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Preliminary screening for endoglucanase, cellobiohydrolase and beta-glucosidase producing fungal isolates from rice husks in husk piles

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Abstract

Cellulases are a group of indispensable enzymes whose market value is projected to increase to billions of dollars in the next few years. This study aimed at screening for the endoglucanase-, cellobiohydrolase-, and beta-glucosidase-producing fungi from rice husks in husk piles. Isolation of the mycoflora of rice husks was done using Potato dextrose agar and carboxy methyl cellulase media. The qualitative screening was done by measuring the zone of hydrolysis after flooding pure colonies plated singly in petri dishes, with Congo red solution. The quantitative screening was done at the end of 120 hours submerged fermentation, by assaying for enzyme production after the growth of colony in mineral media specific for the production of each class of cellulase: endoglucanase, cellobiohydrolase and beta-glucosidase. The genera of fungi isolated were *Absidia, Alternaria, Aspergillus, Cladosporium, Fusarium, Geotrichum* and *Penicillium* species. The most abundant fungi isolated was *Aspergillus flavus* (18.18%) while the least abundant isolates were *Aspergillus restrictus*, *Alternaria* sp. and *Geotrichum* sp., with percentage frequencies of 3.03% each. Eleven of the isolates were found to produce cellulase by zones around colonies, indicating cellulose hydrolysis. The most prolific endoglucanase producer was *Fusarium incarnatum* with 16.60 U/ml at the 120th hour, while the best cellobiohydrolase and beta-glucosidase producer was *Penicillium expansum,* producing 3.10 U/ml of cellobiohydrolase and 44.41 U/ml of beta-glucosidase at its 120th hour of incubation. These isolates show promising potentials for optimization studies and for eventual employment in biotechnological and enzyme-utilizing industries.

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Introduction

The most abundant renewable natural resource in the biosphere is cellulose. It is a biopolymer of insoluble, linear chains of β-1,4-linked glucose units joined by glycosidic bonds and a major constituent of plant cell walls (Dussan *et al*., 2014). Economic interests for cellulose arose for its effective utilization because it serves as a cheap and readily available carbon source. Cellulase is a term for a heterogenous group of enzymes that act in synergy, to breaks down the complex polysaccharide, cellulose. The enzyme complex in fungi is made up of oxidative and hydrolytic enzymes capable of breaking down cellulose, hemicellulose and lignin to mono- and di-saccharide sugars (Imran *et al*., 2016).

Cellulases hydrolyze β-1,4 linkages in cellulose chains by acting on reducing and non-reducing ends, using three major rgroups; endoglucanases, cellobiohydrolase and beta-glucosidases. Endoglucanases (EC 3.2.1.4), (also called carboxy methyl cellulases,cmCase, or 1,4-β-D-glucan glucanohydrolase) randomly cut β-1,4-glycosidic bonds of cellulose chains in amorphous zones, to release oligomers and generate new ends (Shah *et al*., 2017). Cellobiohydrolases (EC3.2.1.91), (also known as exoglucanases or 1,4-β-D-glucan cellobio hydrolases (CBH)) are processive for both reducing and non-reducing ends of cellulose polysaccharide chains and liberate cellobiose or glucose as their main products (Hamdan and Jasim, 2021).

Beta-glucosidases (EC 3.2.1.21) (β-glucosidases) hydrolyze soluble cellodextrins, cellobiose and sometimes cellooligosaccharides, which are generated as product from endoglucanase and cellobiohydrolase hydrolysis, to the glucose. β-glucosidases are responsible for the degradation of cellobiose that are known to inhibit both cellobiohydrolases and endoglucanase and serves as the cellulase fraction that completes cellulose hydrolysis (Yunus and Kuddus, 2021).

Presently, cellulases account for a significant share of the world's industrial enzyme market as it is increasingly being used for a large variety of industrial purposes and in many environmentally friendly and economic industries in today's biotechnological societies—in the textile industries, pulp and paper industries, biofuel and bioethanol-producing industries, food industries for food and feed, as well as detergents-making industries (Imran *et al*., 2016; Bhardwaj *et al*., 2017; Raveendran *et al*., 2018).

