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Identification of endophytic fungi associated in banana (*Musa paradisiaca* L.) and evaluation of its enzymatic abilities

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Abstract Various microorganisms such as fungal endophytes live in *Musa paradisiaca* that grow in internal tissues without harming and causing any diseases on its host plant. These fungi are known for their ability to produce extracellular enzymes. In this study, nine fungal endophytes were identified through cultural, morphological and DNA sequencing of the ITS1 and ITS4 regions. These were *Cladosporium cladosporioides* with 99.14% identity, *Fusarium chlamydosporium* with 99.80% identity, *Fusarium keratoplasticum* with 98.46% identity, *Fusarium solani* strain f2-f6 with 98.87% identity, *Fusarium solani* strain ZB11263612 with 98.07% identity, *Fusarium solani* strain F10-3 with 99.24% identity, *Geotrichum candidum* with 96.32% identity, *Nigrospora oryzae* with 100.00% identity and *Schizophyllum commune* with 99.66% identity. Eight out of nine fungi were able to degrade starch. The endophyte *F. solani* strain f2-f6 obtained highest enzymatic index of 14.16 in amylase production. On the other hand, four out of nine fungal endophytes were able to show cellulolytic activity. *N. oryzae* obtained highest enzymatic index of 13.13 in cellulase activity. Lastly, only *F. solani* strain f2-f6 showed color change for laccase activity. Thus, the study revealed that fungal endophytes from *M. paradisiaca* have the ability to produce these different kinds of enzymes that are of industrial and biotechnological importance.

Keywords: Banana peels, Endophytes, Enzymes

Introduction

Musa paradisiaca commonly called as plantain from Musaceae family is a tropical plant native to India. In the tropics, it is extensively cultivated and considered as a staple crop for over 70 million people in the sub-Saharan Africa and often consumed as unripe fruit or even when ripe (Ahenkora *et al.*, 1997). Aside from being consumed as food, *M. paradisiaca* also possess medicinal properties for it is used for treating diarrhea, dysentery, hysteria and epilepsy (Salawu *et al.*, 2010). In addition, it also has various industrial applications such as in organic fertilizer and biofuel production, in the manufacture of pulp

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and paper and many others. Furthermore, it also used in various biotechnological processes and energy related activities (Ehiowemwenguan *et al.,* 2014; Yousaf and Sajjad, 2015).

Microorganisms such as fungi vary from one species to another because of their ability to produce enzymes (Geethanjali and Reshma, 2014; Waiter *et al.,* 2007). In which, enzymes such as amylase, cellulose and lipase were being produced by various fungal organisms (Mittal, 2018). Additionaly, laccase producing fungi can be found in the family of Ascomycota, Deutromycota and Basiodiomycota (Assavanig *et al.,* 1992). Fungal enzymes such as amylase, cellulose and laccase have different mechanisms. Starch molecules are hydrolyzed into simpler glucose units by the enzyme amylase (Windish and Mhatre, 1965), hydrolysis of cellulose was done by cellulase enzyme (Berry and Paterson, 1990) and the enzyme laccase catalyzes the oxidation of a variety of organic and inorganic compunds (Galhaup *et al.,* 2002).

Therefore, determining the enzymatic abilities of fungi from *M. paradisiaca* will be of great contribution to the industry as well to medical field which is of biotechnological interest. Thus, the study aimed to identify and evaluate the enzymatic abilities of endophytic fungi associated in banana (*M. paradisiaca*) leaves and fruit peels.

Materials and methods

Collection of banana samples

Endophytic fungi were isolated from the healthy leaves and matured unripe fruit peels of *M. paradisiaca.* The leaves and fruit peels were collected from a banana plantation at Villaverde, Nueva Vizcaya, Philippines.

Preparation of Potato Dextrose Agar

Thirty-nine grams of dehydrated Potato Dextrose Agar (PDA) medium in a liter of distilled water was heated up to boiling until totally dissolved. Then, approximately 40 ml of prepared PDA was dispensed into bottles with cotton plug and was autoclaved for 30 min of sterilization at 121˚C at 15 psi.

Isolation from leaves

The collected leaf samples were washed with tap water. Thereafter, it was surface sterilized for one minute using 70% ethanol. Then, the sample was immersed for another one minute in 1% sodium hypochlorite (NaOCl). After

immersion, it was rinsed using sterile distilled water for another minute and allowed to surface dry on filter paper. After drying, the samples were homogenized using a blender (Patel *et al.*, 2013).

Isolation from fruit peels

Collected fruit peels were washed with water, and surface sterilized by soaking them in 1% sodium hypochlorite, 70% alcohol then was rinsed with sterile distilled water for three times. After drying on filter papers, the samples were then homogenized using a blender (El-Gali, 2016).

Serial dilution

About 90 ml of sterile distilled water was prepared and labeled as 10^{-1} where in 10 grams of the homogenized samples were diluted. Six test tubes containing 9 ml of sterile distilled water each were labeled as 10^{-2} to 10^{-7}. From 10^{-1}dilution, 1ml was transferred to 10^{-2} dilution. Then, from 10^{-2}, another 1 ml of sample was transferred to 10^{-3}. The procedure was repeated up to 10^{-7}. From the 10^{-1}, 10^{-3}, 10^{-5} and 10^{-7}dilutions, one ml was dispensed on the sterilized petri plates before pour plating. Triplicates were done. The method was done separately for isolation from leaves and fruit peels. After plating, previously plated petri plates were incubated until the appearance of the fungal growth appeared on the plates at room temperature for 5 to 7 days. Then, inoculating needle was used to get small bits of these growths from periphery which then transferred onto new PDA plates and repetitive re-plating of the fungal colonies were continued until pure cultures were obtained. Differences in color of the fungal colony helped to distinguished between fungal species.

