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Utilization of Agroindustrial Wastes for the Production of Industrially Important Pectinases by *Aspergillus niger* under Submerged Fermentation

Gousiya Begum and Srinivas Munjam*

Department of Microbiology, Kakatiya University, Warangal - 506 009, Telangana, India

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A B S T R A C T

The request for enzymes in the global market is expected to rise at a fast pace in recent years. With this regard, there has been a great increase in industrial applications of pectinase owing to their significant biotechnological uses. This study was undertaken with main objectives of meeting the growing industrial demands of pectinase, by improving the yield without increasing the cost of production. In addition, this research highlights the underestimated potential of agroindustrial residues for the production of biotechnologically important products. In this study, the maximum pectinase production was attained by using wheat bran, among the tested agro residues. These studies deals with to produce pectinases enzyme using *A. niger* in SmF. Agroindustrial residues used as pectin sources were wheat bran, citrus peel, sugarcane bagasse, rice husk, potato peel and orange peel. Best pectinolytic activity based on the diameter of clear hydrolyzed zones on the pectin agar media plates was obtained with *A. niger*. The strains of *A. niger* have good prospect for pectinase production. Wheat bran is a good low-cost fermentation substrate for pectinase production by the investigated fungus. The increased level in the production of pectinases was noticed when the agrowastes were supplemented with additional metal ions was more effective in submerged fermentation.

Key words: *Aspergillus niger,* Pectin, Pectinase, Agrowastes, Submerged fermentation

Microbial pectinase have tremendous potential to offer mankind. Most pectic enzyme preparations are used in the fruit processing industry and pectic enzymes only of the account for about one quarter of the world's food enzyme production. Over the last few decades, pectinases have been used in industrial areas for the degumming and retting of fiber, improving of paper quality, extraction of juice and oil, fermentation of coffee and tea, and the treatment of pectic waste water (Pan *et al.* 2019). Polygalacturonase (E.C. 3.2.1.15) is probably the most important biocatalyst among the pectin hydrolyzing enzymes and is known to be the most effective enzyme for the pectin hydrolysis. The degumming and retting of fibers are significant processes in the textile industry, and the use of polygalacturonase has attracted much attention due to its minimization of fiber damage and its high efficiency of degumming (Zhao *et al.* 2018).

Over the current years, there has been enormous increase in the awareness regarding the effects of population, and public pressure has influenced both industry and government. With the progress of biotechnology; enzymes have found their way into many new industrial processes (Ahlawat *et al.* 2008). In the industrial arena, pectinase, the catch-all idiom that refers to mixtures of primarily three different enzymatic activities [pectin esterase (PE), polygalacturonase (PG) and pectin/pectate lyase (PL/PAL)] is produced by a variety of bacteria and fungi (Sethi *et al.* 2016).

Production of enzymes from agrowastes could be significant because they contain large amounts of cellulose, hemicelluloses and pectin, which could serve as inducers for the production of cellulose, xylanase and pectinase respectively (Bocccas 1994). Submerged fermentation system has been extensively employed for the production of enzymes and to understand physiological aspects of the synthesis of the enzymes. Thus, the biotechnological potential of pectinolytic enzymes from fungi has drawn a

[1]Gousiya Begum, Department of Microbiology, Kakatiya University, Warangal - 506 009, Telangana

[2]*Dr. Srinivas Munjam, Assistant Professor (munjam17@gmail.com), Department of Microbiology, Kakatiya University, Warangal - 506 009, Telangana

prominent deal of attention from various researchers worldwide (Patil and Dayanad 2006). Pectinases have attracted attention globally as biological catalysts in many industrial processes and are used in processing agricultural and agroindustrial waste (Patil and Dayanad 2006, Bai *et al.* 2011). Some agrowastes mainly citrus peel, apple pomace, coffee pulp, wheat bran (Taragano *et al.* 1997), sugarcane bagasse (Solis-Pereyra 1993), 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. Microorganisms are widely accepted as the best sources for the production of enzymes from agrowastes. However bacteria are known to produce industrial enzymes, fungi are desired for the production of enzymes because their nature is generally regarded as safe (GRAS). Recently, the production of pectinases from agrowastes by fungi has been described as more attractive (Couri *et al.* 2000). Therefore, an attempt was made to examine the utility value of pectin rich regional agrowastes for the production of pectinases by *A. niger* in SmF. The effect of the addition of metal ions on the production of pectinases was also discussed in this communication.

MATERIALS AND METHODS

Microorganism

The microorganism used in this work was *A. niger*. This fungus was locally isolated from vegetable waste dump yards soil, Warangal district, India. This strain was preliminary screened for pectinolytic activity on pectin agar media containing 1% pectin as sole carbon source and this was identified as the potential pectinases producer among 30 fungal isolates.