This has encouraged the exploration of microbial enzymes that have high specificity, mild reaction conditions, negligible substrate loss, generation of side products and are environmentally friendly in lignocellulose hydrolysis (Kulkarni and Gupta, 2013).

An approach to this exploration is continuous search for microorganisms which secrete these enzymes in copious amounts and the use of optimization studies on the enzymes produced by them. Screening is an indispensable tool necessary for the selection of desirable traits in any group of enzymes-producing microorganisms that involves testing of the samples for the targeted reaction (Raymond, 2012).

In view of these, this study was carried out to screen for the endoglucanase, cellobiohydrolase and beta-glucosidase producers among the isolates from rice husk piles.

Materials and methods

Sample Collection and Sampling Techniques

Samples were collected from different rice husk piles located at N7.740248 and N5.391513 Ire-Ekiti, Ekiti state, Nigeria and N7.249907 and N5.514090, off Ondo Road, Akure, Ondo state, Nigeria, using proportionate random sampling technique. They were collected in zip lock polybags and transported to the laboratory. They were then dried, pulverized to 20mm mesh size powder and stored at 4°C until use.

Isolation and identification of fungal flora associated with rice husk

Serial dilutions of pulverized husk were plated by pour plate method, on Potato Dextrose Agar (PDA) and minimal mineral media amended with carboxy methyl cellulase (CMC media).

These were homogenized by gentle swirls, left to gel and then incubated (Gallenkamp 9082A incubator) at 28^0C for 72-120 hours. Pure colonies obtained by continuous plating of culture, were stocked on double strength slants and stored at 4^0C. All isolates were identified macroscopically and microscopically, using lactophenol staining, with reference to standard literature on fungal identification (Beneke and Rogers, 1980; Collins *et al*., 1991).

Percentage frequency of isolates

After identification, the isolates were counted numerically per species growth of spore forming unit on media and their percentage frequencies were calculated according to the method of Nwokeoma *et al.* (2017).

$$\text{Percentage frequency of species (\%)} = \frac{\text{Number of isolates of a species (sfu)}}{\text{Total number of fungi counted (sfu)}} \times 100$$

Qualitative screening for cellulase production

Qualitative screening was carried out by plating pure colonies of the isolates singly on carboxy methyl cellulose-enriched media (CMC media) in petri dishes and incubating at 28 ± 2^0C for 72 hours, after which they were flooded with 1% Congo red solution.

This was decanted after 15 minutes, and de-stained with 1M NaCl solution. The NaCl solution was decanted after 15 minutes. The zone of clearance at edge of the colonies represented cellulase hydrolysis and was measured incm. The ratios of clearance diameters against fungal growths were determined and isolates with high zones of clearance were selected for further screening.

Quantitative screening for specific cellulase production

This screening was carried out to ascertain the most prolific specific endoglucanase-, cellobiohydrolase- and beta-glucosidase-producer, among all the enzyme producers. The media were prepared specifically for the group of cellulase screened for. The samples were collected twenty-four hourly for 120hours and assayed immediately.

Quantitative screening for endoglucanase

Endoglucanase was screened for using submerged fermentation in media containing, in g/L: rice husk 10.0, NaCl 1.0, $NaNO_3$ 1.0, K_2HPO_3 1.0, KCl 1.0,$mgSO_4$ 0.5, yeast extract 0.5 g and 10.0mL trace elements, prepared according to the modified method of Al-Kharousi *et al*., (2015). Media was then sterilized at 121^0C for 15minutes, cooled and inoculated with 4 plugs of fungi from 5-day old plates using 10mm diameter sterile cork borer. The flasks were incubated under shaking condition for 120 hours at 28 ± 3^0C and at 120rpm. Sterilized but uninoculated media served as control and mycelia-free broth served as the crude enzyme. Assay for endoglucanase was carried out using 0.5mL of crude enzyme mixed with 0.5mL of 2% carboxy methyl cellulose, (2g/100mL in 0.1M acetate buffer, pH 4.8) and incubated at 50^0C in water bath for 30 minutes. Three milliliters (3mL) of 3,5-dinitrosalicylic acid (DNS) was added and the mixture was boiled for 5 minutes before being cooled on ice. Absorbance was read at 540nm with spectrophotometer after 20mL of distilled water was added. Glucose was used as standard. One unit of endoglucanase was defined as the amount of enzyme that released 1 µmol of reducing sugar per minute under the stated assay conditions.

