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Evaluating the Pectinase Production Potentials of *Aspergillus* species Cultured on Corn Cob and Watermelon Rind

Ogbebor A. S. [1] *, Oshoma C. E. [1]

[1]*Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, P.M.B.1154, Nigeria*
**Corresponding author, Email address: agbonomaire.igbinedion@uniben.edu*

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Abstract: This study aimed to evaluate the pectinase production potentials of *Aspergillus* species using corn cob and watermelon rind. *Aspergillus* species were isolated from soil samples and screened for pectinolytic activity. The screened *Aspergillus* isolates were identified and pectinase production was conducted using the mentioned agro-wastes through submerged fermentation. The presence of glycosyl hydrolase family 28 (GH28) gene was confirmed using polymerase chain reaction. Enzyme activity, changes in pH and protein concentrations were analyzed during fermentation using standard methods. The screened strains of *Aspergillus* with the highest pectinolytic activities on pectin agar were *A. niger* AN1, *A. niger* AN2, *A. niger* AN4 and thus expressed the GH28 gene responsible for polygalacturonase production. *A. niger* AN1 and *A. niger* AN4 exhibited the highest polygalacturonase activity, recording 0.63±0.13 U/mL on corn cob medium and 0.79±0.01 U/mL on watermelon rind medium, respectively. During fermentation, pH values ranged from 3.56±0.00 - 5.71±0.04, while protein concentration varied from 5.45±0.04 mg/mL (*A. niger* AN1) to 15.93±0.00 mg/mL (*A. niger* AN2). Watermelon rind was identified as suitable low-cost substrate for maximum production of pectinase by these strains. The use of cheap agro-waste can reduce the cost of enzyme production. Further research focused on optimization of fermentation parameters could lead to enhanced biotechnological applications and sustainable production processes.

1. Introduction

Agro-industrial wastes are byproducts generated from the processing, production, and harvesting of cereals, vegetables, trees, and fruits. They are produced in large quantities globally and are often repurposed as animal feed or burned in fields (Sarmah, 2009). There is an abundant quantity of lignocellulosic biomass generated from agricultural and forest residues which represents an inexpensive and underutilized renewable feedstock (Bajaj *et al.,* 2014; Ad *et al.,* 2016). These residues are rich in polymeric compounds such as lignin, cellulose and pectin, making them valuable raw materials for industrial applications, including the production of microbial pigments, organic acids, biofuels, aroma compounds, enzymes, protein-enriched feed, and bioactive secondary metabolites (Nigam and Pandey, 2009; Tabaght *et al*., 2023). Due to their high pectin content, these residues can be efficiently utilized for pectinase enzyme production (Bari *et al*., 2010)

The increasing energy demand has focused worldwide attention on the utilization of renewable sources particularly agricultural residues, the major components of which are cellulose, starch, lignin, xylan and pectin. Several microbes are capable of using these substances as carbon and energy sources by producing a vast array of enzymes in different environmental niches (Sethi *et al.*, 2016; Nandiyanto *et al.,* 2022).

Pectinases comprise a consortium of enzymes indispensable for the hydrolysis of pectin (Sethi *et al.*, 2016). Pectin is a class of complex polysaccharides found in the cell walls of higher plants and the cementing material for the cellulose network (Saranraj and Naidu, 2014). Pectinolytic enzymes are classified based on the cleavage of the galacturonan portion of the pectin molecule and they include pectin esterase, pectin lyase and polygalacturonase, which hydrolyzes α (1, 4)-glycosidic linkages between galacturonic acid residues. Polygalacturonases are the most abundant among the family of pectinolytic enzymes (Jayani *et al*., 2005). Depending on the pH optima, pectinases can be categorized into acidic and alkaline types (Ramachandran and Kurup, 2013; Sethi *et al*., 2016).

The pectinolytic enzymes have different roles in nature depending on the organism producing them. They can act as enzymes important for fruit ripening (Rombouts and Pilnik, 1980). Pectinases are of importance in plants for cell wall extension, softening of plant tissues during maturation and storage, decomposition and recycling of waste plant materials. Polygalacturonases are the first cell wall-degrading enzymes produced by fungal pathogens when cultured on isolated plant cell walls or during infections (Mukadam *et al*., 2010; Sethi *et al*., 2016; Saidi *et al.,* 2022).

Polygalacturonases are pectinolytic enzymes that catalyze the hydrolytic cleavage of the polygalacturonic acid chain with the introduction of water across the oxygen bridge. They are the most extensively studied among the family of pectinolytic enzymes. Polygalacturonases catalyze the hydrolysis of α (1,4)-glycosidic linkages in pectic acid (polygalacturonic acid). The polygalacturonases involved in the hydrolysis of pectic substances are endo-polygalacturonase (E.C. 3.2.1.15) and exo-polygalacturonase (E.C. 3.2.1.67). Polygalacturonases have the biological, functional and technical applications in food processing and plant-fungal interactions (Jayani *et al*., 2005; Pedrolli *et al*., 2009). More than 30 different genera of bacteria, yeasts and moulds have been used for the production of polygalacturonases (Banakar and Thippeswamy, 2012). Submerged fermentation has proven to be a useful system for enzyme production at large scale as it is easy to upscale and optimize using various bioprocessing approaches viz., fed batch or continuous mode of nutrition (Ali *et al*., 2025).

Polygalacturonases can act in an endo- and exo-mode. Endo-polygalacturonases are widely distributed among fungi, bacteria and many yeasts. They are also found in higher plants and some parasitic nematodes. They have been reported in many microorganisms including *Fusarium monoliforme, Neurospora crassa, Rhizopus stolonifer, Aspergillus* species and *Paecilomyces clavisporus* (Jayani *et al*., 2005). Endo-polygalacturonase, also known as poly-(1, 4- α-D-galacturonide) glycanohydrolase, catalyzes random hydrolysis of α (1,4)-glycosidic linkages in pectic acid (Tapre and Jain, 2014). In contrast, exo-polygalacturonases occur less frequently. They have been reported in *Erwinia carotovora, Agrobacterium tumefaciens, Alternaria mali, Fusarium oxysporum* and *Bacillus* species. Exo-polygalacturonases can be distinguished into two types: fungal exo-polygalacturonases which produce monogalacturonic acid as the main end product; and the bacterial exo-polygalacturonases, which produce digalacturonic acid as the main end product (Jayani *et al*., 2005). Exo-polygalacturonase, also known as poly (1, 4-α-D-galacturonide) galacturonohydrolase, catalyzes hydrolysis in a sequential fashion of α-1,4-glycosidic linkages on pectic acid (Tapre and Jain, 2014; Amin *et al.*, 2019).

One of the important enzymes in the biotechnological sector with a 25 % share in the global food and beverage enzyme market is pectinases (Jayani *et al*., 2005). These enzymes, explicitly polygalacturonases, have gained significant worldwide application in many industries for the clarification of juices and wines, plant tissue maceration, wastewater treatment, coffee and tea fermentations, oil extraction, plant fibre processing and the enhancement of chromaticity and stability of red wines (Hoondal *et al*., 2000; Revilla and Ganzalez-Sanjose, 2003; Jayani *et al*., 2005; Sethi *et al*., 2016).

Pectinolytic enzymes are widely distributed in higher plants and microorganisms including bacteria, yeasts and fungi. However, the best-acknowledged microbial producers of pectinases are various species of *Aspergillus* (Sethi *et al*., 2016). Agricultural and food processing wastes such as wheat bran, citrus peel, sugar beet pulp, lemon peel and banana peel are the most commonly used substrates for pectinase production (Saranraj and Naidu, 2014; Alavi *et al.,* 2020).

Glycoside hydrolases (GHs) are enzymes that cleave glycosidic bonds in glycosides, glucan, and glycoconjugates (Salam, 2018). GH-coding genes are abundant and present in the vast majority of genomes corresponding to almost half, presently about 47% of the enzymes classified in carbohydrate-active enzymes (CAZy) (Cantarel *et al*., 2009). Due to homologous similarities, the glycoside hydrolases (GH) responsible for pectin degradation belong to family 28 (Pickergill *et al*., 1998). Glycoside hydrolases family 28 (GH28) encompasses a cohort of structurally akin enzymes that catalyze the hydrolysis of homogalacturonan and rhamnogalacturonan constituents of pectin and are critical extracellular enzymes found across plant, fungal, and bacterial species (Abbott and Boratson, 2007). This protein family exhibits functional diversity and varies in gene count among organisms, they fulfill various biological functions, including fruit maturation, biomass recycling and phytopathogenesis. GH28 enzymes fall into the categories of polygalacturonases, rhamnogalacturonases and xylogalacturonases (Sprockett *et al.,* 2011).

