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Isolation, Screening and Identification of Pectinolytic Fungi from the Soil of Decomposed Plant Materials

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Abstract: Pectinases are essential enzymes and command a quarter of all food enzymes sold globally, and are recognized as excellent workhorses in a plethora of industries. These enzymes are mainly sourced from animals, microorganisms and plants, with microbes serving as the main sources, owing to the quality, quantity, ease of extraction and economic considerations. This research study isolated, screened, and identified five pectinase-producing fungi from the soil of decaying fruits and vegetables. The isolated fungal species were cultured and screened for the highest pectinase production using a pectinase screening agar medium containing 1% citrus pectin. Four isolates, identified as *Aspergillus niger*, *Fusarium* species, *Trichoderma* species and *Aspergillus flavus*, exhibited very high values of pectinase hydrolysis based on the clear zone method, which were 25, 23, 20, and 20 mm for the respective isolates. A secondary pectin hydrolysis screening exercise was conducted afterwards to obtain the highest level of reducing sugars and pectinase released by the isolates. *Aspergillus niger*, once again, proved to be the best, as it recorded a maximum reducing sugar of 3.92 mg/mL and pectinase enzyme activity of 36.23 U/mL. Soil has the potential to serve as the primary source of fungal species capable of degrading pectin to release pectinase enzymes, which have numerous applications in the production of pharmaceuticals, food, beverages, animal feed, textiles, detergents, protoplast fusion, pulp and paper and biofuels.

Keywords: pectinase; fungal species; *Aspergillus niger*; pectin; screening

1. Introduction

Pectin is a hecteropolysaccharide that serves as an important component of the cell wall and other internal components of higher plants. It confers mechanical support and flexibility to plants by acting as a cementing material that holds the cell walls of adjacent cells together [1,2]. Pectin is made up of numerous units that consists of D-galacturonic acids with carboxyl groups, mostly esterified with methanol, with the main chain being made up of α-(1, 4) glycosidic bonds. Pectin with its methyl groups removed is called pectate or pectic acid, which is also called polygalacturonic acid [3]. The structure of pectin contains homogalacturonan, which appears as a linear biopolymer and is made up of methyl-esterified or acetylated D-galacturonic acid residues or monomers containing α-(1, 4) glycosidic linkages. Pectin is formed when about 75% of carboxylic acids found in homogalacturonans become methylated. Pectic acid is also formed when less than 75% of the carboxylic acids are methylated polygalacturonic acid results when there is no methyl esterification [4]. Also, protopectin represents the pectic substances that are not soluble in water [5]. Soluble pectic substances can be formed after limited hydrolysis of protopectin [6]. Pectinic substances, which act

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as the methylated form of galacturonic acid are formed when there is enzymatic hydrolysis of protopectin in ripe fruits [7].

Pectin is non-toxic, biocompatible and biodegradable and can dissolve in water. It has crucial stabilizing, hydration, gelling, thickening and swelling properties, which makes it very useful in many commercial applications. A few of the commercial applications include food designs, the production of fruits juices, drinks, and fermented, confectionary pharmaceuticals and health products. Pectin is also utilized as a nano-encapsulator, drug delivery agent, constituent of pro-health formulations, dietary fiber, anti-cancer agent, and hydrogel formulation material. It is also used to lower blood cholesterol and low-density lipoprotein levels, as well as being used in the treatment of diarrhea and constipation [8–10]. The primary sources of pectin have been citrus fruits, apple pomace, mango peels and sugar beet pulp [11–13]. Figure 1 illustrates the structure and powdered form of pectin.

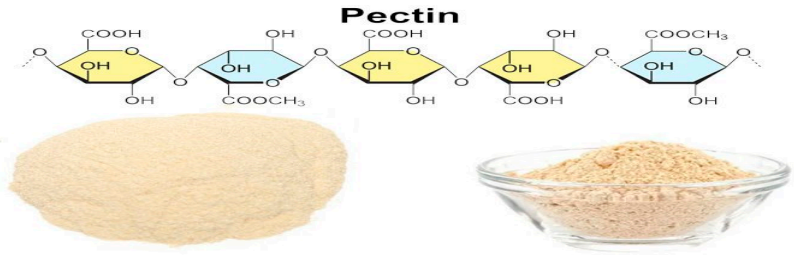

Figure 1. The structure and powdered form of pectin [14].