Quantitative screening for cellobiohydrolase

Quantitative screening for cellobiohydrolase was performed using submerged fermentation in media prepared in g/L by a modified method of Ghori *et al*., 2011: Rice husk- 30g, KNO_3 0.75g, CaCl- 0.04, K_2HPO_4-0.5,$mgSO_4$- 0.2, $FeSO_4$-

0.02, $(NH_4)_2SO_4$- 1.4, Yeast extract- 2.0, Tryptone- 2.0. It was sterilized at 121^{0}C for 15minutes, cooled and inoculated with 4 plugs of fungi from 5-day old plates using 10mm diameter sterile cork borer. The flasks were incubated under shaking condition for 120 hours at 28±3^{0}C and at 120rpm. Sterilized but uninoculated media served as control and mycelia-free broth served as the crude enzyme. Cellobiohydrolase assay was carried out using 1.25 g of avicel suspended in 100mL of 0.1M acetate buffer (pH 4.8) to make 1.25% (w/v). 1.6mL of 1.25% avicel-in-buffer was mixed with 0.4mL of crude enzyme and incubated at 50^{0}C in water bath for 2 hours. The reaction was stopped using ice-cooled water-bath. 1mL of the reaction mixture was then pulled and centrifuged at 13,000rpm for 3 minutes. To 0.7mL of the supernatant, 0.7mL of 5% phenol solution, and then 3.5mL of 98% concentrated tetraoxosulfate VI acid was added. This was thoroughly mixed, cooled and absorbance was read spectrophotometrically at 490 nm. Glucose was used as standard. One unit of cellobiohydrolase was defined as the amount of enzyme that released 1 µmol of reducing sugar per minute under the stated assay conditions.

Quantitative screening for beta-glucosidase

Beta-glucosidase production was screened for using media prepared in g/L according to the method of Choudhari *et al*, 2017: rice husk- 10g, CaCl- 0.4,mgSO_4- 0.3, $(NH_4)_2SO_4$- 1.4, KH_2PO_4- 2.0 , $FeSO_4$- 0.005, $MnSO_4$- 0.0016, $CuSO_4$- 0.0014, $CoCl_2$-0.002, Yeast extract- 3.0, Tryptone- 1.0. This was sterilized at 121^{0}C for 15minutes, cooled and inoculated with 4 plugs of fungi from 5-day old plates using 10mm diameter sterile cork borer. The flasks were incubated under shaking condition for 120 hours at 28±3^{0}C and at 120rpm. Sterilized but uninoculated media served as control and mycelia-free broth served as the crude enzyme. The crude enzyme was assayed using 1.0mL of 5mm *para*-Nitrophenyl-B-D-glucopyranoside (*p*NPG) solution mixed with 1.8mL of sodium acetate buffer (0.1 M, pH 4.8) and 0.2mL crude enzyme. The mixture was incubated at 50^{0}C for 30 minutes in a water bath. 4mL of glycine-NaOH buffer (0.4 M, pH 10.8) was added to stop the reaction. The liberated product of *p*NPG was then measured with spectrophotometer at an absorbance of 430 nm. Crystalline *para*-Nitrophenol (*p*NP) combined at the rate of 20 g/l in acetate buffer (0.1 M, pH 4.8) was used as standard. One unit of β-glucosidase was defined as the amount of enzyme that released 1 µmol of *p*-nitrophenol per minute under the stated assay conditions.