The commercially available pectinase preparations used in food processing are traditionally associations of polygalacturonases, pectin lyases and pectin methyl esterases. These preparations are usually derived from fungi, mainly the genera *Aspergillus* (Lang and Dornenburg, 2000). The microbial pectinase accounts approximately for 25 % of the total worldwide enzyme sale. *Aspergillus niger* is the most commonly used fungal species for industrial production of pectinolytic enzymes (Jayani *et al*., 2005).

There is an increasing demand for pectinases but the application is limited due to the high cost of medium which contributes 30-40 % of production cost (Jahan *et al*., 2017). In Nigeria, approximately N3.6 billion is spent annually on enzyme importation. Despite the intangible demand, enzymes are relatively expensive reagents and this adds to the operational costs of processes that utilize them. Therefore, it is most important to explore alternative, cost-effective, and easily accessible substrates for the production of pectinases (Jahan *et al*., 2017). Shrestha and Rahman (2021) suggest economically suitable carbon and nitrogen sources for pectinase production may include inexpensive agro-industrial wastes.

Corn cob is an agricultural residue that is generated from maize (*Zea mays*) and remains part of the ear on which the kernels grow (Anukam *et al*., 2017). The corn cob is an agricultural waste generated in huge quantities during corn processing. The quantity of corn cobs generated worldwide is approximately 144 million tons per year, and most of it is discarded or burnt, causing serious environmental pollution (Berber-Villamar *et al*., 2018). Pointner *et al*. (2014) described corn cobs as lignocellulosic material composed of cellulose (38.80±2.50 %), hemicellulose (44.40±5.20 %) and lignin (11.90±2.30 %) in dry matter. Due to their chemical composition, corn residues show great

potential as a renewable raw material for producing a variety of added-value chemicals (Inglet, 1970; Ashour *et al*., 2013). Oyeleke *et al*. (2012) revealed that *Aspergillus niger* isolated from corn cobs can produce cellulase and pectinase, hence it may be considered as a source for the production of these enzymes.

Watermelon (*Citrullus lanatus*) rind, a major by-product from fruit processing industries, is mainly structured by a combination of celluloses, hemicelluloses, pectins and lignins (Kavuthodi *et al*., 2015). Watermelon rind can be considered as a valuable source of pectin because it contains a significant amount of this polysaccharide, although it is lower than that of citrus, the main commercial source of pectin (Belkheiri *et al*., 2021). Corn cob and watermelon rind are abundantly present in the environment and could be used as low-cost substrates for the production of polygalacturonases.

2. Methodology

2.1 Sample collection and processing

Fresh watermelon and corn were purchased from markets in Benin City. The samples were washed with 96 % ethanol, thereafter the watermelon fruits were peeled and the corn grains was removed from the cob. All samples were cut into small pieces of uniform sizes. The samples (corn cob and watermelon rind) were oven dried at 70 ^{o}C, then ground into powder (pore size 300 µm) using a sterile blender and sealed in sterile polyethylene bags till further use (Udenwobele *et al*., 2014; Ketipally *et al*., 2019).

2.2 Isolation and identification of fungi

Aspergillus species were isolated from soil samples around decomposing agricultural wastes using potato dextrose agar (PDA). Serial dilution of the samples was carried out by macerating the samples in sterile distilled water. One gram (1.00 g) of the sample were weighed in 9.00 mL of the diluent to provide a 10^{-1} homogenate from which subsequent dilutions of up to 10^{-4} were made. Culturing was carried out using the pour plate method on potato dextrose agar. The plates were incubated at room temperature (28± 2 ^{o}C) for 3 to 5 days (Ketipally *et al*., 2019). The isolates were identified based on cultural and morphological characteristics (Barnett and Hunter, 1972). Colonies were purified by sub-culturing onto freshly prepared media. Spores were grown and maintained on PDA slants.

2.3 Screening of isolates for pectinolytic activity

Inoculum was prepared by harvesting the spores from 12 - 14-day old culture of isolates with 1% Tween 80 solution. Concentration of the spores (1 x 10^{6} spores/mL) was calculated using a hemocytometer (Zaslona and Trusek-Holownia, 2015). Spore suspension (10^{6} spores/mL) of fungal isolates were inoculated on plates containing pectinase screening agar medium (PSAM) with the following composition (g/L): $(NH_4)_2SO_4$, 3.00; KH_2PO_4, 2.00; K_2HPO_4, 3.00; $MgSO_4$. $7H_2O$, 0.10; pectin, 10.00 and agar, 25.00. Plates were incubated at 28±2 ^{o}C for 3 days. After incubation, the plates were flooded with potassium iodide-iodine solution for 5-10 min. Pectinase-producing colonies were detected by the appearance of clearance zones indicating pectinolytic activity. The diameters of the zones of clearance were measured. Three *Aspergillus* species with the highest clear zone diameter on pectin agar plates were selected (Oumer and Abate, 2018; Sudeep *et al.,* 2020).

2.4 Molecular identification of Aspergillus spp.

The fungal isolates (maximum pectinase producer) were grown in potato dextrose broth for 48 hr. The genomic DNA was isolated according to the Sambrook *et al.* (1989) method. A 2-day old mycelium was allowed to drain for about 3 min after being placed on a filter paper. The mycelium was

macerated with pestle and mortar with 500 µl of the extraction buffer containing: 200 mM Tris-HCl (pH 7.50), 25 mM EDTA, 250 mM NaCl and 0.50 % SDS. The squashed sample was transferred to a microfuge tube and again 1 mL of extraction buffer was added to the tube, vortexed and incubated at 55 ºC for 1 hr. The clear lysate was transferred to another fresh tube, to which 100 µl KCl solution was added and incubated on ice for 5 min. The content was centrifuged at 10,000 g for 5 min and the supernatant was transferred to another microfuge tube and 750 µl of phenol: chloroform: isoamylalcohol (25:24:1) mixture was added. The tubes were centrifuged for 10 min at 13,000 g. The upper aqueous phase was mixed with an equal volume of absolute ice-cold isopropanol and incubated for 10 min at room temperature. The tubes were centrifuged at 10,000 g for 10 min. The pellet was washed with 70% (v/v) ethanol. The pellet was air-dried and dissolved in 100 µl TE buffer. The DNA samples were stored at -20 °C for further use.

2.5 Polymerase chain reaction amplification, Purification of PCR products and Sequence identification of 18S rRNA genes

PCR amplification of the 18S rRNA gene of the fungi isolated was carried out using the ITS region primers; ITS1 (5' –TCCGTAGGTGAACCTGCGG- 3') and ITS4 (5' –TCCTCCGCTTATTGATATGC- 3'). The PCR reaction mix contained 100 ng of genomic DNA, 1x*Taq* buffer, 0.20 mM dNTPs, 0.30 µM of each primer, and 1U *Taq* DNA polymerase in a final volume of the reaction mixture was 20 µl. The PCR conditions included initial denaturation at 94 °C for 3 min, denaturation at 94 °C for 45 sec, annealing at 50 °C for 1 min (35 cycles) and extension at 72 °C for 80 sec, final extension at 72 °C for 7 min for amplification of ITS region (Kamalambigeswari *et al*., 2018). The amplified products were analyzed with agarose gel electrophoresis. The nucleotide sequences were identified in the Genbank of the National Centre for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST) (Avwioroko and Tonukari, 2014).

2.6 Detection of the presence of polygalacturonase gene (GH28F)

The detection of the presence of glycosyl hydrolase family 28 (GH28F) gene (endopolygalacturonase) was carried out using specific primers. The primers include forward primer: GH28F-1786F (5'-TRBTGGGAYGGHNWRGG-3') and reverse primer: GH28F-2089R (5'- AAYCARGAYGAYTGYVTBGC -3'). The PCR condition was maintained at initial denaturation (95 ºC for 3 min), second denaturation at 94 ºC for 30 sec, annealing for 30 sec and extension at 72 ºC for 7 min. The PCR product was electrophoresed using 1.50 % agarose gel stained with ethidium bromide (Gacura *et al*., 2016).