Pectin can be hydrolyzed by a group of enzymes called pectinases by splitting the glycosidic bonds existing between pectin molecules [15]. Pectinases are able to break down the pectin oligo-D-galacturonate present in plant cell walls into monogalacturonic acids. Hydrolysis is achieved when pectinolytic enzymes cleave the α-1, 4 glycosidic linkages of polygalacturonic acid [16]. Pectinases can be classified as extracellular and intracellular. Extracellular pectinase enzymes represent those secreted outside of the cell into the surrounding media in which the microbes live, whereas intracellular pectinase enzymes are those secreted and act within the cell membrane [17]. Nevertheless, extracellular and intracellular pectinases are also classified into three broader groups based on their mode of action on pectic substances [17,18], which include protopectinases, esterases, and depolymerases [19–22]. Pectin esterases are able to catalyze the de-esterification of methyl groups in pectin to form pectic acids. Pectin depolymerases, depending on their mechanism of action, are able to cleave the glycosidic bonds found in pectic acids. Also, protopectinases are able to break down protopectin [23]. In terms of their nature, pectinases can be categorized as acidic and alkaline. Acidic pectinases usually enjoy their applications in the wine, beverage and fruit juice industries for clarification and purification of fruit juices and wines [13,24]. Alkaline pectinases, on the other hand, find their applications in textile industries and wastewater treatments, [4,25,26].

Among the various pectinases, extracellular pectinases derived from fungal species have been known to possess excellent abilities compared to pectinases from other sources such as plants, animals and bacteria [27], and their method of extraction is very simple as the integrity of the cells remains intact. Other added advantages include high yields, economic feasibility, stability and easy genetic manipulation. Also, fungal-derived pectinolytic enzymes are generally regarded as having a safe (GRAS) status [23]. For these and many more reasons, extracellular pectinases isolated from fungal species especially that of *Aspergillus* sp. are in high demand in various applications [28,29]. These applications extend across food/feed, beverage, pulp and paper, biofuel, textile, oil extraction and waste and environmental remediation [13,22,30,31]. Pectinases employed in the fruit and wine industries can enhance taste, clarity and overall chromatic appearance compared to untreated ones [25].

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The soil, as part of the natural environment, serves as a huge reservoir for fungal species that can be isolated and employed in the production of pectinase enzymes. Microorganisms isolated from the soil are mesophilic, can tolerate stress and have the ability to synthesize pectinase enzymes in large quantities in a wider spectrum of growth parameters [31]. Soils particularly found in plant food processing locations, markets and organic waste dumpsites have been proven to be very much dominant with pectin-degrading fungus [5]. Recent advances indicate that the trend to identify novel enzymes from microorganisms from such environments is accelerating due to their inherent stability and efficiency [32,33]. It is, therefore, hypothesized herein that, collecting soil composed of decaying organic (plant) matter and isolating from it, the fungal strains capable of degrading the pectin-rich components would be very beneficial to obtaining microbial strains tailored for pectinase production, biofuel production and bioremediation purposes. Also, the ability to identify and characterize such microbes isolated in this work would go a long way in providing more information on fungal strains with special and well-tailored capacities for industrial purposes. Hence, pectinolytic microbes were obtained from the soils exposed to high and low temperatures, and subsequent identification was carried out in this current investigation.

2. Materials and Methods

2.1. Collection of Soil Sample

Soil composed of plant-decayed materials was collected from the dumpsites of fruits, vegetables and plant materials in some markets in Kumasi, as these places contained many spoiled fruits and vegetable wastes and decaying plant materials. All the samples were pooled together, kept in sterile plastic containers and carried to the microbiology laboratories of Kwame Nkrumah University of Science and Technology, where fungal species were isolated from them.

2.2. Preparation of Medium for Isolation of Fungal Species

A sterile nutrient medium containing 1% citrus pectin, $FeSO_4.H_2O$ (5 g/L), $MgSO_4.7H_2O$ (0.02%), $(NH_4)_2SO_4$ (0.14%), and K_2HPO_4 (0.2%) as well as a nutrient solution (0.1%) containing $ZnSO_4.7H_2O$ (1.4 mg/L), $MnSO_4.H_2O$ (1.6 mg/L) and $CoCl_2$ (2.0 mg/L) was prepared, and mixed with 3% agar-agar (the gelling agent) and autoclaved at 121 °C for 20 min [21]. It was then allowed to cool to around 45 °C before being placed into Petri dishes to gel. To ensure sterility, the plates were incubated overnight in an incubator at 37 °C.