Results and discussion

Identification of fungal isolates

A wide variety of fungi were isolated from this study as shown in Table 1. They were of the genus *Absidia* sp*, Alternaria* sp*, Aspergillus* sp*, Cladosporium* sp*, Fusarium* sp*, Geotrichum* sp and *Penicillium* sp. Several species of *Aspergillus* were also encountered. These were *Aspergillus aculeatus, A. allahabadii, A. flavus, A. niger, A. restrictus* and *A. versicolor.* The husks, which were the source of the inoculum, were collected from husk-dumps outside of the rice mills, exposed to the open environment.

This justifies the fact that species encountered were the commonly known environmental fungi. Environmental fungi and their spores are widely distributed in nature and possess a wide range of flexible traits that allows their adamant survival in diverse environments, having adapted to the fluctuating environmental conditions in which they find themselves (Garcia-Rubio *et al.,* 2015). Willetts (2008) explained that the formation of sclerotia by fungi helped them survive different conditions including extreme temperature and desiccation while Garcia-Rubio *et al*. (2015) suggested that the cell wall components, especially the melanin and glycoproteins, aided in fungal cell protection in diverse environments and extreme conditions, like exposure to toxins and UV rays.

Table 1. Macroscopic and Microscopic features of fungal isolates.

Fungi	Macroscopy	Microscopy
Absidia species	White woolly colony with tiny grey spores with white and grey reverse	Medium sized pyriform, sporangia borne on pyriform shaped columella emanating from long sporangiophore
Alternaria species	Moderate growing, light brown, velvety colony with black reverse	Obclavate, septate macroconidia borne on conidiophores. Septation are transverse, sometimes further divided diagonally, and are about four in number
Aspergillus aculeatus	Fast growing white, powdery colony, rapidly becoming brown with visible brown conidial heads borne on phialides. Reverse is pale yellow	Radiate sporangial heads that bears crowded phialides, filling entire vesicle head. Long biseriate phialides bear short chains of two or more conidia, all pointing outwards.
Aspergillus allahabadii	Moderate growing, cream coloured, flat colony with tiny green scanty raised mycelia that develop at the center. Reverse is pale.	Radiate conidial heads that bears crowded phialides, filling three-quarters of vesicle head. Long biseriate phialides bear short chains of two or more conidia, all pointing upwards
Aspergillus flavus	Fast-growing, pale, powdery colony rapidly turning olive-green with visible conidia heads borne on phialides	Radiate conidial heads bearing uniseriate phialides, filling three-quarters of the vesicle. Phialides bear long chains of different lengths conidia, all pointing outwards.
Aspergillus niger	Fast-growing pale, powdery colony, rapidly becoming black with visible black conidia heads borne on phialides. Reverse is pale yellow	Radiate conidial heads that bears crowded biseriate phialides, filling entire vesicle head. Long biseriate phialides bear short chains of more than three linked conidia, all pointing outwards.
Aspergillus restrictus	Slow-growing, dark green flat, powdery colony with flat base, slow growing with no visible out-growing spores	Spores are borne on uniseriate phialides sprouting from one-quarter the head of the vesicle. Spores are one-two layers only and point upwards.
Aspergillus versicolor	Slow-growing, yellow-beige, suede-like colonies with crack-like folds and pale reverse	Radiate conidial heads that bears crowded biseriate phialides, three-quarters of the vesicle. Phialides bear short chains of two to three conidia and are scantier than *A. niger*.
Cladosporium species	Dull green, velvety colonies with feathery edges, becoming powdery with time due to an abundance of spores. Reverse is black	Elliptical-shaped chains of conidia borne on multiple-branching conidiophores
Fusarium incarnatum	Cottony aerial pale pink mycelia, turning chalk pink with time. Reverse is pink	Conidia form in whorls around short conidiophores which are borne on long phialides. Macroconidia are curved, sharp-ended and bear transverse septation.
Geotrichum species	White, flat, cotton colony looking like thick wool. Reserve is white	Branched hyphae fragment into cube-shaped, thin-walled, smooth edged, arthroconidia borne in chains.
Penicillium species	White initial, raised colony, rapidly becoming grey-green, velvety and powdery, with folds and a pale reverse	Conidia are borne on the phialides are also borne on metula, on branched ramuli. Pinnate phialides split into twos or threes as they emerge from metula.