2.7 Polygalacturonase production in submerged fermentation

The screened *Aspergillus* species were studied for polygalacturonase production using powdered agro-wastes; watermelon rind and corn cob as sole carbon sources. Submerged fermentation was carried out at temperature of 28±2 ºC for 10 days in an orbital shaker at a speed of 120 rpm. A 0.50 g of each substrate were weighed into 250 mL Erlenmeyer flasks containing 50 mL of mineral solution with the following composition (g/L): $(NH_4)_2SO_4$, 1.40; KH_2PO_4, 2.00; K_2HPO_4, 6.00; $MgSO_4.7H_2O$ 0.10. The flasks were sterilized at 121 ºC for 15 min and cooled to room temperature. A spore suspension of 2 mL (1 x 10^6 spores/mL) of each isolate was inoculated. The production experiments were carried out in triplicates. Enzyme activity, change in pH of the medium, protein concentration, fungal biomass and reducing sugars were determined at every 2 days intervals (Mrudula and Anitharaj, 2011; Chaitanya and Jaya-raju, 2014).

2.8 Determination of enzyme activity

At the end of the fermentation period, enzyme extraction was carried out by centrifuging the culture broth at 4000 g for 20 min at 4 °C. The supernatants were used as the crude enzyme extract (Mrudula and Anitharaj, 2011). Polygalacturonase activity was determined by measuring the amount of reducing sugar (D-galacturonic acid) liberated from citrus pectin using the dinitrosalicylic acid (DNS) reagent assay. The assay mixture consisted of 0.80 mL of substrate solution (1.0% w/v citric pectin in 0.1M citrate buffer pH 5.00) and 0.20 mL enzyme solution. The reaction mixture was incubated at 50 °C for 20 min in a water bath and terminated by adding 1.50 mL of 3, 5-dinitrosalicylic acid reagent. This was followed by boiling the mixture in a water bath for 10 min for colour development. The reaction was left to cool and absorbance was read at 540 nm. The standard curve was prepared for the reducing group with galacturonic acid. One unit of polygalacturonase activity is defined as the amount of enzyme that releases one micromole (1 µmol) of galacturonic acid per minute under assay conditions (Miller, 1959).

2.9 Determination of pH of medium

The pH measurement of the fermentation medium was carried out using a pH meter (Pancellent Water Quality Test Meter, China) at room temperature (Zaslona and Trusek-Holownia, 2015).

2.10 Determination of protein concentration

Protein concentration was measured according to the Bradford (1976) method. Coomassie Brilliant Blue G-250 and bovine serum albumin were obtained from Molychem and used as supplied. Coomassie Brilliant Blue G-250 (100 mg) was dissolved in 50 mL 95 % ethanol. To this solution, 100 mL 85 % (w/v) phosphoric acid was added. The resulting solution was diluted to a final volume of 1 L. Final concentrations in the reagent were 0.01 % (w/v) Coomassie Brilliant Blue G-250, 4.70 % (w/v) ethanol and 8.50 % (w/v) phosphoric acid. Protein solution containing 10 - 100 ug protein in a volume up to 0.10 mL was pipetted into test tubes. The volume in the test tube was adjusted to 0.10 mL with appropriate buffer. Five millilitres (5 mL) of protein reagent were added to the test tube and the contents mixed by inversion. The absorbance at 595 nm was measured after 2 min and before 1 hr in 3 mL cuvettes against a reagent blank prepared from 0.10 mL of the appropriate buffer and 5.00 mL of protein reagent. The concentration of protein was plotted against the corresponding absorbance resulting in a standard curve used to determine the protein in unknown samples.

2.11 Statistical analysis

The statistical package (SPSS, Version 16.0) was used for the analysis of data obtained (Ogbeibu, 2014). Descriptive statistics were used to analyze experimental data and analysis of variance (ANOVA) was used to establish significant difference between variables. The p-values less than 0.05 were considered statistically significant

3. Results and Discussion

3.1 Screening of Aspergillus species for pectinolytic activity

The 15 species of *Aspergillus* including *A. niger* and *A. flavus* isolated from soil samples were screened for pectinolytic activity on pectin agar as shown in **Figure 1**. The identified isolates were *A. niger* AN1, *A. niger* AN2, *A. niger* AN3, *A. niger* AN4, *A. niger* AN5, *A. niger* AN6, *A. niger* AN7, *A. niger* AN8, *A. niger* AN10, *A. niger* AN11, *A. niger* AN12, *A. niger* AN13, *A. flavus* AF1, and *A.*

flavus AF2 and *A. parasiticus* AP1. The fungal isolates with the diameter of zones of hydrolysis of 15 mm and below were screened out. The isolates with high zones of hydrolysis were *A. niger* AN1, *A. niger* AN2 and *A. niger* AN4. It was observed that there was significant difference in pectinolytic activity among the fungal species ($p < 0.05$). Strains of *Aspergillus niger* and *Aspergillus flavus* were isolated from soil samples. Fungi and bacteria are the major source of organisms to decompose the dead leaves and other organic matter in the soil (Kamalambigeswari *et al*., 2018). *Aspergillus* species isolated from the soil samples were screened for pectinolytic activity on pectin agar. The pectinase producers were identified by flooding the plates with potassium iodide-iodine solution. It was observed that the diameter of zones of hydrolysis ranged from 1.33±1.15 (*A. flavus* AF1) – 18.67±1.15 mm (*A. niger* AN2). Patil and Dayanand (2006) had mentioned about hydrolysis around the colony indicating the zone of clearance. This result is similar to that of Anisa *et al.* (2012), where all tested species of *A. niger, A. flavus* and *A. ochraceous* showed clear zones on treatment with iodine solution indicating pectinolytic activity. Satapathy *et al*. (2019) reported that among fifteen fungal isolates studied, *Aspergillus parvisclerotigenus* was potent for pectinase production next to *Aspergillus niger* in form of halozone of 0.60 mm. Shet *et al.* (2022) reported the isolation of *Aspergillus* species from soil containing decomposed vegetables to produce pectinase enzyme. Pectinase seems to be one of the important enzymes produced by a wide variety of microorganisms contained in the soil (Satapathy *et al*., 2019). According to Anisa *et al.* (2012), members of the fungal genus, *Aspergillus,* are commonly used for the production of polysaccharide degrading enzymes. This genus produces a wide spectrum of cell wall degrading enzymes, permitting complete degradation of the polysaccharide and also tailored modification by using specific enzymes purified from these fungi (Gysler *et al*., 1990).

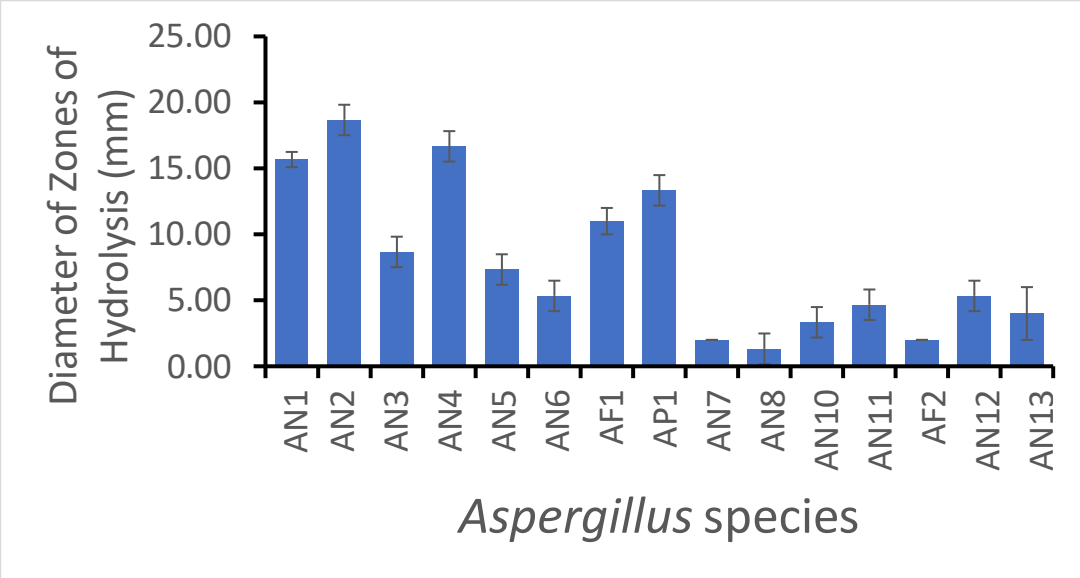

Figure 1. Pectinolytic activity of *Aspergillus* species on pectin agar (AN = *A. niger;* AF = *A. flavus;* AP= *A. parasiticus*)