Identification of isolated fungal organisms

Cultural characteristics like appearance of colony, texture of mycelia and pigmentations were observed on each side of PDA plates after 3–7 days of incubation. Growth rate via colony diameter on PDA was measured wherein six-millimeter cork borer was the standard measure used then it was incubated in darkness for 3 days. Trials were repeated three times and in triplicates (Majid *et al.*, 2015). For microscopic observation, slide culture was used to observe the morphological characteristics for the presence of conidia, conidiophores, branching patterns and chlamydospores. Morphological species characterization was determined following the dichotomous key by Barnett and Barry (1987).

Assessment of enzyme producing efficiencies of endophytic fungi

Amylase activity: Fungal isolate was grown on Glucose Yeast Peptone Agar. One gram each of glucose and yeast extract, 0.5 g of peptone, 16 g agar was dissolved in 1 L of distilled water in pH 6 supplemented with 2% starch as substrate for enzyme activity. After incubation for 7 days at room temperature, the previously inoculated plates were poured with 1% iodine solution. The clear zone formed surrounding the respective fungal colonies were considered positive for amylase activity (Patel *et al.*, 2013).

Cellulase activity: Fungal isolate was grown on Yeast Peptone Agar medium. One gram yeast extract, 0.5 g peptone, 16 g agar were dissolved in 1 L distilled water added with Na-carboxymethyl cellulose (0.5%) as enzyme substrate. Fungal growth was allowed for 7 days at room temperature. After incubation, 0.2% aqueous Congo red reagent was poured. Then, plates destained for 15 min using 1M NaCl. The clear zones formed indicate cellulase activity (Patel *et al.*, 2013).

Laccase activity: Fungal isolate was grown on Glucose yeast peptone agar medium. One g each of glucose and yeast extract, 0.5 g peptone, 16 g agar dissolved in 1 L distilled water amended with 0.005% 1-naphthol as enzyme substrate. Fungal growth was allowed for 7 days at room temperature. On oxidation of 1-naphthol by laccase, the medium changed from clear to blue. The color change indicated the laccase positive test (Patel *et al.*, 2013).

Data analysis for enzymatic activities

Enzymatic index was determined using the colony diameter and the enzymatic halo diameter (EI = Øh/Øc) (Herculano *et al.*, 2011). In which, samples with computed enzymatic index with 2.0 and higher are considered to have a high rate of enzyme production evidenced with the presence of halo or clear zone (Sharma and Sumbali, 2014).

Sequence and phylogenetic analysis

Seven-day old cultures of different fungal isolates grown on PDA medium on test tubes were sent to the Philipine Genome Center for DNA extraction and sequencing. DNA extraction was done by CTAB method. The polymerase chain reaction used universal ITS1 (5’tccgtaggtgaacctgcgg-3’) the forward primer and ITS4 (5’-tcctccgcttattgatatgc-3’) the reverse primer with

annealing temperature of 58℃. Capillary sequencing was carried out on the ABI 3730xl DNA Analyzer using a 50cm 96-capillary array, POP7TM Polymer, and 3730xl Data CollectionSoftware v3.1 and base calling was done on Sequencing Analysis Software v5.4. Trimming and assembling of sequences was done using Codon Code Aligner V8.0.2. Phylogenetic analysis and tree building were done using the software Molecular Evolutionary Genetics Anaysisver 7 (MEGA). Bootstrapping were performed using 1000 replicates. Sequencing was verified by BLAST and aligned Through CLUSTAL W.

Experimental design on enzymatic activities

Screening for amylase, cellulase, and laccase activity were laid out using Completely Randomized Design. Analysis of Variance was used to analyze the gathered data. Duncan Multiple Range Test was used in means comparison at 5% level of significance.

Results

Cultural and morphological characteristics of endophytic fungi

A total of 9 endophytic fungi were isolated from *Musa paradisiaca* leaves and fruit peels namely, *Cladosporium cladosporioides, Fusarium chlamydosporium, F. keratoplasticum* which is a species complex of *F. solani,* three strains of *F. solani* which are f2-f6, ZB11263612 and F10-3, *Geotrichum candidum, Nigrospora oryzae* and *Schizophyllum commune.*

Cladosporium cladosporioides

Colonies of *C. cladosporioides* on PDA were raised, aerial mycelium dense, cottony, grayish green on the center and flat and orange on the margin, reverse dark colored pigmentation around the center to white near the margin, with a colony diameter of 23.40 mm on the 7^{th} day of incubation. Conidia were formed in branches, ovoid and aseptate (Figure 1. A1-A3).

Fusarium chlamydosporium

Colonies of *F. chlamydosporium* on PDA was white, fluffy, aerial mycelia and orange on the reverse side. Colonies grew 44.07 mm diameter after seven days of incubation. Macroconidia of *F. chlamydosporium* are hyaline and septated (Figure 1. B1-B3).

Fusarium keratoplasticum

Colonies of *F. keratoplasticum* on PDA were flat on the enter with entire edge is with aerial mycelium dense, cottony, white, reverse light olivaceous colored pigmentation, with a colony diameter of 45.22 mm on the 7th day of incubation. Microconidia are found on phialides and produce many irregular or swollen conidiophores (Figure 1. C1-C3).

***Fusarium solani* strain f2-f6**

F. solani strain f2-f6 on PDA were fluffy, aerial mycelium cottony, light orange in the obverse while dark red orange colored pigmentation around the center to white near the margin in the reverse, with a colony diameter of 46.55 mm on the 7th day of incubation. The microconidia are globose in shape while the some of the conidiophores are irregular (Figure 1. D1-D3).

***Fusarium solani* strain ZB11263612**

F. solani strain ZB11263612 colonies on PDA spreading flat and the entire surface of the colony and were white with a ring of orange around the margin, reverse side were yellow with white circles reaching a diameter of 56.37 mm after 7 days of incubation. Microconidia are hyaline ovoid and found grouped on poliphialides (Figure 1. E1-E3).

