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Potentials of Rice Bran and Oil Palm Fibre as Substrate for Exo-B-Glucanase Production by *Penicillium chrysogenum*

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ABSTRACT
Glucanases are important industrial enzymes that catalyze the hydrolysis of glucan into oligosaccharides. Commercial β-glucanase are produced from microorganisms such as *Penicillium chrysogenum*, *Aspergillus flavus*, *Pseudomonas fluorescens* etc. The increasing application of glucanases in various industries has called for continuous search for cheaper means of glucanase production. This study explores β-glucanase production from *Penicillium chrysogenum* using rice bran and oil palm fiber as substrates. Fungal isolates of soil samples were characterized and screened for exo- β-glucanase activity using modified Czapek-Dox Agar (MCDA). Production of exo-β-glucanase was carried out through submerged fermentation system. Crude enzyme produced was extracted and assayed for enzyme activity and protein concentration using standard methods Enzyme purification was carried out using ammonium sulphate precipitation and dialysis methods. The purified enzyme was characterized by measuring the effect of temperature, pH, various salts and inhibitors. The optimal conditions for exo-β-glucanase production were found to be 96 hours of incubation, a pH of 4.5 for rice bran and 5.5 for oil palm fiber, and temperatures of 30°C for rice bran and 35°C for oil palm fiber, with ammonium sulphate and oil palm fiber as the best nitrogen and carbon sources respectively. The enzymes were purified with 80% ammonium sulphate and dialysis tubing, resulting in a 1.09 to 1.50-fold increase in purification. The addition of chloride salts, surfactants, and EDTA impacted the activity of the enzyme. Based on its physiochemical properties, the partially purified enzyme has potential industrial and biotechnological applications, such as in beer production and as an additive in laundry detergents and saccharification.

Keywords: Exo-β-glucanases; Oil Palm Fibre; Rice Bran; saccharification; submerge fermentation; *Penicillium chrysogenum*

INTRODUCTION
Enzymes are organic molecules that speed up chemical reactions (Robinson, 2015). Most enzymes are proteins, although a few are catalytic RNA molecules (Wilson and Lilley, 2021). They are very susceptible to environmental conditions and could be isolated from a plant, animal tissue, or microbes. Enzymes produced by microorganisms are economically beneficial because microbial culture is far simpler, grow faster and easier to genetically manipulate than plants and animals. Commercially produced microbial enzymes include amylases, lipases, proteases, β-glucanases and cellulases. β-glucanase degrades β-glucan on the cell wall of cereals and some fungi to glucose monomer or saccharide oligomer (Cosme *et al.*, 2023).
Glucan is a polysaccharide made up of D-glucose molecules linked by glycosidic bonds. β-glucans are unbranched, water-soluble polysaccharides found in the endosperm and aleurone cells of the Poaceae family (Garcia-Gimenez *et al.*, 2019), and are most abundant in oats and barley, but can also be found in smaller amounts in other cereals like corn, rice, rye, wheat, and sorghum (El Khoury *et al.*, 2012).

The concentration and extractability of β-glucans vary depending on the type of grain (Lante *et al*., 2023). β-glucans are important from a medical standpoint and are targeted by antifungal drugs in the echinocandin class. β-glucans, as part of a group of biologically active natural molecules, are increasingly attracting attention not only as a food supplement but also as an immunostimulant and potential drug (Vaclave *et al*., 2019).

β-Glucanases are enzymes that target β-glucans, a key component of plant cell walls, including cellulose (β-1,4-glucans), callose (β-1,3-glucans), cereal β-glucans (β-1,3;1,4-glucans), and β-1,3;1,6-glucans found in the cell walls of some fungal groups (Fesel and Zuccaro, 2016). Some β-glucanases are beta-1,3-glucanase, which breaks down β-1,3-glucans such as callose or curdlan; beta-1,6-glucanase degrades β-1,6-glucans; and cellulase which hydrolyzes 1,4-beta-D-glucosidic linkages in cellulose, lichenin, and cereal β-D-glucans (Parrot *et al*., 2022). These enzymes have multiple industrial applications, including the production of beer (Edison *et al*., 2022), additives in laundry detergents, and in the saccharification of agricultural and industrial waste to produce glucose syrup for animal feed (Caseiro *et al*., 2019).

Rice bran is the cuticle existing between the rice and the husk of the paddy and consist of embryo and endosperm of the seeds of Oryza sativa, family Graminae. It constitutes 8 percent of the weight of the whole grain and contains most of the nutrients. Rice bran, a "little known" food is highly nutritious and delivers a powerhouse of health supporting nutrients which is either thrown away or used for low-level animal feed (Arshied *et al*., 2023). Rice bran contains various antioxidants that impart beneficial effect on human health, it also contains a high level of dietary fibre such as beta-glucan, pectin and gum (Rondanelli *et al*., 2011; Doughari, 2011). Oil palm empty fruit bunch (OPEFB) is obtained after stripping off the fresh oil palm fruit from bunch. About 7.3 million tonnes are generated annually (Rao and Ramakrishna, 2022). Because OPEFB is available in large quantities and has a fairly high cellulose content with an average of 50 % based on an oven dried basis it appears to be a potential substrate for cellulase production. Oil palm fibre can also serve as an alternative source of beta glucan (Tan and Ida, 2013).

Penicillium species produce a wide range of extracellular enzymes that are crucial in breaking down organic matter. These hydrolases include cellulolytic enzymes and polysaccharases such as alpha and beta-glucanases, hemicellulases, and pectic enzymes, as well as lipases and proteolytic enzymes that contribute to the flavor of ripened cheeses. Despite their abundance, only a small number of *Penicillium* species have been utilized for industrial enzyme production. β-glucanase has been manufactured through solid-state fermentation, and the process of extraction involves centrifugation and filtration (Doughari, 2011). Previously known as *Penicillium notatum*, *Penicillium chrysogenum* is a widely studied species of Penicillium that is most famous for being a source of penicillin and several other antibiotics. *Penicillium chrysogenum* can most often be found in indoor environments particularly in those with high humidity, dampness, or previous water damage. It is a widespread fungus that is common in temperate and subtropical regions. It can also be found in soil and vegetation, and can also grow on stored food and damp building materials, making it a common indoor fungus (Andersen *et al*., 2011).

This study focuses on producing, partially purifying, and characterizing β-glucanase from *Penicillium chrysogenum*. Rice bran and oil palm fiber serve as substrates.