2.3. Preparation of Potato Dextrose Agar for Isolation of Fungal Species

Potato Dextrose Agar (PDA) was made from a commercial dehydrated OXOID product. Thirty-nine PDA powder (39.0 g) was dissolved in 1 L of distilled water, and sterilized at 121 °C for 20 min and at a pressure of 1.2 g/cm^2 according to the product recommendations.

2.4. Preparation of 3, 5-Dinitrosalicylic Acid (DNS)

This protocol followed that of Miller [34]. To summarize, 1.0 g of DNS was dissolved by warming in 20 mL of 2N NaOH. A total of 30.0 g of potassium sodium tartarate was dissolved by warming in 50 mL of distilled water. After cooling, the two solutions were combined together and made up to 100 mL with distilled water.

2.5. Isolation and Sub-Culturing of Fungal Specie

The simple dilution plate method was adopted here. One (1) g of soil sample was diluted ten-fold in 9 mL of sterile distilled water. One (1) mL of the 6th dilution (10^{-6}) was flooded onto three replicates of the previously prepared sterile Potato Dextrose Agar (PDA) nutrient medium, which had been supplemented with chloramphenicol antibiotic to inhibit bacterial growth. The plates were then incubated at room temperature (25 °C–28 °C) for 7 days, until visible fungal colonies appeared.

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All morphologically distinct colonies were purified by sub-culturing on different plates and streaking with a sterile inoculation loop. This process proceeded until pure fungal isolates were obtained. Pure cultures were maintained on PDA slants, refrigerated and sub-cultured at regular intervals throughout the study and were used as stock cultures. All pure fungal growths were counted with a colony counter, and identified appropriately. Their frequency of occurrence was subsequently established as well.

2.6. Identification of Fungal Isolates

The identification procedure for all the fungal isolates was based on macromorphological and micromorphological characteristics. Macromorphological identification was conducted by observing colony characteristics such as the color, texture, spore structure and the form of mycelia and/or pattern of growth. The micromorphological characteristics like separation, and spore shapes, among others were determined using the usual lactophenol cotton blue procedure (LPCB). All the fungal isolates had been defined in accordance with the manual for determining the identity of fungi [35] and Illustrated Genera of Imperfect Fungi by Barnett and Hunter, [36]. In summary, a three-day-old pure culture was utilized to prepare slides for microscopic examination. A small amount of mycelia was placed on the slide, along with a drop of lactophenol cotton blue reagent. A cover slip was laid over it, and the specimen was examined under a light microscope at ×400 magnification. Identification of the species was accomplished by comparing features to the micrographs, and the photographs taken included at the end of this section.

2.7. Qualitative (Primary) Screening for Pectinolytic Fungal Species

The process of screening for pectinase synthesis was carried out with the use of the prepared cultivation medium (in the previous section) by applying the spot inoculation method. In brief, pure cultures of the various fungal species were introduced into the already prepared sterile and solidified pectin agar medium. The plates that were inoculated were then incubated at a temperature of 30 °C for four days (96 h), and following that, a clear observable colony of fungi could be seen on every Petri dish. Thereafter, the Petri dishes were soaked with iodine-potassium iodide solutions (a mixture of 1.0 g of iodine and 5.0 g of potassium iodide dissolved in 330 mL of distilled water) for about 10 min. This was done in order to boost visibility for measuring the zone of clearance/carbohydrate hydrolysis [21]. A distinct zone that formed surrounding the pectinase-producing colonies was observed and subsequently measured. The fungal isolates with the most promising clear zone of pectinase hydrolysis were chosen and preserved under 4 °C in the refrigerator on pectin agar and later used in the next step.