***Fusarium solani* strain F10-3**

The colonies of *F. solani* strain F10-3 have color white and dense aerial mycelia with pale yellow at the center after seven days of incubation. This fungus later becomes olive yellow and the texture was powdery. The reverse side was darker at the center then turning into brownish yellow and media cracked as it grows. The colony diameter was 80.11 mm after 7 days of incubation. The conidia were found to be hyaline, one celled, and ovoid in shape (Figure 1. F1-F3).

Geotrichum candidum

The colonies of *G. candidum* on PDA was flat, dense, cottony, white, reverse olivaceous colored pigmentation, with a colony diameter of 21.60 mm on the 7th day of incubation. Conidia were hyaline, aseptate and formed by segmentation of hypae and rod-shaped and truncate ends (Figure 1. G1-G3).

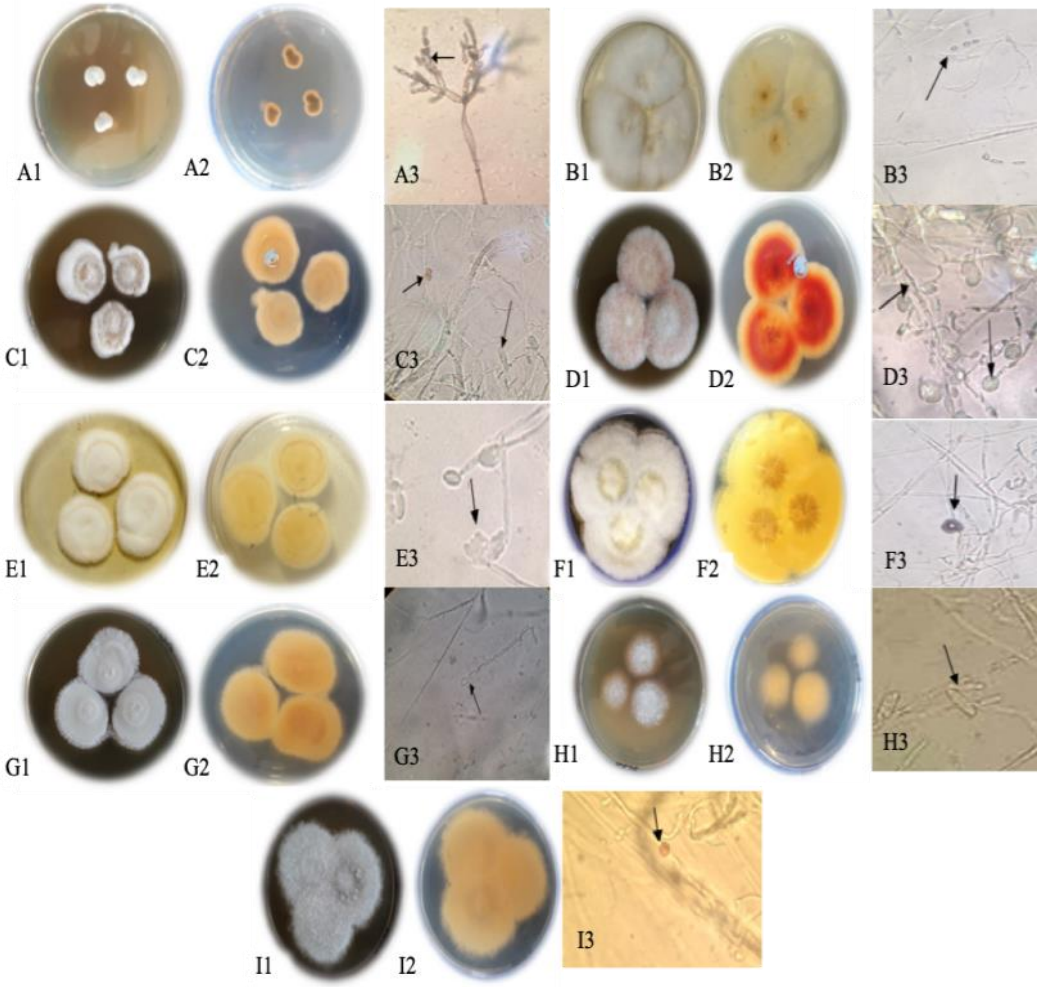

Figure 1. Fungal endophytes isolated from *M. paradisiaca* leaves and fruit peels after seven days of incubation in PDA. *C. cladosporioides* **–** (A1) obverse and (A2) reverse sides, (A3) branches ovoid and aseptate conidia; *F. chlamydosporium* – (B1) obverse and (B2) reverse sides, (B3) hyaline, septated macroconidia; *F. keratoplasticum* **–** (C1) obverse and (C2) reverse sides, (C3) microconidia and many irregular conidiophores; *F.solani* strain f2-f6 – (D1) obverse and (D2) reverse sides, (D3) globose microconidia and some irregular conidiophores; *F. solani* strain ZB11263612 – (E1) obverse and (E2) reverse sides, (E3) hyaline ovoid and found grouped on polyphialid microconidia; *F. solani* strain F10-3 – (F1) obverse and (F2) reverse sides, (F3) hyaline, one celled, and ovoid conidia; *G. candidum* – (G1) obverse and (G2) reverse sides, (G3) rod-shaped and truncate ends of hyaline conidia; *N. oryzae* – (H1) obverse and (H2) reverse sides, (H3) swollen urn-shaped conidia; *S. commune* – (I1) obverse and (I2) reverse sides, (I3) globose brown conidia

Nigrospora oryzae

Colonies of *N. oryzae* on PDA were thick, dense, cottony, and white aerial mycelium, while on reverse brown colored pigmentation around the center to white near the margin, reaching a diameter of 67.15 mm on the 7th day. The conidia were singly and urn-shaped (Figure 1. H1-H3).