The process involves isolating and characterizing *Penicillium chrysogenum* from soil. A crucial step in this research is screening and selecting the isolates for β-glucanase production. Additionally, optimizing production conditions and purifying and characterizing the β-glucanase produced by *Penicillium chrysogenum* are central objectives of this investigation.

MATERIALS AND METHODS

Samples collection

Rice bran and oil palm fiber were collected from rice mill and oil palm mill at Wanokom South Ukelle in Yala local government of Cross river state Nigeria using a sterile plastic container. Soil samples were collected from dump sites of rice mill, oil palm mill of above-mentioned community and were transferred into different MacCartney bottles. The samples were transported to the Department of Microbiology laboratory, of the Modibbo Adama University of Technology, Yola Adamawa State Nigeria for analysis.

Isolation of fungi

One gram of soil sample was aseptically transferred into a glass test tube containing 9 ml of sterilized distilled water to prepare 10 ml sample suspension and shake for 10 minutes at room temperature. From the sample suspension serial dilution up to 10^{-7} were prepared. 0.1 ml of 10^{-6} dilution of soil was inoculated using spread plate method onto Sabouraud Dextrose Agar (SDA) plate and 0.5 ml of chloramphenicol was incorporated in order to inhibit the growth of bacteria. The culture was incubated at 30 °C for 3 - 5 days. The presence of growing fungi on the plate was examined after incubation; the growths were subcultured three times onto Sabouraud Dextrose Agar (SDA) plates, this was to obtain pure fungal isolates (Adeleke *et al*., 2012).

Identification of fungi

On a grease free microscopic slide, a drop of lactophenol cotton blue stain (LPCBS) was placed. Fungal growth from a 3 days old culture was picked with a sterile needle and teased in LPCBS. This was covered with a cover slip and then viewed under x40 objective of the microscope. The slide was examined for hypha arrangement and nature of their fruiting bodies and morphological characterization compared to those in the Atlas of mycology (James and Natalie, 2001).

Fungal isolates screening for exo-β-glucanase production

The process of screening for exo-β-glucanase production among fungal isolates involved growing the isolates on Petri dishes filled with modified Czapek-Dox Agar where beta glucan oat powder was used as the single source of carbon instead of sucrose. After the incubation period, the plates were treated with 50 mM iodine to determine the presence of clear zones surrounding the colonies (Yogesh *et al*., 2009). Fungal strains that exhibited significant clearing zones in the agar containing beta glucan oat were selected as potential exo-β-glucanase producers (Ahmed and Mustafa, 2013).

Analysis of the molecular properties of the screened isolates

The molecular identification process was completed after DNA extraction protocols (Yalcinkaya *et al*., 2016). The DNA of *Penicillium* spp. isolate was amplified using universal primers ITS-1 and ITS 4. The amplified fragments were purified and their integrity was verified on a 1% Agarose gel. The amplified fragments were sequenced using a Genetic Analyzer 3130x1 sequencer from Applied Biosystems using manufacturer manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit and the results were analyzed using MEGA 6 and compared with Type strains in the GenBank database.

The results showed that the isolate was a eukaryote with 80S ribosomes, consisting of large 60S and small 40S subunits, and had two internal transcribed spacer regions between the ribosomal subunits. The isolate was confirmed to be 100% identical and positive as *Penicillium chrysogenum* (Table 1) (Romos-Ibarra *et al*., 2021)

Quantification of fungi spores for fermentation

1 mL of seed culture was transferred into 100 mL flasks with 50 mL of sterile inoculum medium and mixed by shaking at 250 rpm for 10 minutes. The hemacytometer and cover-slip were cleaned and dried, 9 µL of the cell suspension was added to one of the two counting chambers. The chambers were carefully filled without any bubbles, under-filling, or over-filling. The fragments were counted in the corner squares, excluding those touching the borders. The spore count was determined by multiplying the number of fragments counted in the four corner squares by 104 to obtain the number of spores per milliliter according to Sserumaga, (2012).

Substrates preparations

Rice bran was washed, dried, milled with an electric wearing blender and sieved through 20 mm -40 mm mesh sieve. The powder was collected with 0.42 mm and 0.85 mm mesh particle size and used as solid substrate for submerge fermentation. The oil palm fiber was pressed to extract juice using a sugarcane juice extractor and the resulting fiber was left to sun dry for 5 days before being shredded into smaller pieces and sieved to a particle size less than 2 mm. The dried fiber was then stored in airtight plastic bags at room temperature for use in submerged fermentation for enzyme production.

Production of crude exo-β-glucanase

The experiment was performed by homogenizing 6 grams of rice bran with a magnetic stirrer and 200 ml of a 0.5% $(NH_4)_2SO_4$ solution, along with 0.1 g of $MgSO_4.7H_2O$, 0.2 g of KH_2PO_4, and 0.6 g of yeast extract. The pH of the mixture was adjusted to 5.5 using 1N HCl solution, and then transferred to a sterilized 250 ml Erlenmeyer flask, then autoclaved at 121 ºC, and 15lb pressure for 15 minutes. After cooling, the mixture was inoculated with 10 ml of a *Penicillium chrysogenum* suspension, and allowed to ferment for 5 days at room temperature (30-32°C) on an electric rotary shaker at 250 rpm. The process was repeated using oil palm fibre as a substitute for rice bran (Doughari, 2011).

Extraction of exo-β-glucanase

The culture fluids obtained after 5 days fermentation of rice bran and oil palm fibre using the inoculum were filtered separately using whatman filter paper (WFP 1). The filtrates were buffered to pH 5.5 and centrifuged at 5000 rpm for 30 minutes at 4 ºC to obtain the crude enzyme extracts. The crude extracts obtained were filtered again using WFP 1 and buffered to pH 5.5, the clear supernatant was used as the source of the crude exo-β-glucanase (Doughari, 2011; Ahmed and Mustafa, 2013).

Crude enzyme activity assay

The beta-glucanase activity in the culture filtrate was evaluated by measuring the amount of reducing sugar produced in the reaction mixture through the modified Miller method (Miller, 1959) as reported by Doughari, (2011). The reaction mixture consisted of 500 µl of a 3% (wt/vol) beta-glucan substrate in a 50 mM pH 7 phosphate buffer and 500 µl of the crude solution. The spectrophotometer was calibrated using a positive control (enzyme blank) and a negative control (reagent blank). The enzyme blank was created by mixing 500 µl of substrate and 500 µl of crude enzyme solution, while the reagent blank was produced by combining 500 µl of 50 mM pH 7 phosphate buffer with 500 µl of substrate. Both the experimental and control samples were incubated at 37 ˚C for 30 minutes.

