their percentage frequency of occurrence is shown in Table 1. *Mucor piriformis* had the highest frequency of occurrence (50%) followed by *Aspergillus tamarii (*44.4%) while *Aspergillus terreus* had the least (22.2%).

Based on the screening nine of these isolates were active pectinase producers. The screening of the isolates for pectinase production on solid agar shows that *Fusarium compactum, Aspergillus flavus* and *Aspergillus tamari* had the highest diameter of 4.0cm while *Aspergillus terreus* had the least diameter of 0.5 cm on the 6th day of incubation (Table 2).

Polygalacturonase production by submerged fermentation

The polygaluronase production ranged from 0.1153 - 5.8850U/ml in which *Aspergillus tamari* produced the highest at day 3 by submerged cultivation on medium with citrus pectin (Table 3a). On mango peels, the polygalacturonase production by the fungi isolates ranged from 0.0448 – 4.7745 U/ml in which *Aspergillus sclerotioniger* produced the highest on the 6th day of fermentation (Table3a). The polygalacturonase production on watermelon peels ranged from 0.0212 – 2.5153 U/ml in which *Aspergillus parasiticus* produced the highest level of enzymes after 72 hrs of cultivation (Table 3a). On media with banana and plantain peels, the polygalacturonase production ranged from 0.1183 – 2.4683 U/ml and 0.1154 -2.4683 U/ml respectively. *Aspergillus parasiticus* produced the highest quantity of the enzyme in medium with banana peels while *Aspergillus flavus* produced the highest enzyme yield in medium with plantain peels, both on the 6th day of the fermentation (Table 3b). There was a significant difference in poligalacturonase production by the isolates at different times of cultivation (P≥0.05).

Table 3a*. Production of polygalacturonase by the selected fungus strains in submerged fermentation conditions by using pectin rich agro-wastes as substrate*

Strains	**After 3 days of cultivation**					**After 6 days of cultivation**				
	Polyalacturonase production on 1% agro-waste substates, U/ml									
	Citrus pectin	Banana peels	Watermelon peels	Plantain peels	Mango peels	Citrus pectin	Banana peels	Watermelon peels	Plantain peels	Mango peels
Fusarium compactum	0.3507^{j}	0.7977^{j}	ND	1.3859^{d}	2.2329^{b}	0.5624^{j}	1.4750^{h}	1.7859^{d}	1.0566^{j}	1.8850^{g}
Mucor piriformis	1.1507^{f}	1.4850^{d}	0.7272^{e}	1.0850^{i}	0.7742^{f}	1.4095^{h}	1.8095^{c}	1.1035^{h}	1.5742^{g}	2.8213^{c}
Aspergillus flavus	2.4683^{c}	1.1271^{g}	0.9625^{d}	1.1035^{h}	1.3625^{d}	1.4850^{f}	1.0095^{i}	1.7388^{e}	2.4683^{a}	2.8918^{b}
Aspergillus terreus	1.1976^{e}	1.4566^{e}	0.3507^{f}	1.2447^{f}	0.0448^{j}	3.5742^{b}	1.6683^{e}	1.9507^{b}	1.4329^{h}	2.0683^{e}
Aspergillus niger	0.8213^{i}	1.0850^{h}	ND	1.5742^{b}	0.6329^{g}	3.6683^{a}	1.8095^{d}	1.2919^{f}	2.3036^{b}	1.3625^{j}
Aspergillus sclerotioniger	2.6566^{b}	1.0095^{i}	0.2850^{g}	1.1271^{g}	1.2683^{e}	2.4919^{e}	1.4850^{g}	1.9272^{c}	1.6448^{e}	4.7745^{a}
Aspergillus parasiticus	1.0566^{g}	1.5036^{c}	2.5153^{a}	1.2683^{e}	0.4683^{i}	.8918^{d}	2.4683^{a}	1.0095^{i}	1.9742^{d}	1.6919^{h}
Aspergillus piperis	0.8775 ^{h}	1.9977^{a}	0.2566^{h}	0.8448^{j}	2.6563^{a}	1.4329^{g}	1.8329^{b}	1.2683^{g}	1.3624^{i}	1.9742^{f}
Aspergillus tamari	5.8850^{a}	1.2978^{f}	1.6918^{b}	1.7859^{a}	0.5154^{h}	2.9859^{c}	0.8919^{j}	2.2566^{a}	1.6212^{f}	2.2850^{d}