Schizophyllum commune

S. commune colonies on PDA were spreading flat and the entire surface of the colony with globule of mycelial mass in the center and white in color, reverse side was also white reaching a diameter of 69.65 mm after 7 days of incubation. The conidia are globose, brown and aseptate (Figure 1. I1-I3).

Molecular identification of isolated fungi

The endophytic fungi isolated from *M. paradisiaca* were confirmed through the ITS region sequences amplified using ITS1 (5'TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers. The PCR products of the ITS region in the nine different fungi were confirmed to be in range of approximately 600-900bp which is characteristic of internal transcribed spacer region. The results of BLAST analyses presented in Table 1 revealed the species of endophytes and were identified as *C. cladosporioides* with 99.14%, *F. chlamydosporium* with 99.80%, *F. keratoplasticum* with 98.46%, *F. solani* strain f2-f6 with 98.87%, *F. solani* strain ZB11263612 with 98.07%, *F. solani* strain F10-3 with 99.24%, *G. candidum* with 96.32%, *N. oryzae* with 100.00%, and *S. commune* with 99.66% identities.

Table 1. Identities of the cultured fungi using BLAST with NCBI Genbank Accession

Isolate no.	Species	E. Value	Identity (%)	Accession
FP01	*Cladosporiumcladosporioides*	0.00	99.14	KC880082.1
LF01	*Fusariumchlamydosporium*	0.00	99.80	KM278117.1
LF02	*Fusariumkeratoplasticum*	0.00	98.46	KY496176.1
LF03	*Fusariumsolani* strain f2-f6	0.00	98.87	KJ5730076.1
LF04	*Fusariumsolani* strain ZB11263612	0.00	98.07	KX783363.1
LF05	*Fusariumsolani* strain F10-3	0.00	99.24	KF999814.1
FP02	*Geotrichumcandidum*	2e-150	96.32	KX853078.1
FP03	*Nigrosporaoryzae*	0.00	100.00	KX986058.1
FP04	*Schizophyllum commune*	0.00	99.66	JQ409156.1

LF= Isolated from leaf, FP= Isolated from fruit peel

Assessment of enzyme producing efficiencies

Amylase activity

All of the endophytic fungi were able to produce amylase except for *G. candidum*. The endophytic fungi namely *F. chlamydosporium, F. keratoplasticum,* and the three different strains of *F. solani* as well as *S. commune* were able to produce amylase. The highest enzymatic index was from *F. solani* strain f2-f6 (Table 2; Figure 2).

Cellulase activity

A total of four isolated fungal endophytes were able to degrade cellulose these were one strain from *F. solani, G. candidum, N. oryzae* and *S. commune*. The species of fungi that had the largest enzymatic index was *N. oryzae* with an enzymatic index of 13.13 followed by *S. commune* that had 1.98 then *F.solani* with 1.44 and lastly *G. candidum* with 1.10. The production of cellulose was significantly different from other fungal endophytes (Table 3; Figure 3).

Laccase activity

Among the isolated ndophytic fungi, only *F. solani* strain f2-f6 showed positive for the production of extracellular laccase (Table 4; Figure 4).

Table 2. Amylase activity of different isolated fungal endophytes after seven days of incubation

	Amylase		
Fungal endophytes	**Diameter of clear zone (mm)**	**Colony diameter (mm)**	**Enzymatic index (EI)**
S. commune	85.00[a]	85.00	1.00[e]
F. chlamydosporium	85.00[a]	85.00	1.00[e]
F. keratoplasticum	85.00[a]	72.45	1.37[d]
F.solani strain f2-f6	85.00[a]	6.00	14.16[a]
F.solani strain ZB11263612	85.00 [a]	85.00	1.00[e]
F.solani strain F10-3	85.00[a]	85.00	1.00[e]
C. cladosporioides	50.48[b]	6.00	8.37[b]
N. oryzae	20.75[c]	6.00	6.85[c]
G. candidum	0.00[d]	6.00	0.00[f]

Values represent the mean measurements of the clear zones indicating amylase activity produced by the fungalendophytes from *M. paradisiaca.*Means with the same letter superscript are not significantly different at 5% level of significance using DMRT

***EI greater than 2.00 has high rate of enzyme production

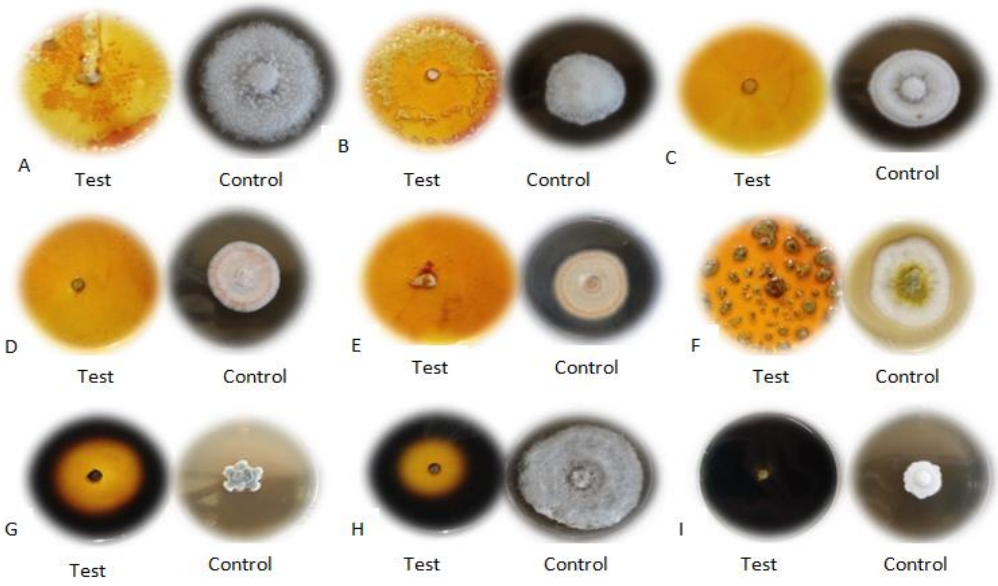

Figure 2. Clear zone produced by different fungal endophytes on amylase activity isolated from *M. paradisiaca;* (A) *S. commune*, (B) *F. chlamydosporium*, (C) *F. keratoplasticum*, (D) *F. solani* strain f2-f6, (E) *F. solani* strain ZB11263612, (F) *F. solani* strain F10-3, (G) *C. cladosporioides*, (H) *N. oryzae* and (I) *G. candidum*