2.8. Quantitative (Secondary) Screening for Pectinolytic Fungal Species

This experiment utilized the protocol established by Kaur and Kaur [37] with some modifications. The four isolates with higher degrees of pectinolytic activity showing larger zones of pectin hydrolysis obtained in the previous experiment were used in a submerged fermentation process. A total of 50 mL of the fermentation medium (without the 3% agar component) prepared earlier underwent inoculation with 1 mL of the spore suspension at a concentration of 1.2×10^6 spores/mL of each of the culture isolates. It was incubated at 30 °C for 7ays on a rotary shaker rotating at 120 revolutions per minute (rpm). Throughout the experiment, triplicate Erlenmeyer flasks (250 mL) were used for each species of fungus. Once the incubation period elapsed, the crude pectinolytic enzyme underwent extraction. The extraction was achieved by mixing 50 mL of sodium acetate buffer with the fermented broth cultures. The mixture was centrifuged for 15 min at 4000 rpm to remove the debris and cells. The supernatants were labeled as crude enzymes. The pectinolytic activities of the enzyme and reducing sugar concentration were measured by estimating the reducing sugars generated from the pectin using the 3, 5-dinitrosalicylic acid (DNS) technique [34]. Glucose was

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employed as the benchmark. Each sample's pectinase activity was defined as the quantity of enzyme required to produce 1 μmol reducing groups per minute.

3. Results and Discussion

3.1. Isolation and Sub-Culturing of Fungal Species

In all, five fungal isolates were obtained from the pooled soil samples collected from various locations. The microbes were designated as FS_1, FS_2, FS_3, FS_4 and FS_5 based on the Petri dishes they were cultured on. This result is clearly in line with recent developments that have involved screening a significant number of microbial species from diverse soil resources, agrowastes and environments. Such developments are aimed at finding microbial species capable of degrading polysaccharides present in plant biomass that would illicit the synthesis of pectinases [5,38–40]. These microbial creatures play crucial roles in the maintenance of nutrient cycles, biodiversity and degradation of organic matter. Microbes have also been shown to be very useful for industrial processes. The fact that, all five isolates are fungal species perfectly agrees with Meyer *et al.*, [41]. According to them, fungal species are the most dominant microbial consortia present in the environment. The dominance is a result of their robustness in their lifecycle and activities [31].

The wide range of fungal species in the natural environment, particularly in plant food processing locations, markets and organic waste dumpsites, is also in strong alignment with Thakur *et al.*, [5]. There is no doubt that soil serves as a reservoir for plant-degrading microorganisms that can be employed to carry out many processes. Previous investigations established by Ezenwelu *et al.*, [42] demonstrated the existence of *Aspergillus niger* as well as *Rhizopus* sp. within the environments composed of decaying plant materials. Ametefe *et al.*, [43] recorded *Aspergillus niger* and *Penicillium* sp. as part of the fungi they isolated from the soil. Aside the soil, decaying fruits have also been implicated in harboring many microbial consortia, especially fungal species for instance. Okonji *et al.*, [31] isolated *Aspergillus fumigatus* from decaying citrus. Mat Jalil *et al.*, [44] also isolated *Aspergillus niger* (LFP-1 strain) from decomposed citrus fruits, and Budu *et al.*, [40], showed the abundance of fungal spp. in decaying cassava peels. Plant-decaying soils (especially that of fruits and vegetables) are attractive to the growth and survival of fungi. This is because, they serve as a rich source of nutrients, and the soil creates a conducive environment that is highly favorable to fungal growth and survival [41].

As a result, such soils have the potential to draw fungi to colonize and flourish in them. Secondly, the physical structure of fruits and vegetables, which is usually characterized by their fibrous and porous nature, provides a generous surface area and soil environment for fungal spores to land, establish their growth and thrive successfully. The physical structure, in addition to texture, pH, oxygen content, cation exchange capacity and redox reactions of the soil, provide synergistic conditions for microbe wellbeing. As such, their presence in organic matter in the soil, as a natural consequence, underscores the roles fungi play as the agents involved in breaking down and recycling organic components and nutrients.

3.2. Identification of Fungal Isolates

Fungal isolates FS_1, FS_2, FS_3, FS_4 and FS_5 were thoroughly identified in accordance with their macromorphological and micromorphological features [34,35]. At the end of this section, *Aspergillus flavus*, *Fusarium* sp., *Aspergillus niger*, *Trichoderma* sp. and *Saccharomyces* sp. were realized. Table 1 demonstrates a summary of the various fungal species successfully obtained and identified as well as their characteristics.