The reaction was terminated by adding 1 ml of 3, 5-dinitrosalicyclic acid and heated in a boiling water bath for 15 minutes. After cooling, the absorbance was measured at 540 nm, and the amount of reducing sugar released was determined using glucose as the reference (Shao-jun *et al*., 2001).

Determination of protein

The protein concentration was determined according to Bradford (1976) and was estimated from the standard curve that utilized bovine serum albumin (BSA) as the benchmark.

Optimization of exo-β-glucanase production

The isolates were grown under different condition to determine the influence of growth medium on exo-β-glucanase production. The effect of Nitrogen, incubation period, temperature and pH were determined according to Thakur *et al*. (2010).

Carbon Sources effects on the production of exo-β-glucanase

Using_Doughari and Onyebarachi, (2019) method, rice bran and oil palm fiber were substituted into the production medium at a 3% (w/v) concentration, replacing agro waste materials (banana peels, orange peels, and yam peels), as well as pure chemicals (glucose, sucrose, maltose and beta glucan oat). The exo-β-glucanase activity in the culture supernatants was then measured.

The effect of Nitrogen Sources and Incubation Time on the production of exo-β-glucanase

For Nitrogen Sources and Incubation Time effect on the production of exo-β-glucanase, Doughari and Onyebarachi, (2019) method was also employed. Peptone, urea, yeast extract and $(NH_4)_2SO_4$ were used as nitrogen sources in the basal medium individually to determine their effect on exo-β-glucanase production. The optimal incubation time for exo-β-glucanase production was determined by incubating the fungal isolate in the production media at 37°C and pH 5.5 for 24, 48, 72, 96, 120, 144, and 168 hours.

Temperature effects on the production of exo-β-glucanase

The production media containing *Penicillium chrysogenum* isolates was incubated at selected temperature: room temperature 30 ˚C, 35 ˚C, 40 ˚C, 45 ˚C and 50 ˚C in order to determine the optimum temperature for the production of the enzyme. The content of the flask was harvested after 5 days of fermentation and exo-β-glucanase activity was assayed (Doughari and Onyebarachi, 2019).

The pH effects on the production of exo-β-glucanase

The fungal isolate was grown in media and incubated using three different buffers with varying buffering capacity: sodium acetate (3.0-5.0), sodium phosphate (5.5-7.5), and sodium carbonate (8.0-9), all at a concentration of 50mM (Doughari and Onyebarachi, 2019).

Partial purification of Exo-β-glucanase

The salting out technique was used to perform protein precipitation with ammonium sulphate fractionation through gentle and constant stirring. The enzyme concentrate was prepared through two ammonium sulphate precipitation steps. The crude enzyme was first saturated with 30% ammonium sulphate and then spun in a centrifuge for 30 minutes at 4°C at 5000 rpm. The supernatant was removed from the pellet, re-suspended in 20 ml of sample buffer, and slowly mixed with 80% ammonium sulphate at a cooled temperature for an hour. The solution was then transferred to a centrifuge tube, spun, and the supernatant was discarded. The pellet was collected, re-suspended in a minimal amount of 50 mM phosphate buffer (pH 7), and the enzyme was dialyzed in 50 mM phosphate buffer (pH 7) (Palanivel *et al*., 2013).

Characterization of the partially purified Exo-β-glucanase

Temperature effect on the partially purified *Penicillium chrysogenum* exo-β-glucanase

The stability of β-glucanase was evaluated using the procedure outlined by Tari *et al*. (2008). The enzyme was pre-incubated in a 50 mM phosphate buffer at temperatures from 30 to 65°C for 30 minutes. Samples were taken and the remaining β-glucanase activity was measured by using β-glucan from oats as described previously (Doughari, 2011).

Effect of pH on the partially purified *Penicillium chrysogenum* Exo-β-glucanase

The activity of exo-β-glucanase was measured using 50 mM of various buffers (ranging from 3 to 9), with β-glucan oat as the substrate, using the method described previously (Doughari,2011). The buffers used in this study were sodium acetate (3.0-5.5), sodium phosphate (6.0-7.5), and sodium carbonate (8.0-9) (Tari *et al*., 2008).

Effect of surfactant and EDTA on the partially purified *Penicillium chrysogenum* Exo-β-glucanase

The experiment involved testing various surfactants (Triton X-100, Tween 80, Cetrimide) and EDTA at 1%, 3%, and 5% concentrations with a β-glucanase enzyme solution. The surfactant/EDTA mixture and the enzyme solution were pre-incubated for 10 minutes at 40 ºC before adding the substrate (3% glucan oat). The residual β-glucanase activity was then measured (Doughari, 2011). A control sample without surfactants and EDTA was also taken and had 100% residual activity (Al-Najada *et al*., 2012).

Effect of chloride salts on the partially purified *Penicillium chrysogenum* Exo-β-glucanase

The experiment involved testing the effect of various chloride salts (NaCl, $CoCl_2$, $CuCl_2$, $MgCl_2$, NH_4Cl, $CaCl_2$, and KCl_2) at concentrations of 1 mM, 2 mM, and 5 mM on the enzyme solution. The mixture of the enzyme and salt solution was incubated for 30 minutes at 40°C before being reacted with 3% glucan oat. The residual activity of the β-glucanase was then measured (Doughari, 2011) and compared to control samples without salt, which were considered to have 100% activity (Ramachandran, 2005; Doughari and Onyebarachi, 2019).

Preparation of the calibration curve

The glucose standard curve was prepared by varying concentration of glucose between 0-1 mg/ml. The absorbance was read at 540 nm and a graph of optical density against glucose concentration was plotted.

Statistical analysis

The impact of various physiochemical factors such as pH, temperature, carbon sources, incubation duration, nitrogen sources, salt, and inhibitors on exo-β-glucanase production and activity was expressed as standard error of the mean and analyzed using GraphPad Prism 5 software.

RESULTS

This study analyzed twelve fungal isolates from natural sources and found that *Penicillium chrysogenum* produced the highest exo-β-glucanase activity on a selective medium containing β-glucan oat. The isolate was confirmed to be *Penicillium chrysogenum* through microscopic and molecular analysis and its genetic profile matched the NCBI strain (Figure 1). The carbon substrates tested, and oil palm fiber had the highest exo-β-glucanase production, followed by rice bran, yam peel, and banana peel. The lowest production was observed with maltose as the carbon source (Figure 2). Both organic and inorganic nitrogen sources were effective for exo-β-glucanase production and maximal production was achieved with peptone and ammonium sulphate for rice bran and oil palm fiber, respectively (Figure 3).