Table 3. Cellulase activity of different isolated fungal endophytes after seven day of incubation

	Cellulase		
Fungal endophytes	**Diameter of clearzone (mm)**	**Colony diameter (mm)**	**Enzymatic Index (EI)**
N. oryzae	79.50[a]	6.00	13.13[a]
G. candidum	76.80[b]	72.20	1.10[d]
F.solani strain f2-f6	71.03[c]	51.05	1.44[c]
S. commune	35.65[d]	18.78	1.98[b]
C. cladosporioides	0.00[e]	15.93	0.00[e]
F. chlamydosporium	0.00[e]	85.00	0.00[e]
F. keratoplasticum	0.00[e]	6.00	0.00[e]
F.solani strain ZB11263612	0.00[e]	6.00	0.00[e]
F.solani strain F10-3	0.00[e]	85.00	0.00[e]

Values represent the mean measurements of the clear zones indicating cellulase activity produced by the fungal endophytes from *M. paradisiaca.* Means with the same letter superscript are not significantly different at 5% level of significance using DMRT
***EI greater than 2.00 has high rate of enzyme production

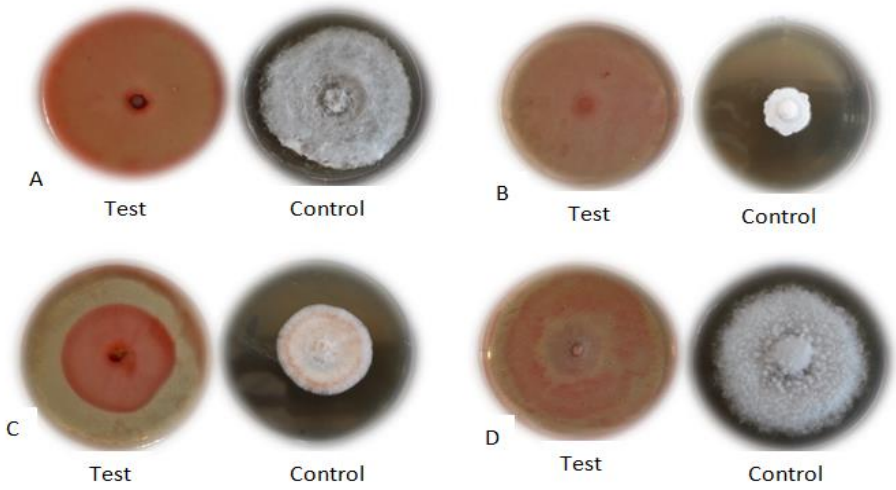

Figure 3. Clear zone produced by different fungal endophytes on cellulase activity isolated from *M. paradisiaca*; (A) *N. oryzae*, (B) *G. candidum*, (C) *F. solani* strain f2-f6 and (D) *S. commune*

Table 4. Laccase activity of different isolated fungal endophytes after seven days of incubation

	Laccase
C. cladosporioides	-
F.chlamydosporium	-
F. keratoplasticum	-
*F.solani*strain f2-f6	+
*F.solani*strain ZB11263612	-
F. solani strain F10-3	-
G. candidum	-
N. oryzae	-
S. commune	-

Note: + presence of color change, – absence of color change.

Figure 4. Color change produced by *F. solani* strain f2-f6

Discussion

A total of nine fungal endophytes were isolated from *M. paradisiaca* namely *C. cladosporioides, F. chlamydosporium, F. keratoplasticum* which may be a species complex of *F. solani,* three strains of *F. solani* which are f2-f6, ZB11263612 and F10-3, *G. candidum, N. oryzae* and *S. commune.*

Cultural and morphological characteristics of these fungi were observed after seven days of incubation. In line with Torres *et al*. (2017) the conidia of *C. cladosporioides* are numerous and bound of up to nine conidia. They are limoniform, ovoid, obovoid to subglobose, aseptate, brown, hilaconspicuous. Supported the observation of Yusuf *et al*. (2010) on the *F. chlamydosporum,* the mycelia were floccose, fairly dense, off white, in some interspersed with yellow pigmentation that formed the outermost ring at the periphery. On the reverse side, which was a dark brown with in the central portion and shades of reddish brown to a dull brown color as the mycelia radiates to the periphery. Then, consistent with Chehri *et al*. (2015) *F. keratoplasticum* has three septate macroconidia produced from sporodochia. The oval-shaped and curved cylindrical conidia were formed on conidiophores in hyphae and terminal and intercalary chlamydospores also are present. For *F. solani* strain f2-f6, the identified fungus causing rot to paprika plant is *Nectria haematoccoca* which is the teleomorph or asexual stage of *F. solani*. The macroconidia were falcate 3-septate and chlamydospores were also produced in single or grouped (Hyeong *et al.*, 2005). Moreover, in keeping with Balali and Iranpoor (2006) on their observation of other strain of *F. solani,* isolates were cream or white and a few are light violet. The macroconidia of the fungi were wider and a conspicuous wall which is sort of the same as macroconidia of *F. oxysporum*. Their apical cell was round, the basal cell was round or foot-shaped, consisting of three to four septa. Consistent with Hatai and Khoa (2005) on the observation of *F. solani* strain F10-3, the aerial conidia was generally produced on the aerial mycelia at three to four day of post-inoculation on SNA and that they were predominantly oval, from ellipsoid to subcylindric. On the other hand, Hafeez *et al*. (2015) described the conidia of *G. candidum* as single celled, arthrosporous, terminal, and aerial on agar surface, hyaline, sub-globose to cylindrical in shape. Furthermore, supported the observation of Abass and Mohammed (2014), *N. oryzae* grew rapidly and produced grayish colonies, initially, and so became brown to dark brown because of the abundance of sporulation after seven days of incubation. Lastly, as reported by Amee *et al.* (2009) the mycelial mat of *S. commune* was purely white in color and initiating near the inoculum and spreading throughout the surface of the medium. It showed a smooth and dull texture with curled or folded surface.