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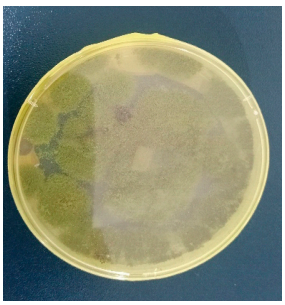
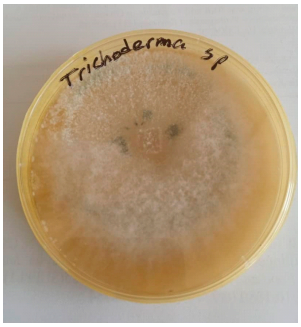
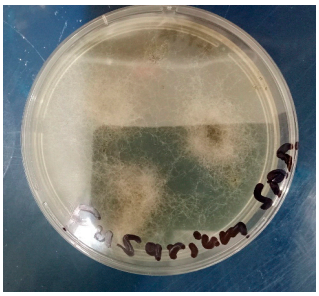
Table 1. Characteristics of the fungal isolates.

Isolate	Characteristics (Macro-and Micromorphological)	Possible Identity
FS_1	Appeared white initially and later turned grey or yellowish brown, whilst pale yellow appeared as the reverse; colonies were fast growing with the texture, appearing velvety or powdery, with a cottony appearance.; Coeonidial heads appeared as compact clusters, typically yellowish-green to yellow in color; large spore-bearing heads, spherical, and densely packed; clear, thin, and short hyphae; greenish mycelia; branched greenish conidia and septate hyphae; dense unbranched conidiophores with rounded or flask-shaped vesicle; elongated Phialides with constricted neck.	 *Aspergillus flavus*
FS_2	The colony had fast and aggressive growth and looked fluffy white initially, turned black later, and generated enormous black spores; reverse appeared pale yellow, wrinkled mycelia with visible dense and lengthy aerial hyphae. The conidia were unbranched; the texture looked velvety or powdery and the conidial produced heads with compact clusters, typically dark green to black in color, The hyphae looked septate, dense and branched; long, unbranched, and terminated conidiophores and gave rise to the conidial heads; rounded or flask-shaped vesicles at the end of conidiophore; Phialides were elongated with constricted necks.	 *Aspergillus niger*.
FS_3	The colony was fast-growing and dominant with a white appearance; sticky green phialoconidia clusters formed within a few days; flask-like Phialides with an enlarged base; woolly colonies formed initially and became compacted over time. As the conidia developed, dispersed blue-green or yellow-green spots appeared visible, the reverse was pale, tan, or yellowish. The conidia usually appeared within a week in dense or loose tufts of green, yellow, or white; a yellow pigment was observed to have been released into the agar medium; an irregular yellow zone without conidia was present around the colony; some concentric circles/rings also appeared.	 *Trichoderma* sp.
FS_4	Colonies grew very fast, and looked pale or brightly-colored with the mycelia appearing like cotton; the thallus looked whitish in color and showed the presence of conidia from slender phialides; conidiophores looked solitary, short, lateral monophialides in the airborne mycelium, but were later observed as thickly branching groups; macroconidia looked fusiform, slightly curved and pointed at the tip and also appeared plentiful, largely non-septate, and straight or curved and looked cylindrically-shaped. The chlamydospores appeared terminal, smooth and rough-walled, while the phialides were short and mainly non-septate.	 *Fusarium* sp.

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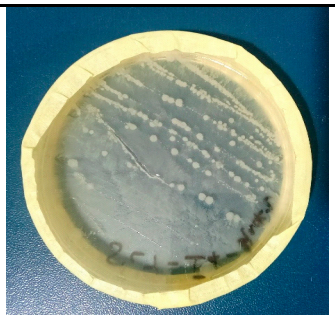
FS_5	Colonies of *Saccharomyces* grew rapidly and matured in three days; they looked small, flat, smooth, moist and creamy or white in color, were raised and clustered, exhibiting oval or spherical to ellipsoidal shapes.	 *Saccharomyces* sp.