The production of exo-β-glucanase increased with duration of incubation, reaching a maximum on day 4 and then declining (Figure 4). The highest production was achieved at pH 4.5 for rice bran and pH 5.5 for oil palm fiber (Figure 5). Maximum production temperatures of exo-β-glucanases were 30 ºC and 35 ºC for rice bran and oil palm fibre respectively (Figure 6). Purification results showed that specific activity of the crude enzyme for *Penicillium chrysogenum* grown on rice bran was 2.39 U/mg which increased to 2.60 after precipitation of ammonium sulphate and finally increased to 2.70 U/mg after dialysis. The yield of crude enzyme was 100 % and decreased to 52 and 13% after precipitation of ammonium sulphate and dialysis respectively giving a purification fold of 1.09 and 1.13 respectively. For oil palm fibre, the specific activity of crude enzyme was 2.06 U/mg. This value increased to 2.34 U/mg after precipitation of ammonium sulphate and to 3.10 U/mg after dialysis. The yield of crude enzyme was 100 % and decreased to 70 and 15 % after precipitation of ammonium sulphate and dialysis, respectively. Purification fold of 1.14 and 1.50 was obtained after precipitation of ammonium sulphate and dialysis (Table 2). The partially purified enzyme showed maximum activities at 55 ºC for both rice bran and oil palm fibre as well as the pH of 5.5 and 5.0 for rice bran and oil palm fibre respectively (Figure 7 and 8). Effect of salts showed that chloride salts influenced the activity of the enzyme at 1 mM, 2 mM and 5 mM concentration (Table 3). Surfactants and EDTA effects on the partially purified *Penicillium chrysogenum* exo-β-glucanases showed that Triton X supported the enzyme activity using both rice and oil palm fibre as substrates while EDTA reduced the activities (Table 4). The glucose standard curve reveals the values of the unknown glucose concentrations released during enzyme assay (Figure 9).

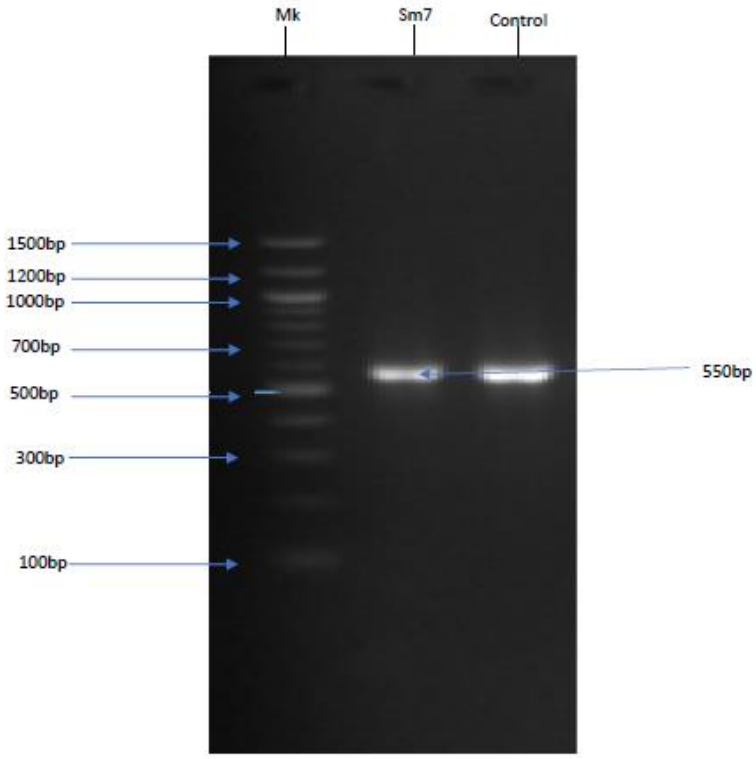

Figure 1. Gel electrophoresis of amplified *Penicillium chrysogenum* DNA. Lane 1: MK DNA maker; Lane 2: (Sm7) *Penicillium chrysogenum*; Lane 3: positive control.

Table 1: Confirmation of *Penicillium chrysogenum* isolate based on BLAST Analysis

Sample ID	Sequence	Accession	Max Score	Total Score	Query Cover	Expect value (E)	Percent Identity	Accession Length	Scientific Name
Sm7	CTGAACCTGCGGAAGGATCATTACCGAGTGA GGGCCCTCTGGGTCCAACCTCCCACCCGTGTT TATTTTACCTTGTTGCTTCGGCGGGCCCGCCT TAACTGGCCGCCGGGGGGCTTACGCCCCCGG GCCCGCGCCCGCCGAAGACACCCTCGAACTC TGTCTGAAGATTGTAGTCTGAGTGAAAATATA AATTATTTAAAACTTTCAACAACGGATCTCTT GGTTCCGGCATCGATGAAGAACGCAGCGAAA TGCGATACGTAATGTGAATTGCAAATTCAGTG AATCATCGAGTCTTTGAACGCACATTGCGCCC CCTGGTATTCCGGGGGGCATGCCTGTCCGAG CGTCATTGCTGCCCTCAAGCACGGCTTGTGTG TTGGGCCCCGTCCTCCGATCCCGGGGGACGG GCCCGAAAGGCAGCGGCGGCACCGCGTCCGG TCCTCGAGCGTATGGGGCTTTGTCACCCGCTC TGTAGGCCCGGCCGGCGCTTGCCGATCAACC CAAATTTTTATCCAGGTTGACCTCGGATCAGG TAGGGATC	MZ093187	1002	1002	99%	0	100.00%	578	*Penicillium chrysogenum*