Identification of pectinolytic fungi

Efficient pectinase producing isolate was sequenced by Macrogen laboratories, South Korea (www.macrogen.com). The resultant fragment was searched against gene bank data base with nucleotide BLAST. The stain were identified as *Aspergillus niger* and sequence deposited in NCBI gene bank with an Accession number MN826319.

Substrates in submerged fermentation (SmF)

Cost of the production enzyme depends mainly on the cost of substrate. To make the process cost effective various types of cheap agro-industrial by products viz. wheat bran, citrus peel, sugar cane bagasse, rice husk, potato peel, and orange peel were added to the production medium at concentration 1% and submerged fermentation was carried out by the selected isolate.

Pectinase production with different agrowastes substrate preparation

The agroindustrial wastes were collected from a local market and were packed in newspapers for further analysis. All of the collected materials were crushed, several times washed with hot water, oven dried (at 70°C), then ground into a mixture by using pestle and mortar. Samples were stored in sterilized containers and used for further experiment.

Quantitative screening (Pectinase production under SmF)

Cultures grown in 250 ml Erlenmeyer flask with 100 ml of pectin broth (pH 7.0), contains 0.2%, $NaNO_3$; 0.1%, K_2HPO_4; 0.05%, $MgSO_4.7H_2O$; 0.05%, KCl; 0.01%, $FeSO_4.7H_2O$; 3%, sucrose; 0.001%, $ZnSO_4$; 0.001%, $CuSO_4$ and 1% different agroindustrial waste residue (wheat bran, citrus peel, sugarcane bagasse, rice bran, potato peel, sugar cane, orange peel) instead of pectin substrate were used for assay of pectinases. After the sterilization, young fungal mycelia of 3 day old cultures at the growing edges were used to inoculate aseptically. Inoculated flasks were incubated in the orbital shaker operating at 120-180 rpm at 28±1°C for 16 days. Ten ml of incubated broth from the culture flasks was withdrawn at different time intervals. The supernatants obtained from the centrifugations were dialyzed and used as enzyme sources for assay and quantification of protein content.

Enzyme recovery

After incubation, the culture medium was filtered to remove mycelium using Whatmann No.5 filter paper, the filtrate was centrifuged at 5000 rpm for 10 min. The clear supernatant was used as the extracellular enzyme source.

Quantitative assay for exopolygalacturonase (Exo-PG)

Exopolygalacturonases, activity was assayed by quantifying reducing sugars using DNS method (Miller 1959). The exo-PGase activity was determined using 1% polygalacturonic acid (PGA) as substrate, prepared in sodium acetate buffer (0.1 M; pH 4.5). The reaction mixture (2 mL) contained equal amounts of enzyme (1.0 mL) and substrate (1.0 mL) and incubating at 50ºC for 30 min in a water bath. The reaction was stopped by addition of 3 ml of 3, 5- dinitrosalicilic acid DNS reagent and the contents were boiled for 15 min. The color thus developed was read at 540 nm. A standard curve of D-galactouronic acid (1 mg/mL) was developed under identical conditions to determine the reducing sugars released. The enzymatic activity was expressed as unit per ml (U/ml), which is defined as the amount of enzyme, which liberates 1 µmole of galacturonic acid (reducing sugar) per mL per minute under assay conditions.

Quantitative assay for endopolygalacturonase (Endo-PG)

Wood's viscometric method (1955) was employed to estimate the endo-PG. Polygalacturonic acid (0.5%) was prepared by dissolving 0.5 g of polygalacturonic acid in 100 ml citrate buffer (pH 5.5). The reaction mixture contained polygalacturonic acid (0.5%) substrate, citrate buffer (pH 5.5) and enzyme source in 4:1:2 ratios. The reaction mixture consisting of 12 ml of substrate, 4 ml of enzyme and 1ml of citrate buffer. The loss of viscosity was measured for every 10 minutes over a period of 30 minutes. The reaction mixture with heat killed (inactivated) enzyme and distilled water served as control. The percentage loss of viscosity was calculated by using the following formula:

$$V = \frac{t_i - t_a}{t_1 - t_0} \times 100$$

Where,

V = percentage of loss of viscosity
t_i = flow time of reaction mixture + inactive enzyme.
t_a = flow time of reaction mixture + active enzyme
t_0= flow time of distilled water + active enzyme at ''O'' time

The relative enzyme activity (REA) of endo PG was calculated by dividing 1000 with time required for 50% loss of viscosity (t50) and in relative viscometric units (RVU).

$$REA = 1000 / tv50$$

Where,
tv50 = time required in minutes taken for 50% loss of initial viscosity

Quantitative assay for pectin methyl esterase activity (PME)

Pectin methyl esterase activity was estimated by the method suggested by Kertesz (1950). PME activity can be measured either by measuring the amount of methanol released or increase in free carboxyl group by monitoring pH changes.