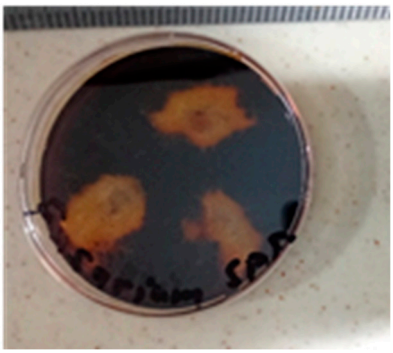
3.3. Qualitative (Primary) Screening for Pectinolytic Fungal Species

One of the numerous ways to show pectinase's production ability of microorganisms, especially fungal species, is the method popularly known as pectin/carbohydrate hydrolysis or zone of clearance. Such procedures have been known to be very effective in determining and selecting the best fungal species needed to carry out pectinase production [20,31,38]. By adopting similar paths, this current study conducted a protocol showing carbohydrate hydrolysis. The aim was to select the isolates with the highest pectin degradation potential, after 4 days of incubation. After subjecting the isolates to pectin hydrolysis, the result showed that, all the fungal species possessed various degrees of pectinolytic activity (Table 2 and Figure 2). *Aspergillus niger* (FS_2) emerged slightly on top as the most efficient microbial species (25.0 mm) among the tested species. *Fusarium* species ($FS_{4)}$ followed second with 23.0 mm, then *Trichoderma* species ($FS_{3)}$ and *Aspergillus flavus* ($FS_{1)}$ produced 20.0 mm each. *Saccharomyces* species (FS_5) gave 7. 0 mm, hence, it was left out in subsequent experiments.

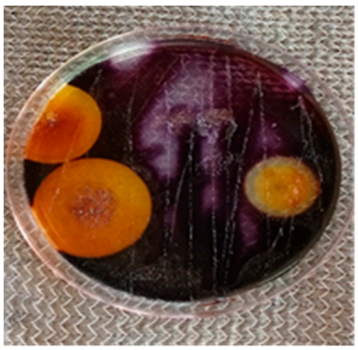
Table 2. Qualitative analysis of fungal species based on the zone of pectin hydrolysis achieved.

Microorganism	Zone of Pectinolytic Activity/mm
Aspergillus niger	25.00
Fusarium sp.	23.00
Trichoderma sp.	20.00
Aspergillus flavus	20.00
Saccharomyces sp.	7.00

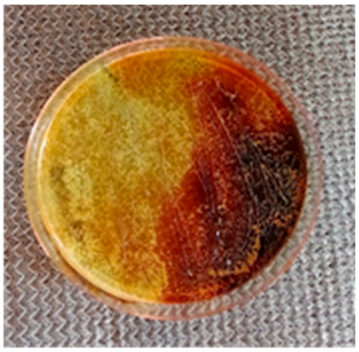
p-value = 0.0511.



A: *Fusarium* species



B: *Aspergillus niger*



C: *Aspergillus flavus*

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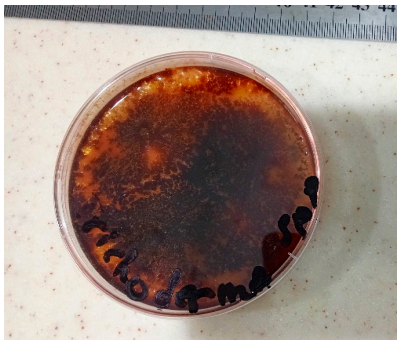

D: *Trichoderma* species

E: *Saccharomyces* species

Figure 2. Qualitative analysis of fungal species based on the zone of pectin hydrolysis: (**A**) *Fusarium* sp.; (**B**) *Aspergillus niger*; (**C**) *Aspergillus flavus;* (**D**) *Trichoderma* sp.; and (**E**) *Saccharomyces* sp.

It is well noted that, the strains of *Aspergillus* spp. have been known to dominate pectinase enzyme manufacturing in various studies and industrial applications [31]. The fact that, fungal species dominated the synthesis of pectinase in terms of the zone of clearance in the current study is favorably supported by El-Ghomary *et al.,* [39]. In their work, they opined that, the majority of commercial pectinase enzymes employed in industries are generated from species of fungal sources, including *Rhizopus stolonifer, Aspergillus* and *Penicillium* sp. Oumer and Abate, [20] and Adedayo *et al.,* [38], also asserted that, commercial pectinase enzyme production has recognized *Aspergillus* and *Penicillium* sp. as the main sources of pectinase enzymes. El-Ghomary *et al.,* [39] isolated four strains of *Aspergillus* spp., which showed clear zones of pectin hydrolysis of 3.62 mm, 3.70 mm, 3.91 mm, and 4.75 mm for the respective strains, with *Aspergillus flavus* emerging as the species with the highest zone of clearance. The current result is also, in agreement with Okonji *et al.,* [31] when a fungal isolate identified as *Aspergillus fumigatus* showed the highest pectin hydrolysis. Other numerous earlier investigations have also indicated the vital role played by *Aspergillus* sp. [13,20,29,44,45].