Values with same letter in a column are not significantly different ($p < 0.05$)

***Table 3b**. Production of polygalacturonase by the selected fungus strains in submerged fermentation at different incubation time*

Strains	After 9 days of cultivation					After 12 days of cultivation				
	Polyalacturonase production on 1% agro-waste substates, U/ml									
	Citrus pectin	Banana peels	Watermelon peels	Plantain peels	Mango peels	Citrus pectin	Banana peels	Watermelon peels	Plantain peels	Mango peels
Fusarium compactum	0.6566^{g}	1.2683^{b}	0.8448^{b}	0.6566^{e}	1.2683^{c}	0.1859^{g}	0.9624^{e}	0.9154^{f}	1.3388^{b}	0.8448^{e}
Mucor piriformis	1.1035^{b}	0.6095^{e}	ND	0.1154^{h}	0.1389^{h}	0.9859^{c}	1.5742^{a}	1.2447^{d}	0.9154^{c}	1.2923^{d}
Aspergillus flavus	1.0328^{c}	0.5859^{e}	1.0095^{a}	0.7977^{d}	0.1624^{g}	0.1153^{h}	0.3271^{g}	1.5507^{a}	0.6850^{f}	0.7507^{f}
Aspergillus terreus	0.8448^{f}	0.4213^{f}	0.6566^{d}	1.4850^{a}	0.9859^{e}	1.2919^{b}	0.8683^{f}	1.3625^{b}	0.8213^{d}	0.1389^{j}
Aspergillus niger	0.3742^{i}	ND	0.3742^{f}	0.2850^{g}	0.6329^{f}	0.4213^{f}	1.1271^{c}	0.6850^{g}	0.3742^{g}	1.3625^{c}
Aspergillus sclerotioniger	1.0095 ^{d}	0.1183^{g}	0.6566^{d}	0.4212	0.1624^{g}	ND	0.3036^{h}	1.3152^{c}	0.2329^{i}	0.3507^{i}
Aspergillus parasiticus	0.8919^{e}	2.0448^{a}	0.5625^{e}	1.0328^{c}	1.2919^{b}	0.7272^{d}	1.4566^{b}	0.4683^{i}	0.3742^{g}	0.4448^{g}
Aspergillus piperis	0.6095^{h}	0.7272^{d}	ND	1.3388^{b}	1.4850^{a}	2.1154^{a}	0.3272^{g}	0.2329^{j}	0.7742^{e}	1.8566^{a}
Aspergillus tamari	0.1859^{j}	0.7272^{d}	0.0212^{g}	0.6566^{e}	1.2447^{d}	0.5154^{e}	0.9849^{d}	1.0328^{e}	0.3507^{h}	0.3977^{h}

Values with same letter in a column are not significantly different ($p < 0.05$)

There was a significant difference in the total protein concentration release on the fermented samples by the different fungi isolates at different times of cultivation days ($P \geq 0.05$) (Table 4). On medium with pure citrus pectin, it ranged from 1.483-22.2259mg/ml in which *Aspergillus terreus* and *Aspergillus sclerotioniger* gave the highest on the 9th day of fermentation. On medium with mango peels, it ranged from 1.2258 – 13.8715 mg/ml in which *Aspergillus tamarii* produced the highest protein concentration released extracellular on day 12 of cultivation (Table 4b). On medium with watermelon peels, it ranged from 1.8926 – 5.2474 mg/ml in which *Aspergillus terreus* gave the highest biosynthesis potential on day 3 of fermentation. The yield of extracellular protein released on medium containing banana peels ranged from 0.9247 – 4.0108 mg/ml in which *Mucor piriformis* had the highest biosynthesis potential on day 3 of submerged cultivation. However, on medium with plantain peels, it ranged from 1.1725 – 8.3441 mg/ml in which *Aspergillus sclerotioniger* had the highest biosynthesis potential on day 3 of cultivation.

Aspergillus spp. dominated *Fusarium* spp. and *Mucor* spp. in polygalacturonase (PG) production. This observation is in agreement with an earlier report by Kutateladze *et al.* 2009. In their collection among pectinase producers representatives of the genera *Aspergillus* and *Penicillium* dominated.

The yield of PG of 5.8850U/ml and 4.7745U/ml obtained in this study is higher than those earlier reported by Soares *et al.* (1999) and Gomes *et al.* (2009) in submerged fermentation. Soares *et al.* (1999) reported a maximum biosynthesis yield of 4.0U/ml.