Pectin esterase activity was measured by increase in free carboxyl group by titrating against NaOH in the presence of a pH indicator like phenolphthalein. For assaying PME activity, 20 ml of 1% pectin (dissolved in 0.15 M NaCl, pH 7.0) and 4 ml of enzyme extract were taken in a beaker and incubated for 1 hour. After incubation, the solution was titrated against the 0.02 N NaOH to reach pH 7.0 using phenolphthalein as indicator (colour change from colourless to pink). The heat killed enzyme extract was used as control.

$$\text{Pectin esterase activity} = Vs - V\,b\ (\text{Normality of NaOH}) \times 100/Vt$$

Where,
Vs-volume of NaOH used to titer sample (ml),
V b-volume of NaOH used to titer blank (ml),
V-volume of incubation mixture (ml), t-Reaction time (min).
PME activity was expressed as milli equivalents of NaOH consumed min^{-1} ml^{-1} of enzyme extract under the assay conditions.

Quantitative assay for Endo-pectin lyase (Endo-PL)

Endo-PL activity was assayed viscometrically as suggested by Wood (1955). 1% pectin was used as substrate in this assay. Four ml of culture supernatant and 0.8 ml of tris HCl buffer pH (8.0) were added to 12 ml of pectin solution. Viscosity changes of reaction mixture were determined by using Ostwald viscometer. Initial reading time and the reading after 30 min of incubation were determined. The loss of viscosity was measured for every 10 minutes over a period of 30 min. The reaction mixture with heat killed (inactivated) enzyme and distilled water served as control. Enzyme activity was expressed in RVU units (relative viscometric units).

Soluble protein assay

The protein content of enzyme source was determined by the Lowry's method, as described by Lowry's (1951) using bovine serum albumin (BSA) as a standard, Absorbance was read at 660 nm using UV-VIS spectrophotometer.

Determination of fungal biomass

After the incubation period of 4, 8, 12, and 16 days the contents of the flasks were aseptically passed through pre-weighed Whatmann No.1 filter paper to separate mycelial mat from culture filtrates. The filter papers, along with mycelial mat were dried at 70°C in an oven overnight and their weight recorded. The difference between the weight of the filter paper bearing mycelia mat and weight of pre-weighed filter paper represented fungal biomass, which was expressed in terms of dry weight of mycelia mat in micrograms.

Statistical analysis

The enzyme activities are presented as Mean±SE of all values. Results obtained in this study were subjected to analysis of variance using one way ANOVA and difference between means were separated by Duncan Multiple Range Test using SPSS software 17.0 version. The results are presented in (Table 1-3).

Optimization of cultural parameters for pectinase production in SmF

The production of high yields of an enzyme using SmF requires optimal environmental conditions to enhance maximum growth of the organisms and increased production of the enzyme. The optimization of medium composition using natural substrates and metal ions were investigated in SmF.

Effect of agroindustrial byproducts and its concentration

The effect of various agroindustrial byproducts viz. wheat bran, citrus peel, sugarcane bagasse, rice bran, potato peel, sugar cane, orange peel on the production of enzyme was studied. The production medium without any agroindustrial byproduct was used as control.

The best agroindustrial byproduct for maximal enzyme production was selected and used at different concentration 0.6%, 0.8%, 1%, 1.2%, 1.4% and 1.6 % w/v in production medium.

Effect of metal ions

Effect of metal ions on pectinase production by selected fungi was investigated by adding various metals calcium chloride, barium chloride, ferric chloride, cobalt chloride, magnesium chloride, sodium chloride, potassium chloride as metal ions at a concentration of 0.1% (w/v) were added to the production medium in SmF. To optimize the concentration of best metal ions for maximal enzyme production it was used at different concentration 0.02%, 0.04%, 0.06%, 0.08% and 0.1%. Enzyme assay were carried out in SmF as described earlier.

RESULTS AND DISCUSSION

Effect of agroindustrial byproducts

Agroindustrial byproducts are inexpensive and abundantly available are considered to be the best substrate for SmF. To make the process cost effective different types of cheap industrial by products like wheat bran, citrus peel, sugarcane bagasse, rice husk, potato peel and orange peel

were added to the production medium at a concentration of 1% and submerged fermentation was carried out by the selected isolate. Pectinase production by *Aspergillus* species was enhanced to some extent with all the agroindustrial byproducts and maximum production was observed with wheat bran, followed by citrus peel. Other substrates such as sugarcane bagasse, potato peel orange peel and rice husk was found to be minimum for pectinase production in *A. niger*.