Furthermore, the relative dominance of *Aspergillus niger*, in the current investigation, after exhibiting the highest activity of pectinase in relation to the zone of clearance (25 mm), is no fluke at all. This assertion is true as its ability to hydrolyze pectin-rich media has also been agreeably demonstrated in a series of previous studies cited in Satapathy *et al.,* [21]. In another closely related study conducted by Adedayo *et al.,* [38], *Aspergillus niger* showed a better zone of clearance of 7.0 mm, whereas *Aspergillus flavus* produced 5.5 mm. In addition, Kamalambigeswari *et al.,* [46], examined three distinct strains of *Aspergillus niger* (F-3, F-4, and F-P), which revealed isolate F-4 as one that exhibited the largest region of pectin breakdown (9 mm).

3.4. Quantitative Screening of Potent Pectinolytic Fungal Species (Secondary Screening)

Due to their promising nature, all four isolates with comparatively higher zones of pectin hydrolysis were selected for further work on pectinase production (secondary screening). The four isolates that were deemed the best at their pectinolytic ability exhibiting a clear zone of clearance were scrutinized for quantitative evaluation of pectinase synthesis utilizing a selective enrichment technique to generate comparative assessments. The secondary pectin hydrolysis screening exercise was then performed to identify the greatest amount of reducing sugars generated by the isolates with their respective enzyme activity via the enzymatic breakdown of pectin. The galacturonic acid released was measured spectrophotometrically. The result (Table 3) showed that among the four (4) fungal isolates, FS_2, which was identified as *Aspergillus niger*, once again, emerged as the most potent fungal species for the production of pectinase after recording the highest values for reducing sugar formation (3.92 mg/mL) and pectinase activity (36.23 U/mL) under submerged fermentation after seven (7) days of incubation.

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Table 3. The figures obtained during the secondary screening.

Microorganism	Reducing Sugar (mg/mL)	Enzyme Activity (U/mL)
Aspergillus niger (FS_2)	3.92	36.23
Aspergillus flavus (FS_1)	3.68	32.52
Trichoderma sp. (FS_3)	3.23	29.74
Fusarium sp. (FS_4)	3.16	28.66

p-value = 0.0260.

This finding fits in with El-Ghomary *et al.,* [39], who found that, fungal isolates tagged as F9 showed a relatively highest titre of pectinase (35.83 U/mL) than that of all the other isolates. Okonji *et al.,* [31] also discovered that *Aspergillus fumigatus* yielded 42 U/mL of pectinase enzyme activity in submerged fermentation conditions. The results achieved in this study and subsequent selection of fungal strains are also in line with Sudeep *et al.,* [29], who, in their research, isolated four strains of fungal species with high pectinolytic activities from the soil. After thorough primary and secondary screening procedures, *Aspergillus* sp. was observed as the most potent fungus for pectinase enzyme production.

4. Conclusions

Soils obtained from the selected markets in Kumasi were found to be a great source of a plethora of diverse pectin-degrading microorganisms, especially fungal species. *A. niger* has proven to be very dominant, probably as a result of its ubiquitous nature and its ability to colonize diverse environments and substrates as it thrives well in many growth media or substrates, as proven by this and other studies. A successful collection of these industrially-relevant strains of microorganisms from the soil could very well contribute to the increasing demand and adequate supply of pectinase enzymes in commercial quantities by related industries. Pectinase enzymes from microbes would also be very useful in the remediation of areas polluted with plant waste. Further researches should be directed towards the mechanisms involved in the molecular regulation of pectinase enzymes production among fungal species, especially *A. niger*. With such knowledge, scientists would be able to optimize the production of pectinase for commercial purposes. Also, subsequent studies could be invested in screening of extreme environments with the aim of finding fungal species with enhanced capabilities that can synthesize pectinase with novel properties.

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