Frequency of occurrence of fungal Isolates

The frequency of occurrence of each of the isolates is shown on Table 2. The most abundant fungi isolated was *Aspergillus flavus* (18.18%). *Absidia* sp was the next most abundant species, being isolated five times and having a 15.15% frequency. The least abundant isolates were *Aspergillus restrictus*, *Alternaria* sp. and *Geotrichum* sp., with percentage frequencies of 3.03. The *Aspergillus* genera were the most occurring genus in this study, with 17 isolates out of the total 33 isolates and a percentage frequency of 51.52%. Like in the result of this research, Martin *et al*, (2012) had isolated the genus *Aspergillus* (56%), *Penicillium* (26%) and *Cladosporium* (18%) that belonged to soil microbiota and the environment in their study while Ja'afaru (2013) had encountered 40.8% *Aspergillus* sp. and 1.8% *Penicillium* sp. whilst screening environmental samples for cellulase and xylanase production.

In a similar study, Rodrigues *et al*. (2020) found *Aspergillus* genera to be the most dominant of all the isolates from the Jansen Lagoon State Park, Maranhão, Brazil and isolated other genera like *Penicillium*, *Absidia*, and *Fusarium* in their work, which was similar to the result derived in this work.

Table 2. Frequency of occurrence of isolates.

Isolates	PDA Media (sfu)	CMC Media (sfu)	Total Occurrence (sfu)	Percentage Frequency (%)
Absidia sp.	0	5	5	15.15
Alternaria sp.	0	1	1	3.03
Aspergillus aculeatus	2	1	3	9.09
Aspergillus allahabadii	0	2	2	6.06
Aspergillus flavus	4	2	6	18.18
Aspergillus niger	1	1	2	6.06
Aspergillus restrictus	0	1	1	3.03
Aspergillus vesicolor	1	2	3	9.09
Cladosporium sp.	2	2	4	12.12
Fusarium sp.	1	1	2	6.06
Geotrichum sp.	0	1	1	3.03
Penicillium sp.	1	2	3	9.09
Total	12	21	33	100.00

Legend:

sfu: spore-forming unit

Qualitative cellulase activity of fungal isolates

The qualitative tests for cellulase and pectinase production is shown in Table 3. The production of these enzymes was shown by clear zone of hydrolysis around the grown fungal mycelia. Of the seventeen fungi isolated, eleven (64.71%), of them were seen to produce cellulase when cultured on minimal media modified with carboxy methyl cellulose. These were *A. allahabadii* (D and I), *A. flavus, A. niger* C, *A. versicolor, Cladosporium, Fusarium, Geotrichum, Penicillium expansum, Penicillium citrinum* and *Penicillium* sp. The highest zone of hydrolysis was 3.00cm and was produced by *Penicillium expansum* while the second highest, 2.23cm, was with obtained with *A. niger* sp. *Penicillium* sp. gave the least zone of hydrolysis, indicating the lowest detectable cellulase production among all the isolates in this study. Several studies like Oyeleke *et al*., (2012), Ja'afaru (2013) and Behera *et al*., (2017) show a variety of fungi genera have been documented to produce these cellulases. Although many fungi were isolated from husk, the screening results showed that only a few were able to produce cellulase. This could mean that the majority that did not produce these enzymes were opportunistic fungi found within that environment or were secondary invaders that tended to feed off the by-products from the degradation of the other fungi (O'Loughlin and Green, 2017). They could also be microbes that produced another set of enzymes outside the scope of this research. Studies show that regardless of the large number of fungi seen to degrade cellulose during plate screening, only few of them can produce copious amounts of enzymes extracellularly for *invitro* hydrolysis (Rathore *et al*., 2014). Environmental fungi thrive as saprophytes by absorbing organic matter like husk, from sources around them (Crowther *et al*., 2012). Thus, the ability to degrade lignocellulose, the most abundant renewable environmental resource, is crucial to the survival of many of them. This ability also helps in nutrient recycling in the ecosystem's energy flow and biogeochemical cycles (Walker and mc Ginnis, 2014).