3.2 Identification of selected strains

The selected strains, *Aspergillus niger* AN1, *Aspergillus niger* AN2 and *Aspergillus niger* AN4 were further identified using genotypic characteristics as presented in **Figure 2** through the amplification of the ITS region in *Aspergillus* species. The 18S RNA sequences of these strains were submitted to GenBank and assigned the accession numbers OQ644795, OQ644796 and OQ644798 as presented shown in **Table 1**. The BLAST search yielded a perfect match (100% similarity) with the *A. niger* sequence, confirming the strains' identities as *A. niger.*

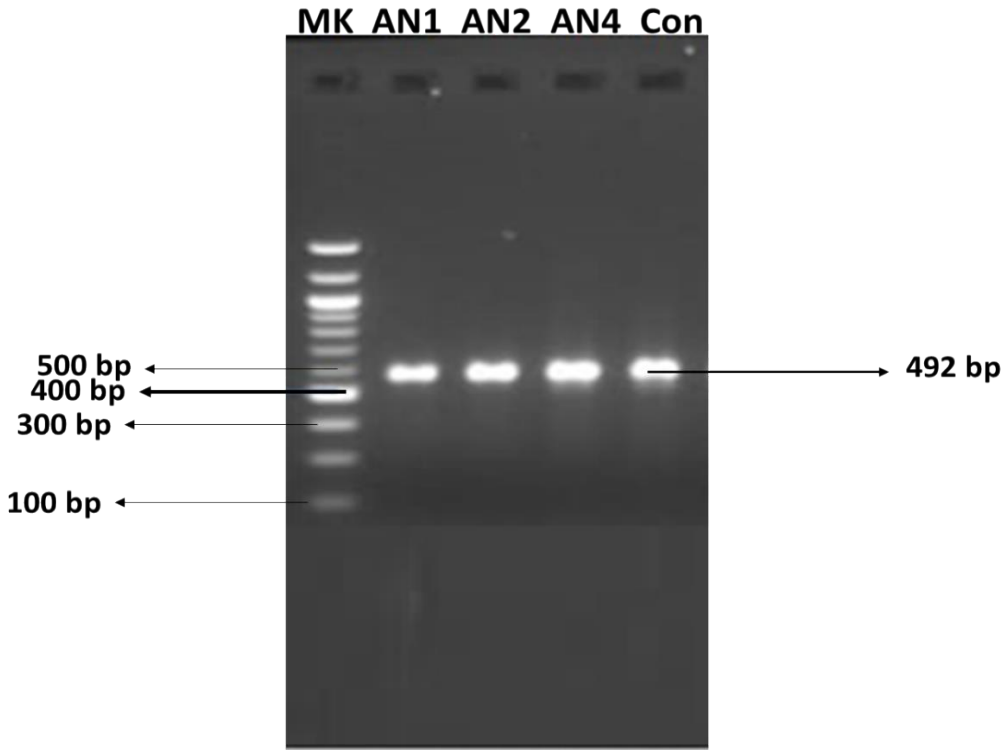

Figure 2. Agarose gel electrophoresis for PCR products using ITS1 and ITS4 primer pair for the amplification of the ITS region in *Aspergillus* species

Table 1. Molecular identification of the isolates

Sample ID	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Identity (%)	Accession No.
AN1	*Aspergillus niger* AN1	909	909	99%	0	100.00	OQ644795
AN2	*Aspergillus niger* AN2	909	909	99%	0	100.00	OQ644796
AN4	*Aspergillus niger* AN4	909	909	99%	0	100.00	OQ644798

3.3 Presence of polygalacturonase gene (GH28F)

The gene responsible for the production of polygalacturonase was detected in the *A. niger* strains as shown in the agar gel electrophoresis in **Figure 3**. All the isolates were observed to possess the gene *GH28F* with a molecular weight of 275bp. Fungi have developed a wide assortment of enzymes to break down pectin, a prevalent polymer in plant cell walls that is important in plant defense and structure. One enzyme family used to degrade pectin is the glycosyl hydrolase family 28 (GH28) (Gacura *et al*., 2016). The presence of glycosyl hydrolase family 28 gene responsible for the production of polygalacturonase was detected in the species. All polygalacturonases are classified into the family 28 of glycoside hydrolases (Polizeli *et al*., 2013; Samanta, 2019). The gene family, glycosyl hydrolase 28 (GH28) codes for a diverse array of hydrolytic enzymes involved in pectin depolymerization, including polygalacturonases, rhamnogalacturonases and xylogalacturonases (Gacura *et al*., 2016). GH28 genes are particularly prevalent in necrotrophic plant pathogen genomes (Sprockett *et al*., 2011) and are important virulence factors in necrotrophic plant pathogens (Zhao *et al*., 2013).

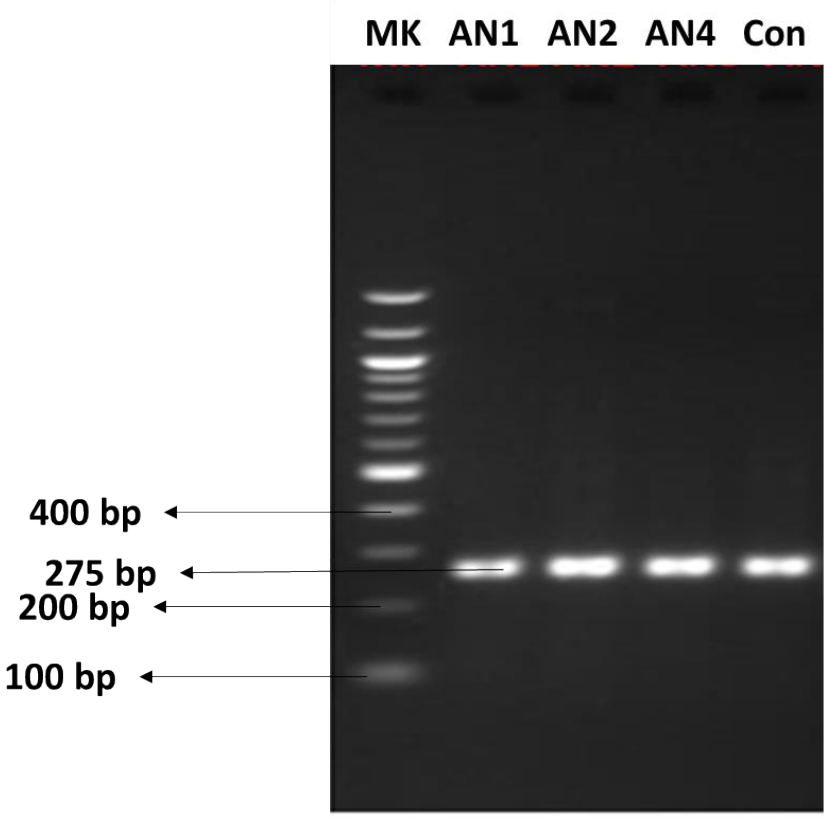

Figure 3. Agarose gel electrophoresis of PCR products of glycosyl hydrolase (GHF28) gene amplified from *Aspergillus* species

3.4 Production of polygalacturonase by Aspergillus species *cultured on the agro-wastes*

The production of polygalacturonase was observed in both agro-waste media. *A. niger* AN1 recorded highest polygalacturonase production on media supplemented with corn cob with an activity of 0.63±0.13 U/mL on day 6. However, polygalacturonase production was lowest in watermelon rind media with the highest activity of 0.50±0.01 U/mL recorded on day 8 (**Figure 4B**). It was observed that there was significant difference ($p < 0.05$) in the enzyme activity based on agro-waste type. Production of polygalacturonase as observed by *A. niger* AN2 in both media revealed lowest production in watermelon rind media with a peak of 0.21±0.00 U/mL on day 2 (**Figure 4B**), whereas, highest production was on corn cob on day 4 with an activity of 0.50±0.00 U/mL (**Figure 4A**). It was observed that there was significant difference ($p < 0.05$) in the enzyme activity based on agro-waste type.