The isolated fungi are all endophytes which is analogous with the previous studies. *C. cladosporioides* was found to be endophytic supported by the study of Guest *et al.* (1998) in which they isolated it from the leaves of *M. acuminata*. Zacaria and Aziz (2018) stated that they isolated the endophytic fungi *F. chlamydosporum* further as *N. sphaerica*, *F. equiseti* and *C. siamense* on the leaves of *Musa* spp. *F. keratoplasticum* which is an endophyte in step with the results of Radiastuti *et al*. (2019). Studies of Paparu *et al.* (2004), Griesbach (2000) and Niere (2001), reported that fungal endophytes *G. candidum*, *Acremonium* sp., *F. concentrichum*, *F. oxysporum* and *F.solani* were isolated from the tissue culture of banana plants. These endophytes showed promising results to regulate the banana weevil from damaging the plant. On the other hand, the study of Photita *et al*. (2001), isolated *N. oryzae* from the young and old tissues of *M. acuminata* from five different sites of Doi Suthep Pui National park at Thailand. Lastly, supported the study of Asuncion *et al.* (2010), the endophyte *S. commune* was isolated from healthy leaves of banana.

Meanwhile, evaluations of the enzymatic abilities of these fungal endophytes were done through amylase, cellulase and laccase tests. All the endophytic fungi were able to produce amylase apart for *G. candidum*. In keeping with to the previous study of Savita *et al.* (2017), *G. candidum* did not successfully produce an amylase. Rensburg *et al*. (2001) stated that it is because of the low percentage of the substrate which caused the fungi to not perform amylolytic activity. The endophytic fungi namely *F. chlamydosporum, F. keratoplasticum,* and therefore the three different strains of *F. solani* further as *S. commune* were able to produce amylase. These fungi had the biggest clear zone with 85mm which were not significantly different from one another. Followed by *C. cladosporioides* and *N. oryzae* which were significantly different from one another. The best production of amylase was recorded from one strain of *F. solani* followed by *C. cladosporioides* and lastly *N. oryzae* which also exhibited higher enzymatic index of extracellular amylase activity. This is identical with the previous studies of Nwagu and Okolo (2011), Kannan *et al.* (2012) and Meenashree *et al.* (2018) that *F. solani, C. cladosporioides* and *N. oryzae* exhibited high production of amylase.

Four isolated fungal endophytes were able to degrade cellulose these were one strain from *F. solani, G. candidum, N. oryzae* and *S. commune*. The species of fungi that had the largest enzymatic index was *N. oryzae* with an enzymatic index of 13.13 followed by *S. commune* that had 1.98 then *F.solani* with 1.44 and lastly *G. candidum* with 1.10. Supported with the study of Meenashree *et al.* (2018), *N. oryzae* showed cellulose degradation. Moreover in the study of Sridhar *et al.* (2005) *Fusarium* spp. was also positive along with the other fungi tested. However, the other *Fusarium* strains failed to produce

cellulase enzyme. As well as the study of Sridhar *et al.* (2005) that seven fungi tested including *Fusarium* spp. was also positive for cellulase activity with pH 7 on the 6^{th} day. However, the other *Fusarium* strains did not produce cellulase enzyme. Consistent with Alconada *et al*. (2010) that their differences in sequential and differential production profile is expounded to the aggressiveness of an isolate. The mechanisms involved in enzymatic activity induction in diverse microorganisms are regulated by certain factors during which may vary from one organism to another. Then, the study conducted by Berrin *et al.* (2017) *G. candidum* may be a promising candidate to further enhance enzyme cocktails utilized in biorefineries like consolidated bioprocessing because of its ability to disrupt cellulose fibers and significantly improved the saccharification of pretreated lignocellulosic. Lastly, the results of Fang *et al.* (1997) showed that six fungi except *Neurospora crassa* are cellulolytic. Under the culture condition, enzyme activities were detected in *C. versicolor*, *F. veultipes*, *G. gibbasum*, *H. erinaceus*, *S. commune* CFCC7139 and *S. commune* are recognized as having high ability in degrading lignocellulosics.

Futhermore for the laccase activity, just one from the strains of *F. solani* showed extracellular laccase activity. According to research of Wu *et al.* (2010) the highest activity of *F. solani* was detected at pH 3.0 and at 70^{O}C. The enzyme retained 46.2–97.2% of its activity within the presence of 20 mM Pb^{2+}, Ni^{2+}, Cr^{3+}, and its activity was enhanced within the presence of 20 mM Hg^{2+}. However, in the previous study of Maria *et al.* (2005) stated that all endophytic fungi tested were not able to produce laccase. One of the reasons for this result is the endophytic nature of these fungi as well as the activeness of laccase which may damage the host plant.