Table 3. Qualitative screening of fungal isolates for cellulase and pectinase producing potential.

Fungi	Cellulase Screening (cm)
Absidia species	0.00±0.00
Alternaria species	0.00±0.00
Aspergillus aculeatus	0.00±0.00
Aspergillus allahabadii D	1.57±0.06
Aspergillus allahabadii I	1.47±0.06
Aspergillus flavus A	1.07±0.06
Aspergillus flavus D	0.00±0.00
Aspergillus flavus E	0.00±0.00
Aspergillus niger	2.23±0.15
Aspergillus restrictus	0.00±0.00
Aspergillus versicolor	1.60±0.20
Cladosporium species	1.70±0.06
Fusarium species	0.50±1.00
Geotrichum species	1.47±0.45
Penicillium expansum	3.00±1.00
Penicillium citrinum	1.50±0.10
Penicillium species	0.80±0.10
Total	11/17
% Frequency of cellulase producers	64.71

Legend:
A. alla. D–*Aspergillus allahabadii* D
A. alla. I–*Aspergillus allahabadii* I
A. nig. -*Aspergillus niger*
F. in -*Fusarium incarnatum*
P. citr -*Penicillium citrinum*
P. exp -*Penicillium expansum*

Quantitative screening for cellulase production

The quantitative screening for the production of specific cellulases; endoglucanase, cellobiohydrolase and beta-glucosidase are shown

in Fig.s 1-3. The Fig.s also display the amount of enzyme produced by each isolate, from which inference can be made for the days of optimum production by each isolate and the highest producer of the various enzymes. All isolates were screened for a period of 144 hours. The samples were all incubated under the same cultural conditions of fixed temperature (30°C) and pH (5.0) and the analyses were run on samples collected daily for all the isolates. Fig. 1 shows the quantitative screening for endoglucanase production. The isolate recorded with the highest production of endoglucanase was *F. incarnatum*; it started with a low value of 1.62 U/ml (the lowest after 24 hours incubation), increased to more than 15 U/ml at the 72nd hour, then increased slightly again to 16.60 U/ml at the 120th hour. *A. niger* was the second highest endoglucanase producer. It started with a high activity (7.85 U/ml) after 24 hours and it increased steadily to 16.00 U/ml at the 120th hour, after which it reduced abruptly within the next twenty-four hours. *Penicillium citrinum* and *Penicillium expansum* were noted to produce the only little endoglucanase compared to all the other isolates. In a study by Dutta *et al*. (2018), *Fusarium* sp was seen to yield copious amounts of cellulases and were recommended as potential candidates for bioconversion of cellulose into fuel and other enzyme-catalyzed industrial processes because of their high activity and stability over a wide range of cultural parameters that affect their enzyme production rate like, temperature, pH, inhibitors oxidizing agents, ions and surfactants. Sakai *et al*. (2020), included the added advantage of their broad spectrum on cellulose complexes.

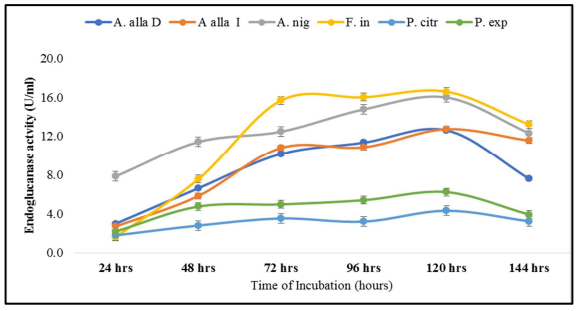

Fig. 1. Quantitative screening of isolates for endoglucanase production at 30^{0}C.