A. niger AN4 recorded highest polygalacturonase production on watermelon rind media on day 4 with an activity of 0.79±0.01, however the lowest production (0.54±0.01 U/mL) was recorded in corn cob media on day 6 (**Figure 4A and B**). It was observed that there was significant difference ($p < 0.05$) in the enzyme activity based on agro-waste type. The selected strains; *Aspergillus niger* AN1, *Aspergillus niger* AN2 and *Aspergillus niger* AN4 from initial screening were subjected to polygalacturonase production under submerged fermentation for a period of 10 days. They were selected for the production of polygalacturonase on corn cob and watermelon rind media. Production of polygalacturonase was observed in both agro-waste media. *Aspergillus niger* is the most commonly used fungal species for industrial production of pectinolytic enzymes (Gummadi and Panda 2003; Jayani *et al*., 2005). Agricultural wastes contain an abundance of carbon and nitrogen in the form of polysaccharides and proteins that can be broken down into their assimilable forms via cell-wall-

degrading enzymes, including pectinases, proteases and cellulases (Wayne *et al*., 2009). Agro-wastes have been used increasingly for enzyme production because they contain large amounts of cellulose, xylan, hemicelluloses and pectin, which could serve as inducers for the production of cellulase, xylanase and pectinase respectively (Patil and Dayanand, 2006; Sethi *et al*., 2016).

Maximum polygalacturonase production by the three strains ranged from 0.50 - 0.79 U/mL. Among the strains, it was observed that *A. niger* AN4 had the highest polygalacturonase activity of 0.79 U/mL on corn cob media. This was followed by *A. niger* AN1 and *A. niger* AN2 with activities of 0.63 U/mL and 0.50 U/mL on watermelon rind and corn cob media respectively. Amande and Adebayo-Tayo (2012) reported the polygalacturonase yield by *Aspergillus* spp. ranged from 0.0212 - 5.8850 U/mL with *Aspergillus parasiticus and Aspergillus flavus* producing the highest quantity of the enzyme in medium with banana peels and plantain peels respectively. A maximum pectinase activity of 3.52 U/mL was achieved by *A. fumigatus* on orange peels (Okonji *et al*., 2019). Sethi *et al*. (2016) observed that *A. terreus* NCFT 4269.10 was superior in pectinase production and banana peel was found suitable for noticeable production of pectinase (550±70.71 U/mL) which was attributed to the high pectin content of banana peels. Shet *et al*. (2022) reported two fungi (*Aspergillus cervinus* ARS2 and ARS8) isolated from decaying fruit peels and soil containing decomposing vegetables to have activities of 41.88±1.57 IU/mL and 39.27±1.14 IU/mL. Ahmed and Rahman (2021) observed the highest pectinase activity (0.48 U/mL) for orange peels as the carbon source with *Pencillium chrysogenum* MF318506. Maximum production of polygalacturonase was observed on day 4 of fermentation in watermelon rind and corn cob media for strains *A. niger* AN2 and *A. niger* AN4 with activities of 0.50 U/mL and 0.79 U/mL respectively. Mrudula and Anitharaj (2011) and Sethi *et al*. (2016) obtained similar observation as maximum pectinase production was achieved on day 4. However, maximum polygalacturonase production by *A. niger* AN1 on day 6 was observed on corn cob media with activity of 0.63 U/mL. Amande and Adebayo-Tayo (2012) also observed that the maximum yield of polygalacturonase was obtained after day 6 of cultivation on medium with in mango peels, plantain and banana peels. Amande *et al*. (2022) reported that almost all fermenting organisms produced a greater enzyme yield on the day 3 and 6. Maximum activity of pectinase by *A. fumigatus* was recorded by Okonji *et al*. (2019) after 144 hr (day 6) of incubation. In contrast, Onofre *et al.* (2017) reported that the highest yield of endopolygalacturonase was achieved after 30 hr of incubation by *Penicillium brevicompactum* in solid state fermentation**.** Amande and Adebayo-Tayo (2012) also reported that maximum level of pectinase was achieved at 72 hr of submerged cultivation on medium with pure citrus pectin. Martin *et al.* (2004), Patil and Dayanand (2006) and Kamalambigeswari *et al*. (2018) had obtained similar observations. They stated that maximum pectinase production was achieved at 72 hr irrespective of the type of agro-waste employed. The cause of decrease in enzyme production after certain time interval during incubation might be due to the exhaustion of essential supplements and/or accumulation of toxic metabolites in the culture medium, high fungal cell density and decreased oxygen concentration (Phutela *et al.,* 2005; Bakri *et al*., 2022).

Several studies have reported that pectinolytic fungi produce pectinase maximally between 3 and 6 days of incubation (Okonji *et al*., 2019). Okafor *et al*. (2010) reports that an incubation time of 72 hr is ideal from an economic viewpoint which is based on the enzyme production rate and the low cost of the substrates. Sudeep *et al.* (2020) suggested that a shorter fermentation period of 48 hr could be advantageous for production of pectinase at industrial scale. Fermentation period is dependent upon the composition of the medium, its concentration and the organism grown (Patil and Dayanand, 2006). Amande and Adebayo-Tayo (2012) agree that this variation could be due to the composition of the substrate and length of lag phase of the fungi strains employed.

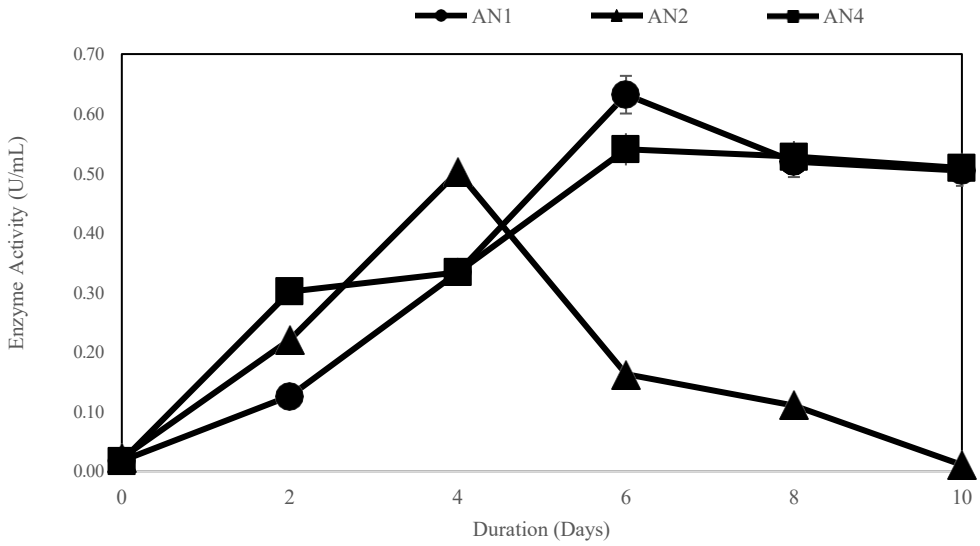

Figure 3.A Activity of polygalacturonase of *A. niger* strains using corn cob as substrate in the course of fermentation.

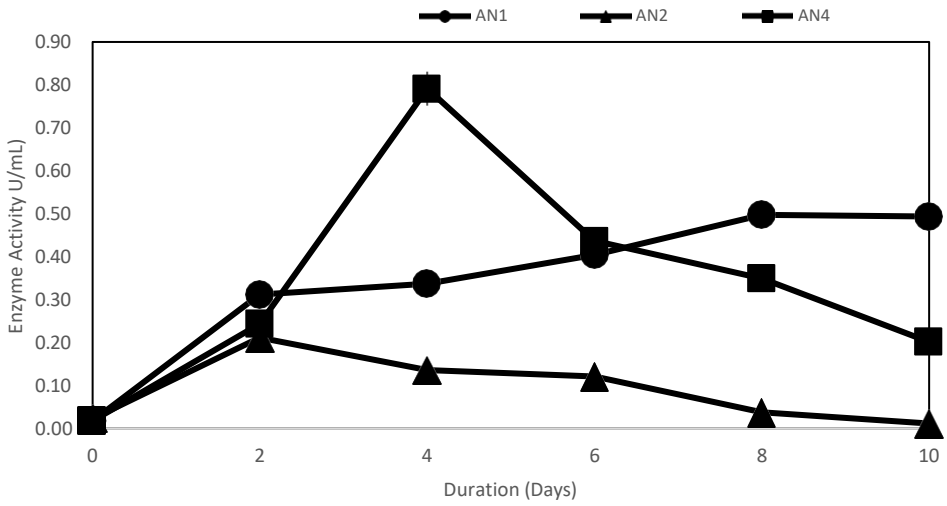

Figure 3.B Activity of polygalacturonase of *A. niger* strains using watermelon rind as substrate in the course of fermentation.