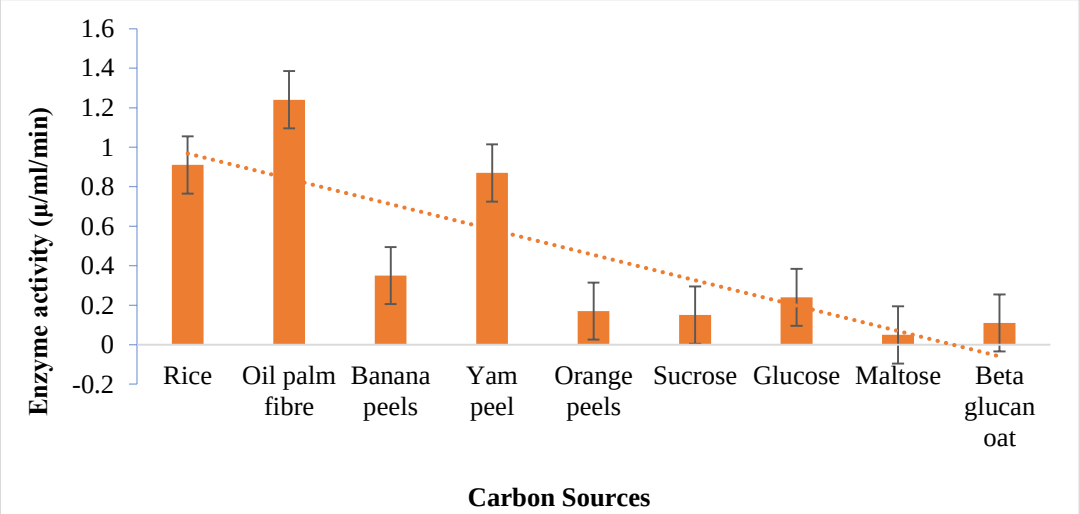

Figure 2: Effect of carbon source on *Penicillium chrysogenum* crude exo-β-glucanase production.

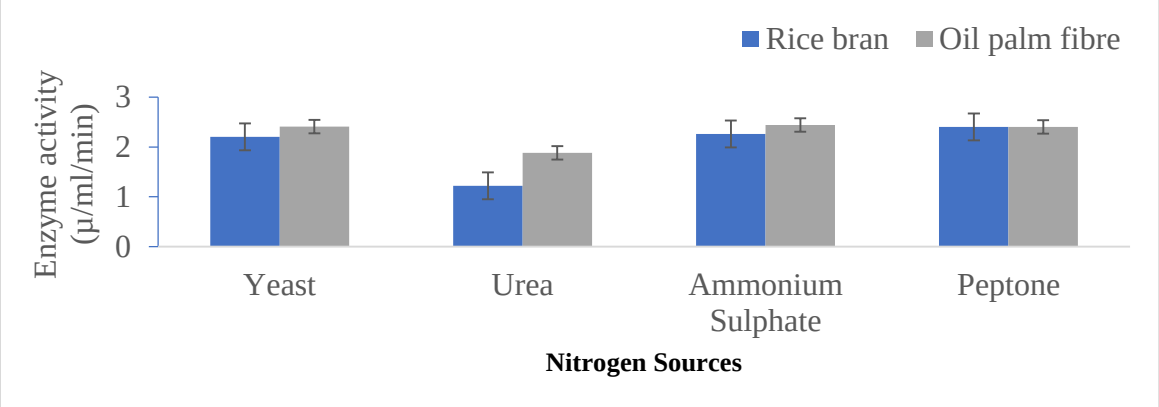

Figure 3: Effect of nitrogenous source on *Penicillium chrysogenum* crude exo-β-glucanase production

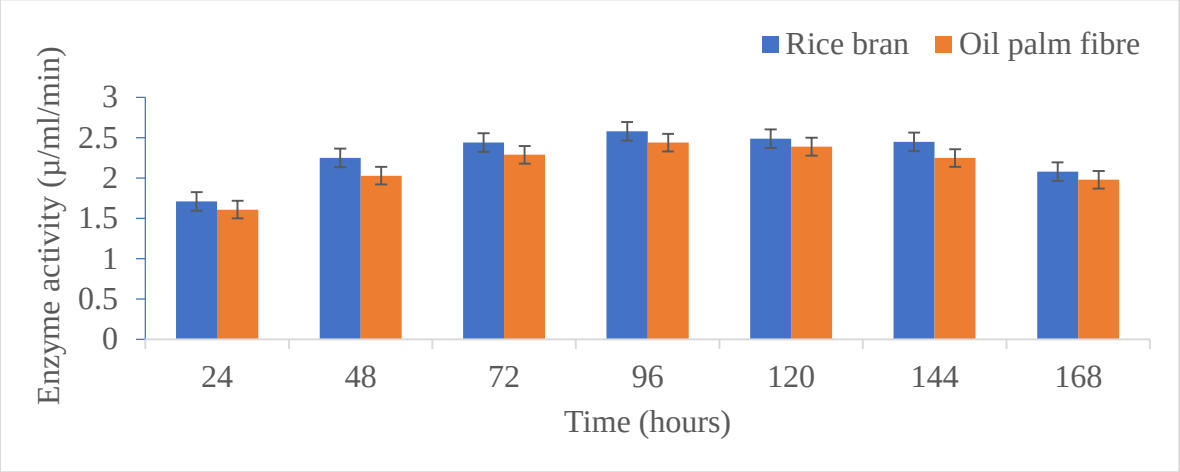

Figure 4: Effect of length of incubation period on *Penicillium chrysogenum* crude exo-β-glucanase production

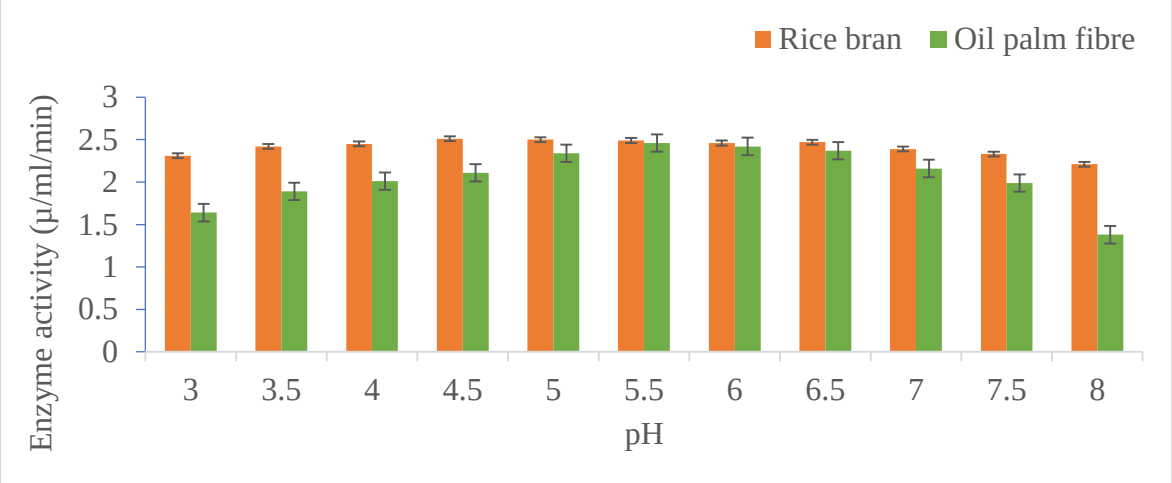

Figure 5: Effect of pH on *Penicillium chrysogenum* crude exo-β-glucanase production