The utilization of agro-wastes as substrates for pectinolytic enzymes production are important based on its abundance and availability in Nigeria. The use of agro waste for enzyme production is a useful way of converting wastes to raw materials for our industries. A similar opinion is shared by Bayoumi *et al.* (2008).

The maximum PG production was obtained on cultivation on medium with pure citrus pectin,

followed by mango peels and watermelon peels. A maximum value of 2.4683U/ml of PG was produced on media with plantain and banana peels by *Aspergillus* spp. This could be due to the fact that banana and plantain (cooking banana) belongs to the family *Musaceae* and share most physicochemical characteristics such as pectin content.

***Table 4a**. Total extracellular proteins produced by selected fungal strains by submerged cultivation on media with pectin rich agro-wastes as substrates*

Strains	After 72 hrs of fermentation					After 144 hrs of fermentation				
	Citrus pectin	Banana peels	Watermelon peels	Plantain peels	Mango peels	Citrus pectin	Banana peels	Watermelon peels	Plantain peels	Mango peels
Fusarium compactum	15.4625^{d}	1.6237^{h}	3.5055^{f}	2.6559^{h}	11.2904^{a}	1.483^{i}	3.6883^{a}	4.0430^{b}	2.9248^{f}	3.1614^{e}
Mucor piriformis	13.9678^{e}	4.0108^{a}	4.4302^{d}	4.6559^{c}	4.0865^{f}	6.3334^{c}	2.7098^{f}	4.1613^{b}	3.6559^{b}	6.0969^{b}
Aspergillus flavus	16.4301^{c}	2.2904^{f}	2.9145^{h}	3.2474^{g}	4.7958^{d}	3.2797^{g}	3.1184^{e}	4.2151^{b}	2.4947^{g}	5.0324^{c}
Aspergillus terreus	20.0865^{a}	1.3871^{i}	5.2474^{a}	4.2367^{e}	4.8066^{c}	5.7418^{d}	2.3118^{h}	4.6453^{a}	5.0215^{a}	9.0435^{a}
Aspergillus niger	7.0754^{h}	3.0969^{c}	5.1285^{b}	4.5915^{d}	2.9575^{h}	6.6668^{b}	3.3549^{c}	2.9355^{c}	3.3334^{c}	2.1285^{g}
Aspergillus sclerotioniger	12.3012^{f}	3.1829^{b}	4.9463^{c}	8.3441^{a}	4.5268^{e}	4.2904^{e}	3.1285^{d}	2.9462^{c}	2.9356^{e}	1.9248^{h}
Aspergillus parasiticus	9.8715^{g}	2.1075^{g}	4.3657^{e}	4.8763^{b}	3.0435^{g}	2.8173^{h}	3.3657^{b}	4.3227^{b}	2.3872^{h}	1.7313^{i}
Aspergillus piperis	5.3011^{i}	2.6238^{e}	2.6989^{i}	4.5807^{d}	8.9575^{b}	3.8715^{f}	2.2043^{i}	5.5377^{a}	3.2152^{d}	4.0865^{d}
Aspergillus tamari	19.5699^{b}	2.6883^{d}	3.1614^{g}	3.9463^{f}	2.4625^{i}	8.9355^{a}	2.6238^{g}	4.5268^{a}	1.4947^{i}	2.8926^{f}

Values with same letter in a column are not significantly different ($p < 0.05$)

***Table 4 b**. Total protein content produced by fungi isolates by using pectin rich agro-wastes as substrate in submerged fermentation*