3.5 The pH of changes of the medium

The pH values during fermentation ranged from 3.56±0.00- 5.71±0.04. The initial pH of all media was set at 5.50. It was observed that in all media, there was an initial drop in pH. However, pH gradually increased between days 4 and 6 of incubation as shown in **Figure 5**. It was observed that the correlation between enzyme activity and pH in all media was a weak negative type. The pH of medium regulates the growth of the culture and exerts impact upon the catalytic activity of the enzyme (Sethi *et al.,* 2016). The pH of the both media was set at 5.50, however there was an initial drop which could be due to glucuronic acid being liberated in the medium because of the action of pectinolytic enzyme and also the production of other acids due to the fermentation of the carbon source. There was a gradual increase in pH values between day 4 and 6 for all fermenting media. This could be as a result of the utilization of organic acids or the production of alkali components (Onofre *et al*., 2017). The alkalization or acidification of a culture medium reflects the substrate consumption (Zeni *et al*., 2011).

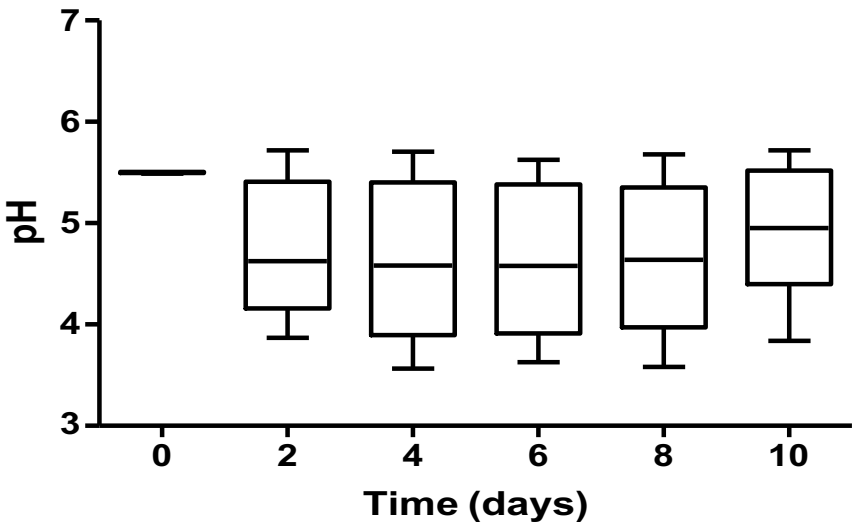

Figure 5. pH changes during production of polygalacturonase by strains of *A. niger* using corn cob and watermelon rind

3.6 Protein production

In corn cob media, protein concentration ranged from 3.83±0.04 mg/mL (*A. niger* AN1) – 5.45±0.02 mg/mL (*A. niger* AN4) (**Figure 6A**), however on watermelon rind media, protein concentration ranged from 10.81±0.06 mg/mL (*A. niger* AN4) – 15.93±0.00 mg/mL (*A. niger* AN2) (**Figure 6B**). It was observed that there were no significant differences in the protein concentrations of the media fermented by the three strains ($p > 0.05$). It was also observed that there was positive correlation between enzyme activity and protein production. It was observed that protein concentration increased during the course of fermentation for all media with a maximum of 5.45 mg/mL and 15.93 mg/mL on corn cob and watermelon rind for *A. niger* AN4 and *A. niger* AN2 respectively. Amande and Adebayo-Tayo (2012) reported that protein concentration ranged from 1.2258 – 13.8715 mg/mL in which *Aspergillus tamarii* produced the highest protein concentration on mango peel media. However, it ranged from 1.8926 – 5.2474 mg/mL on watermelon peel media by *Aspergillus terreus*. The protein production observed in this study could be an array of the proteinous metabolites generated during the growth and metabolism of fungal strains.

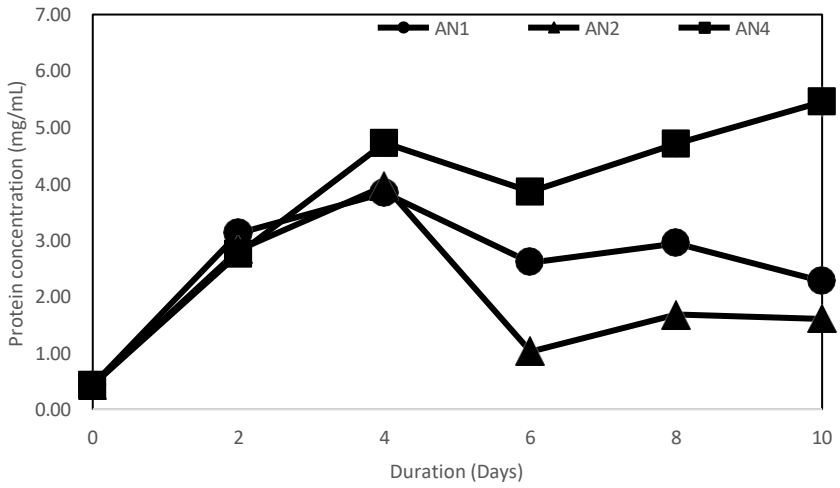

Figure 6A. Protein production by *A. niger* strains on corn cob (A) as substrate

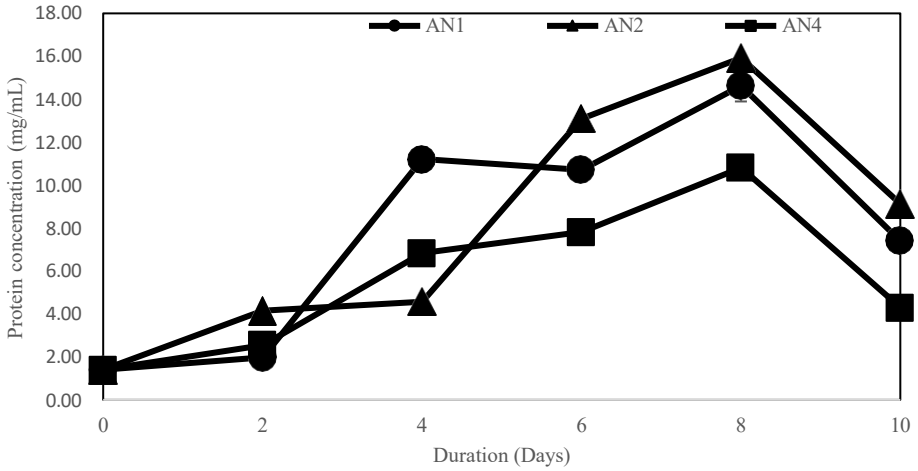

Figure 6B. Protein production by *A. niger* strains on watermelon rind (A) as substrate

Conclusion

This study reports the production of polygalacturonase using *A. niger* strains isolated from soils in Benin City. Corn cob and watermelon rind were identified as suitable low-cost substrate for maximum production of the enzyme by these strains. The use of cheap agro-waste can reduce the cost of enzyme production. Their use can also help reduce environmental pollution. However, the observed yield suggests further optimization of fermentation parameters including pH, temperature and substrate concentration which could significantly enhance production and enzyme recovery methods. Scale up of this process is crucial as further research could lead to enhanced biotechnological applications and optimized sustainable enzyme production processes.

Disclosure statement: *Conflict of Interest:* The authors declare that there are no conflicts of interest.
Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

References

Abbott, D.W., Boraston, A.B. (2007) The structural basis for exopolygalacturonase activity in a family 28 glycoside hydrolase, *J. Mol. Biol.,* 368(5), 1215-1222. https://doi.org/10.1016/j.jmb.2007.02.083

Ad C., Benalia M., Djedid M., Elmsellem H., Ben Saffedine F., Messaoudi A., Kadmi Y., Ouzidan Y., Hammouti B. (2016), A new lignocellulosic material based on Luffa cylindrica for Nickel(II) adsorption in aqueous solution, *Mor. J. Chem.* 4(4), 1096-1105

Ahmed, N.N.E., Rahman, H.M.A. (2021) Optimizing the production of pectinase of orange peel waste by *Penicillium chrysogenum* MF318506 using response surface methodology in submerged fermentation, *J. Microbiol. Biotechnol. Food Sci.,* 11(1), e3931 https://doi.org/10.15414/jmbfs.3931

Alavi, A., Tahghighi Nia, F., Pajoom Shariati, F. (2020). Polygalacturonase production by *Aspergillus niger* solid state fermentation on barley bran and sugar beet pulp mixture, *Adv. J. Chem., Sect. A,* 3(3), 350-357. https://doi.org/10.33945/SAMI/AJCA.2020.3.13

Amande, T., Adebayo-Tayo, B. (2012) Screening of new isolates fungal strains for polygalacturonase production in submerged fermentation, *Innov. Rom. Food Biotechnol.,* 11, 15-22.