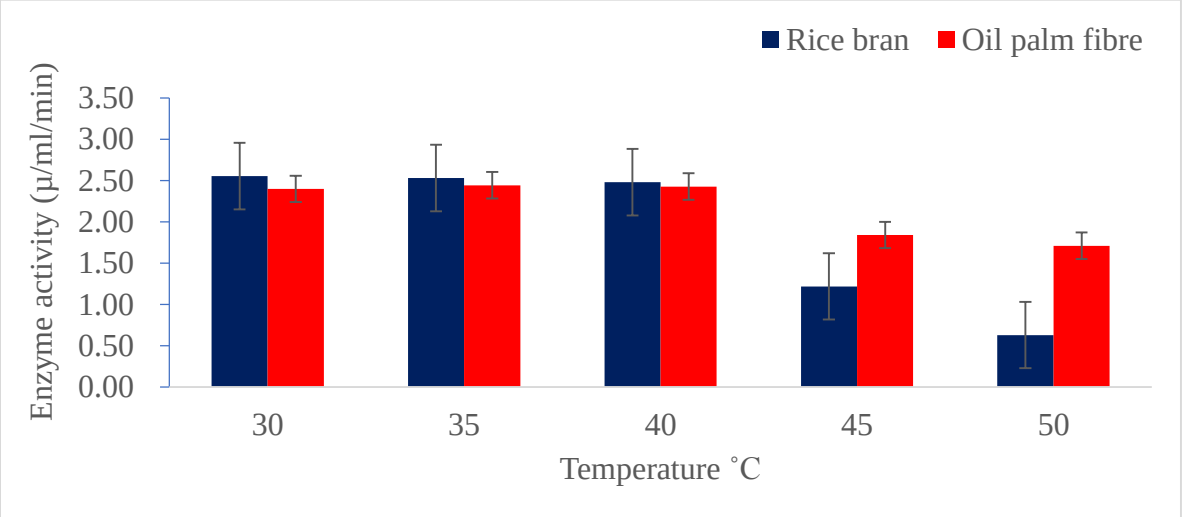

Figure 6: Effect of temperature on *Penicillium chrysogenum* crude exo-β-glucanase production

Table 2: Partial purification of *Penicillium chrysogenum* Exo-β-glucanase

Parameter	Purification Steps					
	Rice bran substrate			Oil palm fibre substrate		
	Crude	$(NH_4)_2SO_4$ Precipitation	Dialysis	Crude	$(NH_4)_2SO_4$ Precipitation	Dialysis
Volume (ml)	200	80	40	200	80	40
Protein Concentration (mg/ml)	0.88	0.42	0.10	0.99	0.61	0.10
Total Protein (mg)	176.00	33.60	4.00	198	48.8	4.8
Activity (U/ml)	2.11	1.09	0.27	2.04	1.43	0.31
Total Activity (U)	422.00	87.20	10.80	408	114.40	12.40
Specific Activity (U/mg)	2.39	2.60	2.70	2.06	2.34	3.10
Yield (%)	100	52	13	100	70	15
Purification fold	1.00	1.09	1.13	1.00	1.14	1.50

Table 3: Effect of Salts on partially purified *Penicillium chrysogenum* exo- β-glucanase

	Exo- β-glucanase activity (U/mg) by substrate per salt concentration (mM)					
	Rice bran			Oil palm fibre		
Salt	1mM	2mM	5mM	1mM	2mM	5mM
Control	0.913±00	0.913±00	0.913±01	0.322±02	0.322±00	0.322±02
NaCl	1.054±04	0.610±06	0.569±00	0.294±06	0.271±08	0.176±00
$CoCl_2$	0.933±13	1.066±11	1.346±05	0.154±02	0.174±03	0.196±11
$CuCl_2$	1.111±11	0.807±06	0.502±12	0.213±00	0.165±00	0.160±09
$MgCl_2$	0.770±12	0.695±03	0.640±00	0.161±03	0.150±03	0.143±04
$CaCl_2$	0.805±01	0.926±06	0.657±14	0.182±00	0.219±11	0.161±09
NH_4Cl	0.873±01	0.789±00	0.582±08	0.270±09	0.172±00	0.135±00
KCl_2	0.870±09	0.870±11	0.709±21	0.206±05	0.141±00	0.141±00

Table 4: Effect of surfactant and EDTA on partially purified *Penicillium chrysogenum* exo-β-glucanase

	Exo- β-glucanase activity (U/mg)					
	Rice bran			Oil palm fibre		
Surfactant/EDTA	1(%)	3(%)	5(%)	1(%)	3(%)	5(%)
Control	1.042±07	1.042±07	1.042±11	0.586±12	0.586±02	0.586±06
Triton X	1.533±01	2.201±16	2.399±15	1.455±06	1.915±09	1.892±07
Tween 80	0.834±12	0.668±03	0.755±04	0.759±04	0.653±10	0.840±06
Cetrimide	2.232±16	1.942±05	0.810±05	2.358±15	1.559±09	1.170±07
EDTA	0.870±05	0.642±03	0.342±01	0.963±02	0.508±02	0.350±07