According to (Table 1) it is evident that the best exo-PG was obtained by *A. niger* in wheat bran (1.597^{a}±0.002 U/ml) followed by citrus peel (1.443^{b}±0.002 U/ml) orange peel (1.056^{d}±0.005 U/ml) on 12th day of incubation. Control (0.965^{d}±0.005 U/ml), and sugarcane peel (0.850^{ef}±0.005 U/ml) produced moderate levels of exo-PG activity. Potato peel (0.828^{ef}±0.005 U/ml) and rice husk (0.756^{g}±0.005 U/ml) produced lesser level of exo-PG activity. Jahan *et al.* (2017) results indicates that 1% wheat bran was the most suitable concentration for PGase production by *Bacillus licheniformis* KIBGE-IB3. Kalaichelvan (2012) also found that wheat bran in comparison to rice bran produced more enzymes from *Bacillus* species.

Table 1 Effect of agroindustrial products on production of pectinases by *A. niger* under submerged fermentation

Agroindustrial byproducts	Final pH	Incubation day	Dry weight (gm)	Exo-PG (U/ml)	Endo-PG (RVU)	Endo-PL (RVU)	PME (meq of NaOH consumed/min/ml)	Protein con. (mg/ml)
Wheat Bran	6.0	8th	1.2	0.005^{b}±0.003	200^{a}±0.002	333^{a}±0.002	0.044^{a}±0.001	1.5
	6.0	12th	0.9	1.597^{a}±0.002	35^{b}±0.001	33.3^{b}±0.005	0.015^{b}±0.001	1.5
Citrus peel	6.0	8th	1.1	0.082^{a}±0.003	155^{b}±0.002	300^{b}±0.002	0.025^{bc}±0.001	1.5
	6.0	12th	0.8	1.443^{b}±0.002	38^{b}±0.001	38^{a}±0.005	0.019^{a}±0.001	1.1
Sugar cane	6.0	8th	1.0	0.618^{bc}±0.003	88.7^{c}±0.002	70.0^{de}±0.002	0.025^{bc}±0.001	1.3
peel	6.0	12th	0.6	0.85^{ef}±0.002	33.3^{c}±0.001	25.2^{c}±0.005	0.003^{f}±0.001	0.9
Rice husk	6.0	8th	0.8	0.609^{bc}±0.003	41^{f}±0.003	25.0^{g}±0.002	0.017^{d}±0.001	1.4
	6.0	12th	0.7	0.756^{g}±0.002	33.3^{c}±0.001	20.3^{e}±0.005	0.033^{c}±0.001	0.8
Potato peel	6.0	8th	0.7	0.484^{e}±0.003	66.6^{de}±0.003	68.0^{de}±0.002	0.019^{c}±0.001	1.3
	6.0	12th	0.5	0.828^{ef}±0.002	28.2^{d}±0.001	25^{c}±0.005	0.009^{e}±0.001	1.0
Orange peel	6.0	8th	0.7	0.79^{f}±0.003	60^{de}±0.003	200^{c}±0.002	0.025^{bc}±0.001	1.2
	6.0	12th	0.6	1.056^{c}±0.002	25^{e}±0.001	22^{de}±0.005	0.015^{d}±0.001	0.9
Control	6.0	8th	0.5	0.587^{cd}±0.003	41^{f}±0.003	50.0^{f}±0.002	0.017^{d}±0.001	1.0
	6.0	12th	0.5	0.965^{d}±0.002	33.3^{c}±0.001	20^{e}±0.005	0.006^{f}±0.001	0.7

Results from the (Table 1) shows the appreciable amount of endo-PG activity was recorded by *A. niger* in wheat bran (200^{a}±0.002 RVU) followed by citrus peel (155^{b}±0.002 RVU) on 8th day of incubation. Sugar cane peel (88.7^{c}±0.002 RVU), potato peel (66.6^{de}±0.003 RVU) and orange (60.0^{de}±0.003 RVU) produced moderate activity. Control (41.0^{f}±0.003 RVU) and rice husk (41.0^{f}±0.005 RVU) recorded lesser activity. Barman *et al.* (2015) found optimsation of pectinase production by *A. niger* using banana (*Musa balbisiana*) peel as substrate. Results of Giese *et al.* (2008) were different who observed that orange peel produced maximum pectinase from *Botryosphaeria rhodina* MAMB- 05.