Strains	After 216 hrs of fermentation					After 288 hrs of fermentation				
	Citrus pectin	Banana peels	Watermelon peels	Plantain peels	Mango peels	Citrus pectin	Banana peels	Watermelon peels	Plantain peels	Mango peels
Fusarium compactum	14.4732^{b}	2.0538^{c}	2.7849^{f}	3.6238^{a}	5.0108^{a}	2.7098^{g}	1.4194^{b}	3.1506^{b}	2.6559^{c}	1.5377^{h}
Mucor piriformis	6.8496^{f}	2.6883^{a}	2.5807^{h}	2.2367^{f}	2.5161^{f}	3.2367^{e}	1.4087^{c}	2.1725^{g}	1.1725^{i}	5.1829^{c}
Aspergillus flavus	5.6453^{g}	1.9356^{d}	3.3334^{c}	2.8602^{c}	2.2904^{h}	11.1937^{a}	1.4732^{a}	1.8926^{h}	3.4947^{a}	2.0324^{g}
Aspergillus terreus	22.2259^{a}	1.6023^{f}	3.6129^{b}	2.258^{e}	2.6238^{e}	8.1614^{c}	1.0865^{g}	0.8926^{i}	2.4839^{d}	1.2258^{i}
Aspergillus niger	12.6559^{c}	1.2043^{i}	2.7743^{g}	2.1505^{g}	3.2474^{b}	1.6559^{i}	1.2152^{e}	2.4517^{e}	2.0645^{e}	2.6883^{e}
Aspergillus sclerotioniger	22.2259^{a}	2.1725^{b}	4.0215^{a}	2.1506^{g}	2.2367^{i}	8.4194^{b}	0.9356^{h}	2.8818^{d}	1.4947^{h}	5.9145^{b}
Aspergillus parasiticus	2.7743^{h}	1.5055^{h}	2.9677^{e}	1.8173^{h}	2.6989^{d}	5.6559^{d}	1.0968^{f}	3.0754^{c}	1.7098^{g}	2.2043^{f}
Aspergillus piperis	9.9248^{d}	1.5269^{g}	3.3119^{d}	2.9677^{b}	2.8065^{c}	2.7958^{f}	0.9247^{i}	2.3334^{f}	1.7849^{f}	4.3549^{d}
Aspergillus tamari	9.666^{e}	1.6559^{e}	2.4722^{i}	2.4086^{d}	2.4947^{g}	1.8715^{h}	1.3657^{d}	3.3442^{a}	3.0109^{b}	13.8715^{a}

Values with same letter in a column are not significantly different ($p < 0.05$)

The maximum level of pectinase was achieved at 72 hr of submerged cultivation (day 3) on medium with pure citrus pectin. Patil and Dayanand (2006) had obtained similar observations. In their report, the maximum pectinase production was achieved at 72 hrs irrespective of the type of agrowaste employed. However, the maximum yield of PG was obtained after day 6 of cultivation on medium with in mango peels, plantain and banana peels. This variation could be due to the composition of the substrate and length of lag phase of the fungi strains employed. Similar observations were made also by Martin *et al.* (2004), Bayoumi *et al.* (2008) and Gomes *et al.* (2009).

The protein production observed in this study could be due to an array of proteinous metabolites generated during the growth and metabolism of the fungi isolates in submerged fermentation.

Conclusion

Fungi isolated from contaminated tropical fruits can be important candidates for the production of polygalacturonase by submerged fermentation by used liquid media containing agro wastes as nutritive sources and enzymes biosynthesis inductors. *Aspergillus* spp. are strains of choice for polygalacturonase production. The polygalacturonase production on mango peels (4.7745 U/ml) competed favorably with that on pure citrus pectin (5.8850 U/ml) suggesting that mango peels can be used for the large scale production of useful quantities of polygalacturonase by submerged fermentation.

References

Aehle, W., (2007) *Enzymes in industry* (3rd edn.). Wiley-VCH VerlagGMbH& Co. KGaA

Aguilar, G., Huitron. C., (1987) Stimulation of the production of extracellular pectinolytic activities of *Aspergillus* sp. by galacturonic acid and glucose addition. *Enzyme Microb. Technol.*, 9, 690-696

Bayoumi, R. A., Yassin, H.M., Swelim, M.A., Abdell-All, E.Z. (2008) Production of Bacterial pectinase(s) from Agro-Industrial Wastes under Solid State Fermentation Conditions,. *Journal of Applied Sciences Research*, 4 (12), 1708 – 1721

Cavalitto, S. F. Arcas, J. A., Hours, R.A, (1996) Pectinase production profile of *Aspergillus foedidus* in solid state cultures at different acidities, *Biotechnology Letter*,18, 251-256

Domsch, K.H., Gam, W., Anderson, T.H., (1980), *Compendium of soil fungi*, vol. 1, Academic Press, London

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 Technol. Biotechnol.*, 44(2), 221-227

Favela-Torres, E., Aguiler, C.N., Contraras-Equivel, J.C., Viniegra-Gonzalez, G., (2005) Pectinase. In :*Enzyme Technology*, A. Pandey, C. Webb, C.R. Soccol, C. Larroche (Eds.). Asiatech Publishers Inc., New Delhi, India, 265-287

Gomes, E., Leite, R.S.R., da Silva, R., Silva, D., (2009) Purification of an exo polysaccharide from *Penicillium viridicatum* RFC3 produced in submerged fermentation, *International Journal of Microbiology*, 2009,1-8

Gupta, R., Singh, S., (2004) Apple juice clarification using fungal pectinolytic enzymes and gelatin, *Indian Journal of Biotechnology*, 3, 573 – 576

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.