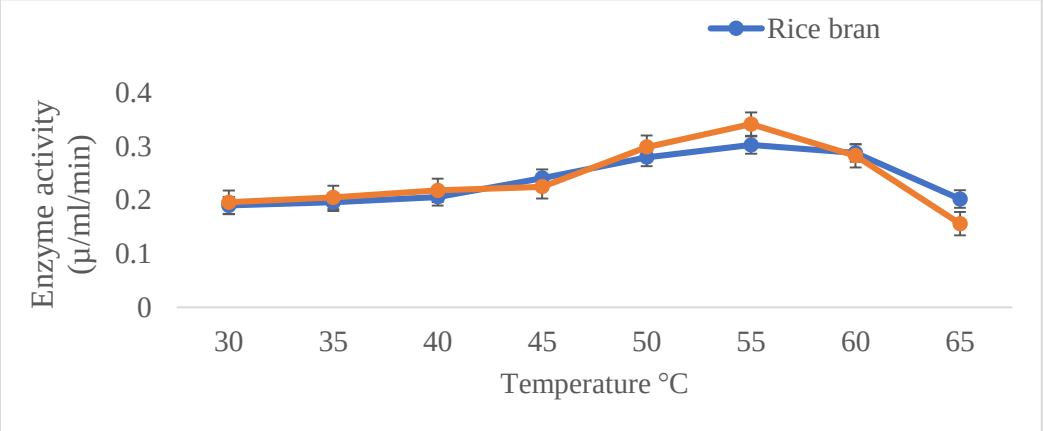

Fig. 7: Effect of temperature on partially purified *Penicillium chrysogenum* exo-β-glucanase

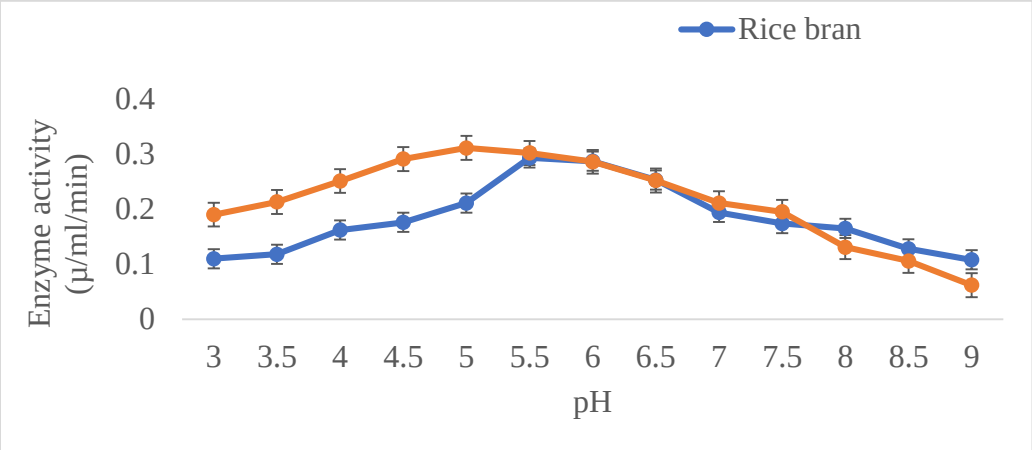

Figure 8: Effect of pH on partially purified *penicillium chrysogenum* exo-β-glucanase

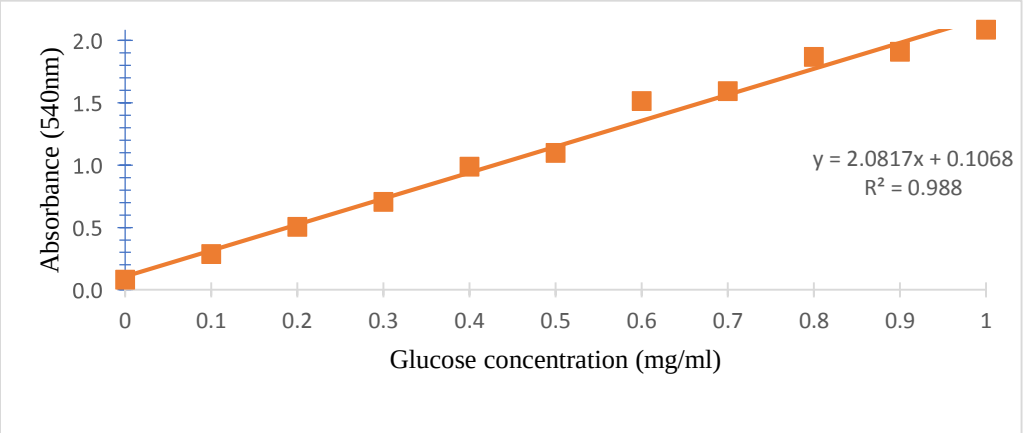

Figure 9: Effect of varying concentration of glucose on the initial reaction velocity.

DISCUSSION

The ability of *Penicillium chrysogenum* to break down glucan, as shown by the production of exo-β-glucanase, forms the basis for determining its glucanase activity. The variations in enzyme yield between the organic waste and commercial carbon sources used as substrate for exo-β-glucanase production could be due to variations in the ratio of amylose to amylopectin in different substrates. It has been reported that the ratio of amylose to amylopectin typically varies from one starch/polysaccharide source to another (Doughari and Onyebarachi, 2019). Organic waste products such as yam and banana peels have also been reported to induce higher exo-β-glucanase production among fungi, they contain polysaccharides derive from glucose monomers thus more carbon content (Varki *et al*., 2009). It has also been reported that rice bran and oil palm fibre contain large amount of glucan which act as inducer for the production of glucanase (Doughari, 2011). Carbon sources play a major role for biosynthesis in microorganisms through enzyme synthesis regulation by induction and repression (Sanchez and Demain, 2008**;** Singh and Mandal, 2012). The ability of *Penicillium chrysogenum* to grow on various carbon sources is an indication of their utilization by the fungus. This finding agrees with Fierro *et al*. (2022), Rondanelli *et al*. (2011) and Doughari (2011) who reported oil palm fibre and rice bran as good carbon sources which can be utilized by fungi for enzyme production. This result is beneficial for the utilization of agricultural waste and implies that lower cost for exo-β-glucanase production is possible. It has been reported that yeast extracts may have the potential to induce exo-β-glucanase. Result indicated maximum production of β-glucanase by *Penicillium chrysogenum* in yeast supplemented rice bran as substrate. This is similar with the report by Xing-Jun, (2004) who reported high production of β-glucanase by *Bacillus subtilis* grown on barley flour as substrate in the presence of yeast extract. Abundant vitamins, minerals and amino acids are contained in yeast extract which are necessary for cell growth and enzyme synthesis, thus inducing β-glucanase production (Zheng *et al*., 2011).