The best endo-PL was obtained by *A. niger* in wheat bran (333^{a}±0.002 RVU) followed by citrus peel (300^{b}±0.002 RVU) and orange (200^{c}±0.005 RVU) in their 8th day of incubation. Sugar cane peel (70.0^{de}±0.005 RVU), potato peel (68.0^{de}±0.005 RVU) and control (50^{ef}±0.005 RVU) produced moderate level of activity. Rice husk (25.0^{g}±0.005 RVU) produced lesser level of endo-PL activity (Table 1). The results of Atalla *et al.* (2019) showed that pea peel and sugarcane bagasse were most promising for enzyme activity and produce (58.22 and 57.38 U/ml) respectively followed by banger peel produce 56.96 U/ml. The other wastes showed moderate to low activity. High production of pectinase by these raw materials may be due to the reason that solid substrate not only supplies the nutrient to the microbial cultures growing in it but also serves as anchorage for the cells allowing them to utilize the substrate effectively (Pandey *et al*. 2000).

Results from the (Table 1) shows that the good amount of PME was recorded by *A. niger* in wheat bran (0.044^{a}±0.001 meq of NaOH consumed/min/ml) followed by citrus peel (0.025^{bc}±0.001 meq of NaOH consumed/min/ml), orange peel (0.025^{bc}±0.001 meq of NaOH consumed/min/ml) and sugarcane peel (0.025^{bc}±0.001 meq of NaOH consumed/min/ml) in their 8th day of incubation. Potato peel (0.019^{c}±0.001 meq of NaOH consumed/min/ml) recorded moderate level, control (0.017^{d}±0.001 meq of NaOH consumed/min/ml)) and rice husk (0.017^{d}±0.001 of NaOH consumed/min/ml) with lesser level of PME activity. Similarly, Mrudula and Anitharaj (2011) revealed that the orange bagasse was the most significant for the pectinase production in *A. niger*. The universal stability of wheat bran as substrate might be due to the fact that it contained sufficient nutrients that it able to remain free even in high moist condition providing large surface area (Archana satyanarana 1997).

It was observed that 8th day of incubation was found to be favorable for the production of endo-PG, endo-PL and PME while 12th day was for exo-PG. Results shows that the dry weight was obtained in the range of 0.5-1.2 gm on 8th day of incubation and on 12th day dry weight was observed in the range of 0.5-0.9 gm. Maximum dry weight was reported in wheat bran was 1.2 gm on 8th day of incubation. The final pH 6.0 was recorded in all the pectinases

production. The protein concentration was obtained in the range of 1.0-1.5 mg/ml on 8th day of incubation and on 12th day protein concentration was observed in the range of 0.7-1.5 mg/ml. Highest protein concentration was reported in wheat bran was 1.5 mg/ml on 12th day of incubation. Among the different substrates used for the production of pectinases, wheat bran powder revealed excellent production.

The results from the (Table 2) showed that the varying amount of enzyme activity was observed in different wheat bran concentrations (0.50%, 1.00%, 1.50%, 2.00% and 2.50%). Increased exo-PG activity (1.129^{a} ± 0.005 U/ml) and endo-PG activity (75.0^{a} ± 0.001 RVU) was observed in 1.00% respectively in wheat bran. Similar results were also recorded in endo-PL (76.7^{a} ± 0.003 RVU) and PME (0.055^{a} ± 0.001 meq of NaOH consumed/min/ml) in 1.00% wheat bran on 8th and 12th days of incubation period. Pectinases activity was started at 0.50% wheat bran concentration, showed optimum at 1.00% and declined in subsequent concentrations. Sethi *et al.* (2016) reported enhanced production of pectinase by *A. terreus* NCFT 4269.10 using banana peels as substrate. Khan *et al.* (2012) reported that the combination of wheat straw and mosambi bagasse gave higher yields. It has been reported that wheat bran was proved the best substrate for *Moniliella sp*., *Penicillium sp*. (Martin *et al.* 2004) and *Streptomyces lydicus* (Jacob and Prema 2006) for the maximal activity value of PGase in submerged fermentation.

Table 2 Effect of wheat bran concentration on production of pectinases by *A. niger* under submerged fermentation

Wheat bran (%)	Exo-PG (U/ml)		Endo-PG (RVU)		Endo-PL (RVU)		PME (meq of NaOH consumed/min/ml)	
	8th day	12th day	8th day	12th day	8th day	12th day	8th day	12th day
0.50%	0.505^{b}±0.001	0.800^{b}±0.005	56.0^{b}±0.001	38.50^{b}±0.001	45.0^{c}±0.003	27.5^{b}±0.001	0.020^{d}±0.001	0.001^{d}±0.001
1.00%	0.759^{a}±0.001	1.129^{a}±0.005	75.0^{a}±0.001	46.0^{a}±0.001	76.7^{a}±0.003	30.50^{a}±0.001	0.055^{a}±0.001	0.030^{a}±0.001
1.50%	0.495^{c}±0.001	0.675^{c}±0.005	47.0^{c}±0.001	20.0^{cd}±0.001	55.8^{b}±0.003	20.1^{c}±0.001	0.042^{b}±0.001	0.022^{b}±0.001
2.00%	0.348^{d}±0.001	0.540^{d}±0.005	25.0^{d}±0.001	20.0^{cd}±0.001	36.7^{d}±0.003	7.6^{d}±0.001	0.030^{c}±0.001	0.020^{c}±0.001
2.50%	0.193^{e}±0.001	0.490^{e}±0.005	10.5^{e}±0.001	18.5^{e}±0.001	20.0^{e}±0.003	5.0^{e}±0.001	0.011^{e}±0.001	-