Thus, fungal enzymes are important for they are utilized in food, beverages, confectionaries, textiles and leather industries to simplify the processing of raw materials. Enzymes from a fungal source are often more stable than enzymes derived from other sources. The enzymes produced by endophytes are degraders of the polysaccharides available within the host plants. With the utilization of simpler solid media, it permits the rapid screening of enormous populations of fungi in order to evaluate the presence or absence of specific enzymes that are of importance (Srinivas *et al.*, 2013).

References

Abass, M. H. and Mohammed, N. H. (2014). Morphological, molecular and pathological study on *Nigrospora oryzae* and *Nigrospora sphaerica*, the leaf spot fungi of date palm. Basra Journal of Date Palm Researches, 13:1-2.

Ahenkora, K. M., Kye, A., Marfo, K. and Banful, B. (1997). Nutritional composition of false horn Aponte pa plantain during ripening and processing. African Crop Science Journal, 5:243-248.

Alconada, T. M., Hours, R. A. and Kikot, G. E. (2010). Extracellular enzymes of *Fusarium graminearum* isolates. Brazilian Archives of Biology and Technology, 53:779-783.

Amee, P., Nagadesi, P. K., Susy, A. and Arya, A. (2009). Morphology, anatomy and cultural characters of two wood decaying fungi *Schizophyllum commune* and *Flavadon flavus*. Journal of Mycology and Plant Pathology, 39:27-31.

Assavanig, A., Amornkitticharoen, B., Ekpaisal, N., Meevootisom, V. and Flegel, T. W. (1992). Isolation, characterization and function of laccase from *Trichoderma*. Applied Microbiology and Biotechnology, 38:198-202.

Asuncion, M. M. C., Cavalcanti, M. H. C. Q. and Menezes, M. (2010). *Schizophyllum commune* isolated as endophytic leaf fungus de bananaeira (*Musa* spp.) in Pernambuco, Brazil. Agrotropic, 22:67-70.

Balali, G. R. and Iranpoor, M. (2006). Identification and genetic variation of *Fusarium* species in Isfahan, Iran, using pecticzymogram technique. Iranian Journal of Science and Technology, 30.

Barnett, H. L. and Barry, H. (1987). Illustrated genera of imperfect fungi 4th ed. New York: Mac Millan Publishing co.

Berrin, J. G., Simon, L., Haon, M., Villares, A., Cathala, B., Grisel, S., Gimbert, I. H. and Henrissat, B. (2017). The yeast *Geotrichum candidum* encodes functional lytic polysaccharide monooxygenases. Biotechnology for Biofuels, 10:215.

Berry, D. R. and Paterson, A. (1990). Enzymes in food industry. In Enzyme Chemistry, Impact and Applications, 2:306-351.

Chehri, K., Salleh, B. and Zakaria, L. (2015). Morphological and phylogenetic analysis of *Fusarium solani* species complex in Malaysia. Microbial Ecology, 69:457-471.

Ehiowemwenguan, G., Emoghene, A. O. and Inetianbor, J. E. (2014). Antibacterial and phytochemical analysis of banana fruit peel. Journal of Pharmacy, 4:18-25.

El-Gali, Z. I. (2016). Isolation and identification of fungi associated with fruits sold in local markets. International Journal of Research Studies in Biosciences, 4:61-64.

Fang, J., Qu, Y. and Gao, P. (1997). Wide distribution of cellobiose-oxidizing enzymes in wood-rot fungus indicates a physiological importance in lignocellulosics degradation. Biotechnology Techniques, 11:195-197.

Galhaup, C., Goller, S., Peterbauer, C. K., Strauss, J. and Haltrich, D. (2002). Characterization of the major laccaseiso enzyme from *Trametes pubescens* and regulation of its synthesis by metal ions. Microbiology, 148:2159-2169.

Geethanjali, P. A. and Reshma, K. (2014). Potentiality of soil fungi to produce protease through solid substrate fermentation technique. International Journal of Emerging Engineering Research and Technology, 2:327-332.

Griesbach, M. (2000). Occurrence of mutualistic fungal endophytes in bananas (*Musa* spp.) and their potential as biocontrol agents of banana weevil *Cosmopolites sordidus* (Germany) in Uganda (Ph.D Thesis). University of Bonn, Bonn, Germany.

Guest, D. I., Brown, K. B. and Hyde, K. D. (1998). Preliminary studies on endophytic fungal communities of *Musa acuminata* species complex in Hong Kong and Australia. Fungal Diversity, 1:27-51.

Hafeez, R., Akhtar, N., Shoaib, A., Bashir, U., Haider, M. S. and Awan, Z. A. (2015). First report of *Geotrichum candidum* from Pakistan causing postharvest sour rot in loquat (*Eriobotrya japonica*). The Journal of Animal & Plant Sciences, 25:1737-1740.

Hatai, K. and Khoa, L. V. (2005). First case of *Fusarium oxysporum* infection in cultured kuruma prawn *Penaeus japonicus* in Japan. Fish Pathology, 40:195-196.

Herculano, P. N., Lima, D. M. M., Fernandes, M. J. S., Neves, R. P., Souza-Motta, C. M. and Porta, A. L. F. (2011). Isolation of cellulolytic fungi from waste of castor (*Ricinus communis* L.). Current Microbiology, 62:1416-1422.

Hyeong, J. J., Kyung, Y. R, Chang, K. S. and Ki, W. N. (2005). Occurrence of stem and fruit rot of paprika caused by *Nectria haematococca*. Plant Pathology, 21:317-321.

Kannan, K. P., Amirita, A., Sindhu, P., Swetha, J. and Vasanthi, N. S. (2012). Enumeration of endophytic fungi from medicinal plants and screeening of extracellular enzymes. World Journal of Science and Technology, 2:13-19.