Kurowski, W. M., Dunleav, J. A., (1976) Pectinase Production by Bacteria Associated with Improved Preservative Permeability in Sitka Spruce: Synthesis and Secretion of Polygalacturonate Lyase by *Cytopha gajohnsonii*, *Journal of Applied Bacteriology*, 41,119- 128

Kutateladze, L., Zakariashvili, N., Jobava, M.,Urushadze, T., Khvedelidze,R., Khokhashvili, I., (2009) Selection of Microscopic Fungi - Pectinase Producers, *Bulletin of the Georgian National Academy of Sciences*, 3(1), 136-141

Kwi, S.K, Fred, B., Wolfgang Z., Amin, F., (1994) Pectinolytic enzymes from Actinomycetes for the degumming of ramie bastfibres, *Applied and Environmental Microbiology*, 60(6), 2107 – 2112

Martin, N., Souza, S.R.D., Silva, R.D. and Gomes,E ., (2004) Pectinase Production by Fungal strains in solid state fermentation using Agro-Industrial Biproducts, *Brazilian Archives of Biology and Technology*, 47(5), 813-819

Martins,E.S., Silva, D. , Da Silva, R. , Gomes, E., (2002) Solid-state production of thermostable pectinases from thermophilic *Thermoascus aurantiacus*, *Process Biochem.*, 37, 949–954

Miller, G. L., (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Anal Chem.*, 31, 426-428

Oliveira, K.F., Malavolta, L., Souza, C.S., Vincente, E.J., Laluce, C., (2006) Pectinolytic activity secreted by yeasts isolated from fermented citrus molasses, *Journal of Applied Micobiology*, 100, 633-640

Patil , S.R., Dayanand, A., (2006) Exploration of regional agrowastes for the production of pectinase by *Aspergillus niger*, *Food Technol. Biotechnol.* 44(2), 289-292

Rangaraja, 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

Samson, R. A., Houbraken, J.A.M.P., Kuijpers, A.F.A., Frank, J.M., Frisvad, J.C., (2004) New Ochratoxin A or sclerotium producing species in *Aspergillus* section *Nigri*, *Studies in Mycology*, 50, 45-61

Silva, D., Martins, E. S., Silva, R. and Gomes, E., (2002) Pectinase production from *Penicillium viridicatum* RFC3 by solid state fermentation using agricultural residues and agro-industrial by-product, *Braz. J. Microbiol.*, 33, 318-324

Singh, K., Frisvad, J.C., Thranes, U., Mathur, S.B., (1991) An illustrated manual on the identification of some seedborne *Aspergilli*, *Fusaria*, *Penicillia* and their mycotoxins, Danish government institute of seed pathology for developing countries, Hellerup, 31-69

Soares, M.M. C. N.; Silva, R. and Gomes, E., (1999) Screening of bacterial strains for pectinolytic activity characterization of the Polygalacturonase produced by *Bacillus* species, *Rev. Microbiol.*, 30, 229-303

Steel, R. G. D. and Torrie, J. H. (1980) *Principles and Procedures of Statistics*. McGraw – Hill, New York, New York, USA

Teixeira, M.F.S., Lima Filho, J.L. , Durán, N. (2000) Carbon sources effect on pectinase production from *Aspergillus japonicus* 586, *Braz. J. Microbiol.*, 31, 286–290

Wu, Q., Dobozi, M., Hemmat, M., and Hrazdina, G., (1993) Endopolygalacturonase in apples (*Malus domestica*) and its expression during fruit ripening, *Plant Physiol*., 102, 219-225

Yandav, S., Shastri, N.V., (2005), Partial purification and characterization of a pectin lyase produced by *Penicillium oxalicum*in solid state fermentation, *Indian Journal of Biotechnology*, 4, 501-505

Zheng, Z.X. ,Shetty, K., (2000) Solid-state production of polygalacturonase by *Lentinus edodes* using fruit processing wastes, *Process Biochem.*, 35, 825–830.