Effect of metal ions

Mineral nutrition is very much necessary for the better growth and the metabolism of microorganisms. The effect of metal ions on pectinases, dry weight and protein production by *Aspergillus niger* using wheat bran in SmF shown in (Fig 1- 6).

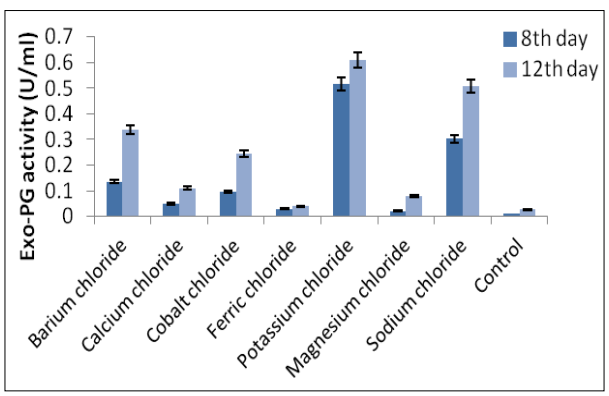

Fig 1 Effect of metal ions on exo-PG production by *A. niger* under SmF using wheat bran

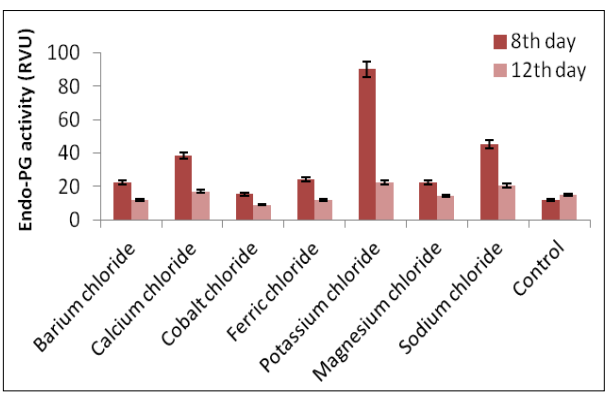

Fig 2 Effect of metal ions on endo-PG production by *A. niger* under SmF using wheat bran

Results from the (Fig 1) showed that KCl (0.610 U/ml), NaCl (0.506 U/ml), $BaCl_2$ (0.340 U/ml), $CoCl_2$ (0.245 U/ml), $CaCl_2$ (0.110 U/ml), were recorded optimum in enzyme production, whereas $MgCl_2$ (0.08 U/ml), $FeCl_3$ (0.04 U/ml), control (0.027 U/ml) have more inhibitory effect on exo-PG production. Jahan *et al.* (2017) was studied by adding KH_2PO_4 + K_2HPO_4 separately and also in combination into the medium and was found that increased PGase production was achieved in medium containing KH_2PO_4. The media containing both salts (KH_2PO_4 and K_2HPO_4) produced comparatively low enzyme. Sakellaris *et al.* (1989) reported the effect of various metal ions in polygalacturonase production and observed that Mg^{2+} and Mn^{2+} were the activators of polygalacturonase and NaCl, $BaCl_2$, $FeCl_3$ and $ZnCl_2$ were the potent inhibitors of polygalacturonase.

Data from the (Fig 2) showed the effect of metal ions on endo-PG production in SmF. It was observed that KCl (90.33 RVU), NaCl (45.33 RVU) recorded the highest amount of endo-PG followed by $CaCl_2$ (38.33 RVU), $FeCl_3$ (24.10 RVU), $MgCl_2$ (22.33 RVU) and $BaCl_2$ (22.33 RVU), whereas $CoCl_2$ (15.4 RVU) and control (12.0 RVU) have

little impact on 8th day of incubation. Meager amount of enzyme production was observed at 12th day for all the enzymes studied. Oumer and Abate (2018) were reported that, $CaCl_2.2H_2O$, $CoCl_2.6H_2O$, $MgSO_4.7H_2O$ and NaCl enhanced pectinase production on submerged fermentation. Both $CaCl_2.2H_2O$ and $MgSO4.7H_2O$ significantly increased pectinase production by three folds. Saad *et al*. (2007) also reported that metal ions such as Mn^{2+} increased polygalacturonase activity by up to 37%.