Anisa, S.K., Ashwini, S., Girish, K. (2013) Isolation and screening of *Aspergillus* spp. for pectinolytic activity, *Electron. J. Biol.,* 9(2), 37-41.

Anukam A.I., Goso B.P., Okoh O.O., Mamphweli S.N. (2017) Studies on characterizing of corn for application in a gasification process for energy production, *J. Chem.,* Article ID 6478389, 9pp. https://doi.org/10.1155/2017/6478389

Ashour A., Amer M., Marzouk A., Shimizu K., Kondo R., El-Sharkawy S. (2013) Corncobs as a potential source of functional chemicals, *Molecules,* 18, 13823-13830 https://doi.org/10.3390/molecules181113823

Avwioroko, O.J., Tonukari, N.J. (2014) Isolation and molecular identification of *Aspergillus* species associated with the spoilage of cassava in Nigeria, *Niger. J. Sci. Environ.,* 13(1), 55-68.

Bajaj, B.K., Sharma, M., Rao, R.S. (2014) Agricultural residues foor production of cellulase from *Sporotrichum thermophile* LAR5 and its application for saccharification of rice straw, *J. Mater. Environ. Sci.* 5(5), 1454-1460.

Banakar, S.P., Thippeswamy, B. (2012) Isolation, production and partial purification of fungal extracellular pectinolytic enzymes from the forest soils of Bhadra Wildlife Sanctuary, Western Ghats of Southern India, *J. Biochem. Technol.* 3(5), 138-143.

Barnett, H. L., Hunter, B. B. (1972) *Illustrated Genera of Imperfect Fungi.* (3rd edition) Burgess Publishing Co., Minneapolis. 241pp.

Bari, M. R., Alizadeh, M., Farbeh, F. (2010) Optimizing endopectinase production from date pomace by *Aspergillus niger* PC5 using response surface methodology, *Food Bioprod. Process.,* 88(1), 67-72. https://doi.org/10.1016/j.fbp.2009.08.009

BCC Research. (2018) *Global Markets for Enzymes in Industrial Applications.* BCC Research, Wellesley, MA, USA. 184pp.

Belkheiri, A., Forouhar, A., Ursu, A.V., Dubessay, P., Pierre, G., Delattre, C., Djelveh, G., Abdelkafi, S., Hamdami, N., Michaud, P. (2021) Extraction, characterization, and applications of pectins from plant by-products, *Appl. Sci.,* 11(14), 6596. https://doi.org/10.3390/app11146596

Berber-Villamar, N.K., Netzahuatl-Muñoz, A.R., Morales-Barrera, L., ChaÂvez-Camarillo, G.M., Flores-Ortiz, C.M., Cristiani-Urbina, E. (2018) Corncob as an effective, eco-friendly, and economic biosorbent for removing the azo dye Direct Yellow 27 from aqueous solutions, *PLoS ONE,* 13(4), e0196428. https://doi.org/10.1371/journal.pone.0196428

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Anal. Biochem.,* 77, 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Cantarel, B.L., Coutinho, P.M., Rancurel, C., Bernard, T., Lombard, V., Henrissat, B. (2009) The Carbohydrate-Active EnZymes database (CAZy): an expert resource for glycogenomics, *Nucleic Acids Res.,* 37, 233-238. https://doi.org/10.1093/nar/gkn663

Chaitanya M., Jaya-raju K. (2014) Production of pectinase enzyme under solid state fermentation using banana pseudostem by *Aspergillus niger* NCIM 616, *Sciences J. Chem. Biol. Phys. Sci.,* 4(4), 3900-3908.

Gacura, M.D., Sprockett, D.D., Heidenreich, B., Blackwood, C. (2016) Comparison of pectin-degrading fungal communities in temperate forests using glycosyl hydrolase family 28 pectinase primers targeting Ascomycete fungi, *J. Microbiol. Methods,* 123, 108-113. https://doi.org/10.1016/j.mimet.2016.02.010

Gummadi, S. N., Panda, T. (2003) Purification and biochemical properties of microbial pectinases. A review, *Process Biochem.,* 38, 987-996 https://doi.org/10.1016/S0032-9592(02)00203-0

Gysler, C., Harmsen, J.A., Kester, H.C., Visser, J., Heim, J. (1990) Isolation and structure of the pectin lyase D-encoding gene from *Aspergillus niger*, *Gene,* 89, 101-108. https://doi.org/10.1016/0378-1119(90)90222-N

Hoondal G.S., Tiwari R.P., Tiwari R., Dahiya N., Beg Q.K. (2000) Microbial alkaline pectinases and their applications: a review, *Microbiol. Biotechnol.,* 59(4-5), 409-418 https://doi.org/10.1007/s002530051648

Inglett, G. (1970) *Corn: Culture, Processing and Products*. AVI Publishing Co., Westport, CT, USA.369pp.

Jahan, N. Shalid, F., Aman, A., Mujahid, T.Y., Qader, S.A.U. (2017) Utilization of agrowaste pectin for the production of industrially important polygalacturonase, *Heliyon,* 3(2017) Article No-eD00330, 13pp. https://doi.org/10.1016/j.heliyon 2017.e00330

Jayani, R. S., Saxena, S., Gupta, R. (2005) Microbial pectinolytic enzymes: A review, *Process Biochem.,* 40, 2931-2944. https://doi.org/10.1016/j.procbio.2005.01.026

Kamalambigeswari R., Yadav, S.A., Sivaswamy N., Ushani U. (2018). Isolation, identification, screening and optimization of pectinases producing soil fungi (*Aspergillus niger*), *Int. J. Res. Pharm. Sci.,* 9(3), 762-768

Ketipally, R., Kumar, G.K., Ram, M.R., (2019) Polygalacturonases production by *Aspergillus nomius* MR103 in solid state fermentation using agro-industrial wastes, *J. Appl. Nat. Sci.,* 11(2), 305-310. https://doi.org/10.31018/jans.v11i2.2053

Lang, C., Dörnenburg, H., (2000) Perspectives in the biological function and the technological application of polygalacturonases, *Appl. Microbiol. Biotechnol.,* 53, 366-75. https://doi.org/10.1007/s002530051627

Miller, G.L. (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar, *Anal. Chem.,* 31(3), 426-428. https://doi.org/10.1021/ac60147a030

Mrudula, S., Anitharaj, R., (2011) Pectinase production in solid state fermentation by *Aspergillus niger* using orange peel as substrate, *Glob. J. Biotechnol. Biochem.,* 6(2), 67-71.

Mukadam, D.S., Chavan, A.M., Taware, A.S., Taware, S.D., (2010) Isolation, cloning and molecular characterization of polygalacturonase I (*pga*I) gene from *Aspergillus niger* isolate from mango, *Indian J. Biotechnol.,* 9, 153-159.

Nandiyanto A.B.D., Ragadhita R., Ana A., Hammouti B. (2022), Effect of Starch, Lipid, and Protein Components in Flour on the Physical and Mechanical Properties of Indonesian Biji Ketapang Cookies, *International Journal of Technology*, 13(2), 432-443; https://doi.org/10.14716/ijtech.v13i2.5208

Nigam, P.S., Pandey, A. (2009) *Biotechnology for Agro-Industrial Residues Utilisation.* Springer, Netherlands. 466pp. https://doi.org/10.1007/978-1-4020-9942-7

Ogbeibu, A. E., (2014) *Biostatistics: A Practical Approach to Research and Data Handling*, (2nd edition). Mindex Publishing Co. Ltd., Benin City, Nigeria. 285pp.

Okafor U.A., Okochi V.I., Chinedu S.N., Ebuehi O.A.T., Onygeme-Okerenta B.M. (2010) Pectinolytic activity of wild-type filamentous fungi fermented on agro-wastes, *Afr. J. Microbiol Res.,* 4(24), 2729-2734.