Fig. 2 shows the quantitative screening for cellobiohydrolase production. The cellobiohydrolase values were much lower than those obtained from endoglucanase. *Penicillium citrinum* and *Penicillium expansum* were noted to produce close values of cellobiohydrolase until the 72nd hour of incubation (0.71:.079; 1.43:1.11; and 1.46:1.68 U/ml, respectively). At the 120th hour, *Penicillium expansum* was recorded to yield the highest activity of cellobiohydrolase (3.10 U/ml) while the least activity of cellobiohydrolase (0.22 U/ml) was recorded at the 24th hour for *F. incarnatum*.

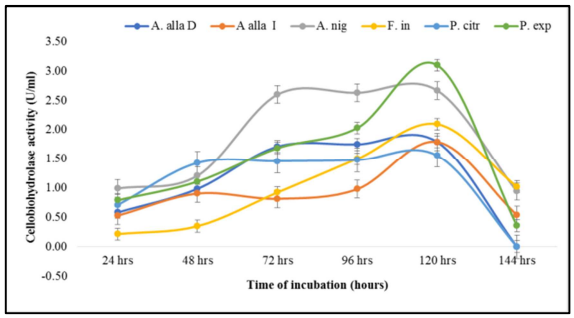

Fig. 2. Quantitative screening of isolates for cellobiohydrolase production at 30^{0}C.

Quantitative screening for beta-glucosidase production among the isolates was displayed in Fig. 3. Beta-glucosidase values were the highest recorded for all the enzymes. The values for all the isolates increased to reach optimum values at the 120th hour, with *P. expansum* yielding the highest (44.41 U/ml) while *A. allahabadii* D (1.96 U/ml) yielded the least activity at the 24th hour of incubation. All the beta-glucosidase values peaked at the 120th hour and lessened at the 144th hour.

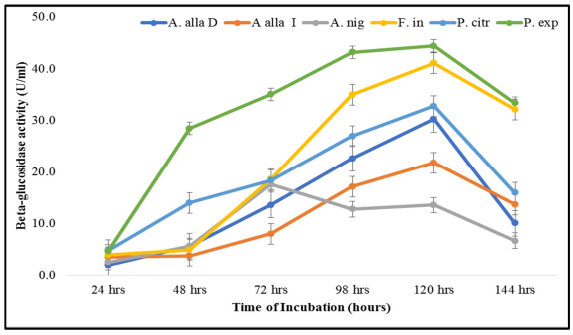

Fig. 3. Quantitative screening of isolates for beta-glucosidase production at 30^{0}C.

According to the results in this study, *Penicillium expansum* was the best producer of both cellobiohydrolase and beta-glucosidase. Studies by Mesa *et al.* (2016), Prasanna *et al*. (2016) and Vaishnav *et al.* (2018) have shown *Penicillium* spp. to be good producers of cellulolytic enzymes. Similar to the result obtained in this study, Prasanna *et al*. (2016) reported that the beta-glucosidase produced by *Penicillium* sp. had a higher activity than the exoglucanase and endoglucanase it had produced in their study. In a review on *Penicillium* sp., Gusakov and Sinitsyn (2012) highlighted that the extracellular multi-enzyme complex of *Penicillium* sp. were highly efficient at cellulose conversion and concluded that these species of fungi show great capacity for industrial scale lignocellulose conversion and for biotechnological applications requiring cellulases complexes.

Conclusion

Cellulase-producing fungi dwell among the abundant variety of mycobiota in the environment and among cellulosic wastes. Screening fungi for specific endoglucanase-, cellobiohydrolase- and beta-glucosidase-producers present a stream-lined approach for the selection of enzyme-producing strains for further enzyme studies. *F. incarnatum* and P*. expansum* were the most prolific endoglucanase, cellobiohydrolase and beta-glucosidase producers in this study.

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