Majid, A., Zahran, Z., Hafis, A., Rahim, A., Ismail, N., Rahman, W., Zubairi, K., Dieng, H. and Satho, T. (2015). Morphological and molecular characterization of fungus isolated from tropical bedbugs in Northern Peninsular Malaysia, *Cimex hemipterus* (Hemiptera: cimicidae). Asian Pacific Journal of Tropical Biomedicine, 5:707-713.

Maria, G. L., Sridhar, K. R. and Raviraja, N. S. (2005). Antimicrobial and enzyme activity of mangrove endophytic fungi of southwest coast of India. Journal of Agricultural Technology, 1:67-80.

Meenashree, B., Rajagopal, K., Binika, D., Joshila, D., Tulsi, P. S., Arulmathi, R., Kathiravan, G. and Tuwar, A. (2018). Mycodiversity and biotechnological potential of endophytic fungi isolated from hydrophytes. Current Research in Environmental and Applied Mycology, 8:172-182.

Mittal, P. (2018). List of fungi benefits. Retrieved from Sciencing. https://sciencing.com/listfungi-benefits-8606974.html.

Niere, B. (2001). Significance of non-pathogenic isolates of *Fusarium oxysporum* Schlecht: Fries for the biological control of the burrowing nematode *Radopholus similis* (Cobb) Thorne on tissue cultured banana (Ph.D Thesis). University of Bonn, Bonn, Germany.

Nwagu, T. N. and Okolo, B. N. (2011). Extracellular amylase production of a thermotolerant *Fusarium* sp. isolated from Eastern Nigerian soil. Brazilian Archives of Biology and Technology, 54:649-658.

Paparu, P., Dubois, T., Gold, C. S., Adipala, E., Niere, B. and Coyne, D. (2004). Inoculation, colonization and distribution of fungal endophytes in *Musa* tissue culture plants. Uganda. Journal of Agricultural Sciences, 9:583-589.

Patel, C., Yadav, S., Rahi, S. and Dave, A. (2013). Studies on biodiversity of fungal endophytes of indigenous monocotaceous and dicotaceous plants and evaluation of their enzymatic potentialities. International Journal of Scientific and Research Publications, 3:1-5.

Photita, W., Lumyong, S., Lumyong, P. and Hyde, K. D. (2001). Endophytic fungi of wild banana (*Musa acuminata)* at Doi Suthep Pui National Park, Thailand. The British Mycological Society, 12:1508-1513.

Radiastuti, N., Bahalwan, H. and Susilowati, D. (2019). Phylogenetic study of endophytic fungi associated with *Centella asiatica* from Bengkulu and Malaysia accessions based on the ITS rDNA sequence. Biodiversitas, 20:1248-1258.

Rensburg, P. V., Strauss, M. L. A., Jolly, N. P. and Lambrechts, M. G. (2001). Screening for the production of extracellular hydrolytic enzymes by non- *Saccharomyces* wine yeasts. Journal of Applied Microbiology, 91:182-190.

Salawu, L., Bolarinwa, R. A., Adegunloye, A. B. and Moraine, H. A. (2010). HBs Ag, anti-HCV, anti-HIV and VDRL in blood donors: prevalence and trends in the last three and a half years in a tertiary health care facility in Ile-Ife, Nigeria. International Journal of Medical Science, 2:335-341.

Savita, P. D., Suvarna, V. C., Annu, T., Balakrishna, A. N., Kanchanashri, B. and Yallappa, M. (2017). Characterization and identification of phytate solubilizing yeasts isolated from food grains. International Journal of Current Microbiology and Applied Sciences, 6:1184-1192.

Sharma, S. and Sumbali, G. (2014). Isolation and screening of cellulolytic fungal species associated with lower denomination currency notes, circulating in Jammu City (India). International Journal of Recent Scientific Research, 5:596-600.

Sridhar, K. R., Maria, G. L. and Raviraja, N. S. (2005). Antimicrobial and enzyme activity of mangrove endophytic fungi of southwest coast of India. Journal of Agricultural Technology, 1-14.

Srinivas, C., Sunitha, V. H. and Nirmala, D. (2013). Extracellular enzymatic activity of endophytic fungal strains isolated from medicinal plants. World Journal of Agricultural Sciences, 9:01-09.

Torres, D. E., Rojas-Martinez, R. I., Mejia, E. Z., Fefer, P. G., Guzman, G. J. M. and Martinez, C. P. (2017). *Cladosporium cladosporioides* and *Cladosporium pseudocladosporioides* as potential new fungal antagonists of *Puccinia horiana* Henn., the causal agent of chrysanthemum white rust. PLoS ONE, 12: e0170782.

Waiter, M. J., Morgan, N. L., Rockey, J. S. and Higton, G. (2007). Microbial enzymes. In.industrial microbiology (1st edition.). Blackwell publishing, New Delhi, pp. 113-114.

Windish, W. W. and Mhatre, N. S. (1965). Microbial amylases. Advances in Applied Microbiology, 7:273-304.

Wu, Y. R., Luo, Z. H., Chow, R. K. K. and Vrijmoed, L. L. P. (2010). Purification and characterization of an extracellular laccase from the anthracene-degrading fungus *Fusarium solani* MAS2. Bioresource Technology, 101:9772-9777.

Yousaf, M. M. and Sajjad, S. (2015). Application of thermally and chemically modified banana peels waste as adsorbents for the removal of iron from aqueous system. Journal of Environmental Analytical Chemistry, 2:1-12.

Yusuf, U. K., Siddiquee, S. and Zainudin, N. A. I. M. (2010). Morphological and molecular detection of *Fusarium chlamydosporum* from root endophytes of *Dendrobium crumenatu.* African Journal of Biotechnology, 9:4081-4090.

Zacaria, L. and Aziz, W. N. (2018). Molecular identification of endophytic fungi from banana leaves (*Musa* spp.) Tropical Life Sciences Research, 29:201-211.

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