Okonji, R.E., Itakorode, B.O., Ovumedia, J.O., Adedeji, O.S. (2019) Purification and biochemical characterization of pectinase produced by *Aspergillus fumigatus* isolated from soil of decomposing plant materials, *J. Appl. Biol. Biotechnol.,* 7(3),1-8. https://doi.org/10.7324/JABB.2019.70301

Onofre, S.B., Bertoldo, I.C., Abatti, D., Refosco, D., Tessaro, A.A., Tessaro, A.B., (2017) Characterization of polygalacturonases produced by the endophytic fungus *Penicillium brevicompactum* in solid state fermentation, *Int. J. Adv. Eng. Res. Sci.,* 4(9), 94-101. https://doi.org/10.22161/ijaers.4.9.14

Oumer, O.J., Abate, D. (2017) Characterization of pectinase from *Bacillus subtilis* strain Btk 27 and its potential application in removal of mucilage from coffee beans, *Enzyme Res.,* 2017(1), p.7686904. https://doi.org/10.1155/2017/7686904

Oyeleke, S.B., Oyewole, O.A., Egwim, E.C., Dauda, B.E.N., Ibeh, E.N., (2012) Cellulase and pectinase production potentials of *Aspergillus niger* isolated from corn cob, *Bayero J. Pure Appl. Sci.,* 5(1):78-83. https://doi.org/10.4314/bajopas.v5i1.14

Patil, S.R., Dayanand, A., (2006) Production of pectinase from deseeded sunflower head by *Aspergillus niger* in submerged and solid-state conditions, *Bioresour. Technol.,* 97(16), 2054-2058. https://doi.org/10.1016/j.biortech.2005.09.026

Pedrolli, D.B., Monteiro, A.C., Gomes, E., Carmona, E.C. (2009) Pectin and pectinases: production, characterisation and industrial application of microbial pectinolytic enzymes, *Open Biotechnol., J.* 3, 9-18. https://doi.org/10.2174/1874070700903010009

Pickersgill, R., Smith, D., Worboys, K., Jenkins, J. (1998) Crystal structure of polygalacturonase from *Erwinia carotovora* ssp. *Carotovora*, *J. Biol. Chem.,* 273(38), 24660-24664. https://doi.org/10.1074/jbc.273.38.24660

Pointner, M., Kuttner, P., Obrlik, T., Jager, A., Kahr, H. (2014) Composition of corncobs as a substrate for fermentation of biofuels, *Agron. Res.,* 12(2), 391–396.

Polizeli, M.L., Jorge, J.A., Terenzi, H.F., (1991) Pectinase production by *Neurospora crassa*: purification and biochemical characterization of extracellular polygalacturonase activity, *Microbiology,* 137(8),1815-1823. https://doi.org/10.1099/00221287-137-8-1815

Phutela, U., Dhuna, V., Sandhu, S., Chadha, B.S., (2005) Pectinase and polygalacturonase production by a thermophilic *Aspergillus fumigatus* isolated from decomposting orange peels, *Braz. J. Microbiol.,* 36, 63-69. https://doi.org/10.1590/S1517-83822005000100012

Ramachandran, S., Kurup, G., (2013) Screening and isolation of pectinase from fruit and vegetable wastes and the use of orange waste as a substrate for pectinase production, *Int. Res. J. Biol. Sci.,* 2(9), 34-39.

Revilla, I., Ganzalez-Sanjose, M.L. (2003) Addition of pectolytic enzymes: an ecological practice which improves the chromaticity and stability of red wines, *Int. J. Food Sci. Technol.,* 38, 29-36. https://doi.org/10.1046/j.1365-2621.2003.00647

Rombouts, F.M., Pilnik, W. (1980) Pectic enzymes. pp 227-282. In: *Microbial Enzymes and Bioconversions.* A.H. Rose (ed.). Academic Press. London.

Saidi N., Azzaoui K., Ramdani M., Mejdoubi E., Jaradat N., Jodeh S., Hammouti B., Sabbahi R., Lamhamdi A. (2022) Design of Nanohydroxyapatite/Pectin Composite from Opuntia Ficus-Indica Cladodes for the Management of Microbial Infections. *Polymers,* 14, 14, 4446. https://doi.org/10.3390/polym14204446

Samanta, S. (2019) Microbial pectinases: a review on molecular and biotechnological perspectives, *J. Microbiol. Biotechnol. Food Sci.,* 9(2), 248-266. https://doi.org/10.15414/jmbfs.2019.9.2.248-266

Sambrook, J., Fritsch, E. F., Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Nova York.

Saranraj, P., Naidu, M. A. (2014). Microbial pectinases: a review, *Glob. J. Tradit. Complement. Altern. Med. Syst.,* 3(1), 1 – 9.

Sarmah, A. K., (2009) Potential risk and environmental benefits of waste derived from animal agriculture. In: *Agriculture Issues and Policies Series-Agricultural Wastes,* G. S. Ashworth and P. Azevedo (eds.). Nova Science Publishers Inc., New York, USA.

Satapathy, S., Behera, P.M., Tanty, D.K., Srivastava, S., Thatoi, H., Dixit, A., Sahoo, S.L. (2019) Isolation and molecular identification of pectinase producing *Aspergillus* species from different soil samples of Bhubaneswar regions, *BioRxiv,* 2019, p.837112. https://doi.org/10.1101/837112

Sethi B.K., Nanda P.K., Sahoo S. (2016) Enhanced production of pectinase by *Aspergillus terreus* NCFT 4269.10 using banana peels as substrate, *3 Biotech,* 6, 36-50. https://doi.org/10.1007/s13205-015-0353-y

Shet, A.R., Muhsinah, A.B., Alsayari, A., Achappa, S., Desai, S.V., Mahnashi, M.H., Muddapur, U.M., Shaikh, I.A., Mannasaheb, B.A., Khan, A.A. (2022) Media optimization by response surface methodology for the enhanced production of acidic extracellular pectinase by the indigenously isolated novel strain *Aspergillus cervinus* ARS2 using solid-state fermentation, *Fermentation,* 8, 485-509. https://doi.org/10.3390/fermentation8100485

Sprockett, D.D., Piontkivska, H., Blackwood, C.B., (2011) Evolutionary analysis of gylcosyl hydrolase family 28 (GH28) suggests lineage specific expansions in necrotrophic fungal pathogens, *Gene,* 479(1-2), 29-36. https://doi.org/10.1016/j.gene.2011.01.029

Sudeep K.C., Upadhyaya J., Joshi D.R., Lekhak B., Chaudhary D.K., Pant B.R., Bajgai T.R., Dhital R., *et al*. (2020) Production, characterization and industrial application of pectinase enzyme isolated from fungal strains, *Fermentation,* 6(59), 1-10. https://doi.org/10.3390/fermentation6020059

Tabaght FE., Azzaoui K., El Idrissi A., Jodeh S., Khalaf B., Rhazi L., Bellaouchi R., Asehraou A., Hammouti B., Sabbahi R. (2023). Synthesis, characterization, and biodegradation studies of new cellulose-based polymers, *Scientific Reports*, 13, 1673, https://doi.org/10.1038/s41598-023-28298-5

Tapre, A.R., Jain, R.K. (2014) Pectinases: enzymes for fruit processing industry, *Int. Food Res. J.,* 21(2), 447-453.

Udenwobele D.I., Nsude C.A., Ezugwu A.L., Eze S.O.O., *et al*. (2014) Extraction, partial purification and characterization of pectinases isolated from *Aspergillus* species cultured on mango (*Magnifera indica*) peels, *Afr. J. Biotechnol.,* 13(24), 2445-2454 https://doi.org/10.5897/AJB2014.13805

Wayne M., Jurick I. I., Vico I., McEvoy J.L., Whitaker B.D., Janisiewicz W., Conway W.S. (2009) Isolation, purification and characterization of a polygalacturonase produced in *Penicillium solitum*-decayed 'golden delicious' apple fruit, *Phytopathology,* 99(6), 636-641. https://doi.org/10.1094/PHYTO-99-6-0636

Zaslona, H., Trusek-Holownia, A., (2015) Enhanced production of polygalacturonase in solid-state fermentation: selection of the process conditions, isolation and partial characterization of the enzyme, *Acta Biochim. Pol.,* 62(4), 651-657. https://doi.org/10.18388/abp.2015_1112

Zeni, J., Cence, K., Grando C.E., Tiggermann L., Colet R., Lerin L.A., Cansian R.L., Toniazzo G., de Oliveira D., Valduga E. (2011) Screening of pectinase-producing microorganisms with polygalacturonase activity, *Appl. Biochem. Biotechnol.,* 163(3), 383-392. https://doi.org/10.1007/s12010-010-9049-1

Zhao, Z., Liu, H., Wang, C., Xu, J. (2013) Comparative analysis of fungal genomes reveals different plant cell wall degrading capacity in fungi, *BMC Genomics,* 15, 1-5. https://doi.org/10.1186/1471-2164-15-6