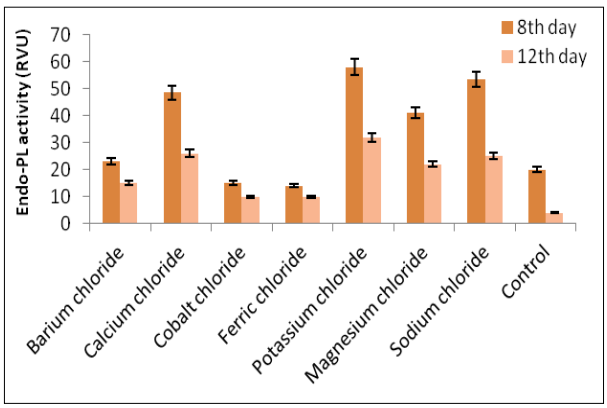

Fig 3 Effect of metal ions on endo-PL production by *A. niger* under SmF using wheat bran

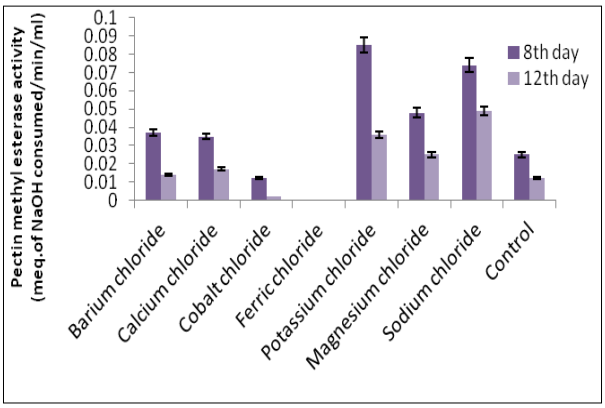

Fig 4 Effect of metal ions on PME production by *A. niger* under SmF using wheat bran

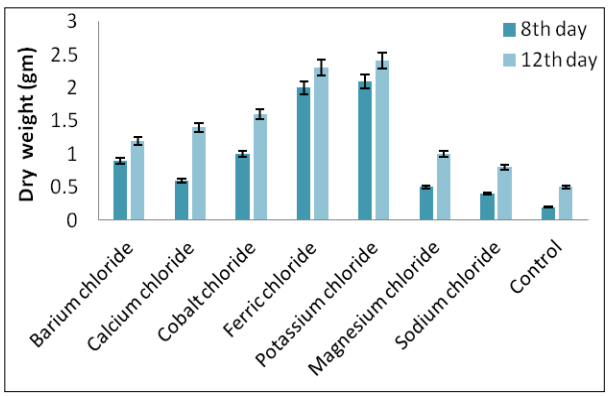

Fig 5 Effect of metal ions on dry weight by *A. niger* under SmF using wheat bran

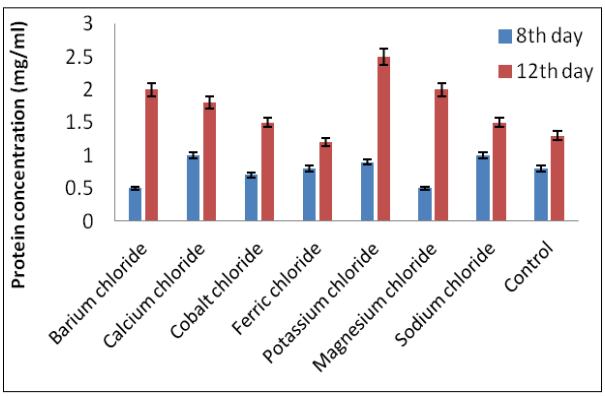

Fig 6 Effect of metal ions on protein production by *A. niger* under SmF using wheat bran

The present study revealed the effect of metal ions on endo-PL production and results were shown in (Fig 3). It was expressed that KCl (58 RVU) followed by NaCl (53.5 RVU), $CaCl_2$ (48.5 RVU) produced highest amount of enzyme activity, while $MgCl_2$ (41 RVU), $BaCl_2$ (23.0 RVU) showed intermediate activity. Control (20.0 RVU) played a little role in promoting the enzyme production. $CoCl_2$ (15.0 RVU), and $FeCl_3$ (14.0 RVU), have more inhibitory effect on endo-PL production 8th day of incubation. All the enzymes showed minimum amount of enzyme activity on 12th day. Thakur *et al*. (2010) studied that addition of metal ions such as Mn^{+2}, Co^{+2}, Mg^{+2}, Fe^{+3}, Al^{+3}, Hg^{+2}, and Cu^{+2} had inhibitory effect on polygalacturonase production. Demir *et al*. (2011) also observed that there is enhanced production of pectinase in the presence of chloride ions.