Using oil palm fibre as a substrate, maximum production of exo-β-glucanase by *Penicillium chrysogenum* was obtained in the presence of ammonium sulphate. This finding indicates that ammonium ion in the fermentation medium enhanced the growth of *Penicillium chrysogenum* as it serves as precursors for the biosynthesis of amino acids and various essential cellular proteins. Both organic and inorganic nitrogen sources were suitable for exo-β-glucanase production. Enzyme production was at its highest when there were enough nutrients, but decreased as nutrients were depleted. This was also seen in pectinase production in submerged fermentation (Yogesh *et al*., 2009). However, β-glucanase production was at its highest in solid-state fermentation after 7 days at pH 5.0 (Yang *et al*., 2014). The results showed that a moderate acidic pH was favorable for the growth of *Penicillium chrysogenum* and the production of exo-β-glucanase. However, an increase in pH caused a decrease in production, as enzymes are sensitive to changes in pH and higher temperatures can denature enzymes (Ju and Kabir, 2023).

Optimal temperature for β-glucanase production was found to be 50°C in solid-state fermentation using Rhizomucor miehei and 28°C in another study, while activity decreased at temperatures higher than 40°C. Klecius *et al*. (2006) reported an optimum temperature of 60 °C for β-glucanase production using *Bacillus halodurans* C-125. Microbial product formation is also greatly influenced by temperature.

This study's purification results showed that the exo-β-glucanase from *Penicillium chrysogenum* can be purified to homogeneity using ammonium sulphate precipitation and dialysis membrane tubing. The protein concentration of the crude enzyme in rice bran was found to be 0.88 mg/ml and decreased after ammonium sulphate precipitation, indicating that more protein was precipitated. Further decrease in activity was observed after dialysis using membrane tubing, showing that more of the protein of interest was precipitated and impurities were removed.

The specific activity of the crude enzyme was 2.39 U/mg, which increased after ammonium sulphate precipitation and further increased after dialysis. For oil palm fibre, protein concentration of crude enzyme assayed was found to be 0.99 mg/ml which decreased after ammonium sulphate precipitated indicating that protein was precipitated and further decrease was recorded after dialysis using membrane tubing. This showed that more protein of interest was precipitated and other protein impurities have been removed. Crude enzyme specific activities were 2.06 U/mg. This value increased after ammonium sulphate precipitation and further increased after dialysis. This result agrees with the report by Doughari and Onyebarachi (2019) that for a successful purification procedure, the specific activity of the desired enzyme must be higher after the process of purification than it was before. However, the increase in specific activity is a measure of purification achieved.

The effect of various pH on the partially purified exo-β-glucanase showed that exo-β-glucanase of *Penicillium chrysogenum* is an acidic enzyme as optimum activities were recorded at pH ranges of 5 to 5.5. This is also similar with the report of Doughari (2011) who stated that optimum β-glucanase activity was observed at pH of 5.5 and 6.0. Sena *et al* (2011) also reported maximum β-glucanase activity at pH 5.0 with *Moniliphthora perniciosa*. However, among the β-glucanase obtained from different microbial sources, it has been indicated that the optimum pH range is from 3.5 – 6.5 (Doughari, 2011). This finding agrees with the report of Yang *et al.* (2014) that optimum pH 4.5 was recorded for β-glucanase activity with *Rhizomucor miehei.* The deviation of the pH value from the ideal condition leads to a decrease on enzyme activities and eventually stops. There is a basic region in the active site, and acidic region. The acidic region is where the enzyme catalyzes the chemical reaction. The basic region helps to stabilize the enzyme (Mahapatra, 2005).

Optimum activity of purified exo-β-glucanase using rice bran and oil palm fibre as substrates were at the temperature range of 40 - 45 °C. Activity of the enzyme increased with increase in temperature (40 - 45 °C). But at higher temperature of above 50 °C, there was loss of activity. Further increase in this loss of activity at higher temperature is an indication that *Penicillium chrysogenum* exo-β-glucanase is thermostable enzymes. This agrees with reports by Yang *et al*. (2014) that optimum temperature of β-glucanase activity was at 55 °C with *Rhizomucor miehei*. However, Masatake *et al*. (2005) reported optimal temperature glucanase activity at 60 °C with *Bacillus halodurans*.

As the temperature rises, the activity of enzymes increases. But at excessively high temperatures, the rate drops again due to denaturation which results in loss of enzyme function. The best enzyme activity is seen at its optimal temperature. When the temperature goes beyond the optimum, there is a rapid decline in activity as the enzyme's active site undergoes structural changes, resulting in denaturation (Ju and Kabir, 2023). In this study, *Penicillium chrysogenum* exo-β-glucanase activity decreases with increase in the concentration of NaCl (1, 2 and 5 mM).

High amount of NaCl affect the structure of protein thus influences the activity. This was reported by Sena e*t al.* (2011) where glucanase activity was high at a low concentration of NaCl and decrease with increase in concentration of the salt. The activity of the enzyme increases with increase in concentration of $CoCl_2$. The findings are similar to those reported by Fukuda *et al.* (2008) who showed that the purified glucanase activity from the fruiting body of the edible mushroom (*Flammulina velutipes)* increased in the presence of $CoCl_2$. However, the enzyme activity decreased with an increase in the concentration of $CuCl_2$ and $CaCl_2$. According to Doughari (2011), the activity of β-glucanase from *Penicillium oxalicum* and *P. citrinum* was slightly diminished by $CuCl_2$.

The activity of β-glucanase was weakly suppressed by $MgCl_2$, NH_4Cl and KCl_2, as reported by Yasir *et al*. (2013). Effect of surfactant and EDTA on *Penicillium chrysogenum* purified exo-β-glucanase using rice bran and oil palm fibre as substrate showed that for the rice bran, activity decreased in the presence of 3 % Tween 80. An increase in EDTA concentration caused a decrease in enzyme activity, similar to Regmi *et al*. (2020). However, Yazdi *et al*. (2013) found a decrease in enzyme activity with 3% Tween 80. In the case of oil palm fiber, this study found that Triton X increased enzyme activity at all concentrations (1, 3, and 5%), with the highest activity observed at 3%. This is in line with Wang *et al*. (20 20) report that Triton X increases enzyme activity.

CONCLUSION

The study revealed that rice bran and oil palm fiber can be effectively utilized as substrates in the production of exo-β-glucanase using *P. chrysogenum* in a submerged fermentation system. The optimum temperature and pH for exo-β-glucanase production from rice bran and oil palm fiber are 30°C and 4.5, and 30°C and 5.5, respectively. The purified enzyme has potential industrial and biotechnological applications such as being an effective additive in laundry detergents and beer production. Rice bran and oil palm fiber are cheap and readily available, thus reducing the production cost of exo-β-glucanase and minimizing environmental pollution.

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