The data from (Fig 4) showed the effect of metal ions on PME production. Highest activity was recorded in KCl (0.085 meq of NaOH consumed/min/ml), NaCl (0.074 meq of NaOH consumed/min/ml) while $MgCl_2$ (0.048 meq of NaOH consumed/min/ml), played an intermediate role in promoting the enzyme production. Rest of the metal ions showed less impact on 8th day of incubation. No production was seen in $FeCl_3$, have more inhibitory effect on PME production. Less enzyme activity was observed on 12th day of incubation. Couri *et al*. (2000) demonstrated that addition of Fe^{2+} and/or Zn^{2+} ions was significantly positive to the enzyme production. On the other hand, Cu^{2+} and Mn^{2+} ions had almost no effect on these parameters. Other workers have earlier conducted studies on the use of different salts on different strains.

(Fig 5) shows that the dry weight was obtained in the range of 0.2-2.1 gm on 8th day and 0.5-2.4 range on 12th day of incubation. Maximum dry weight was reported in KCl followed by $FeCl_3$ on 12th day of incubation. Results from (Fig 6) shows that the protein production was recorded in the range of 0.5-1.0 mg/ml and 1.2-2.5 mg/ml on 8th day and 12th day of incubation respectively. Highest protein concentration was observed in KCl (2.5 mg/ml) on 12th day of incubation. Pectinase production was increased by the fungi in the presence of k^+, Na^{+2}, Mg^{+2} and Ca^{+2}. In contrast

the pectinase production was strongly inhibited by Co^{+2}, Fe^{+3} and Ba^{+2}.

The data presented in (Table 3) shown increasing level of enzyme activity in KCl at different concentrations (0.02%, 0.04%, 0.06%, 0.08% and 0.10%) up to 12 days of incubation. Exo-PG and endo-PG activities showed significantly highest level of 0.165^{a}±0.001 U/ml and 45.60^{a}±0.002 RVU respectively at 0.06% of KCl. Maximum endo-PL activity of 28±0.003 RVU was observed in 0.06% KCl and maximum PME activity of 0.039^{a}±0.005 meq of NaOH consumed/min/ml was observed in 0.06% KCl.

The inhibition of the enzyme production by these ions may be due to inhibitory effect on the growth of the fungi and enzyme mainly metal ions may be interacting with the sulphydryl groups, suggesting that there is a very important cysteine residue in or close to the active site of the enzymes. The metal salts are related directly to the microorganism metabolism, stimulating or inhibiting enzyme production. KCl and NaCl were reported as the best inducers of pectinase production by *A. niger* SmF.

Table 3 Effect of KCl concentration on production of pectinases by *A. niger* under SmF using wheat bran

KCl (%)	Exo-PG (U/ml)		Endo-PG (RVU)		Endo-PL (RVU)		PME (meq of NaOH consumed/min/ml)	
	8^{th} day	12^{th} day	8^{th} day	12^{th} day	8^{th} day	12^{th} day	8^{th} day	12^{th} day
0.02%	0.091d±0.001	0.098d±0.001	31.6c±0.002	25c.0±0.005	28±0.003	12.5±0.002	0.020±0.005	--
0.04%	0.0120b±0.001	0.136b±0.001	33.4b±0.002	27b.8±0.005	26.5±0.003	13.5±0.002	0.035±0.005	--
0.06%	0.150a±0.001	0.165a±0.001	45.6a±0.002	29a.0±0.005	25.5±0.003	17.0±0.002	0.039±0.005	--
0.08%	0.105c±0.001	0.110c±0.001	17.7d±0.002	18d.0±0.005	17±0.003	15.0±0.002	0.025±0.005	--
0.10%	0.007e±0.001	0.005e±0.001	15.0e±0.002	14.0e±0.005	15.3±0.003	12.5±0.002	0.018±0.005	--

In conclusion, strain of pectinolytic fungi *A. niger* have been isolated from vegetable waste dump yards had been identified as efficient strain in pectinase production. This strain efficiently utilized wheat bran as low-cost substrate for pectinase production. Higher levels of pectinase activity were obtained by SmF in the presence of several agroindustiral wastes as substrates. Pectinase production was increased by the fungus in the presence of K^{+}, Na^{+2}, Mg^{+2} and Ca^{+2}. In contrast the pectinase production was strongly inhibited by Co^{+2}, Fe^{+3}, and Ba^{+2}. The use of agrowastes for pectinase production will not only reduce the production costs of the enzyme but also help decrease pollution-load due to the agroindustrial waste.

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