AMYLOLYTIC POTENTIALS OF MOULDS ISOLATED FROM SOIL SAMPLES

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ABSTRACT

Enzymes are biological catalysts that play vital roles in bakery, brewing, alcoholic, cheese, textile, detergent, foods, paper and leather industries. A total of eight (8) soil samples were collected from sugar cane and cassava plantation. Moulds were isolated from the samples using spread plating technique. The isolates were identified on the basis of their cultural and microscopic characteristics. The amylolytic activities of the isolates were determined using direct inoculation technique. Twenty two (22) moulds were identified and they belong to *Aspergillus*, *Mucor*, *Rhizopus* and *Penicillium* genera. They showed varied degrees of amylolytic activities on starch agar medium. The *Mucor*, *Rhizopus* and *Penicillium* species did not show any visible amylolytic potential. Only *Aspergillus tubingensis*-SSC$_3$, *Aspergillus tubingensis*-SSB$_4$, *Aspergillus tubingensis*-SSE$_3$, *Aspergillus tubingensis*-SSB$_1$ and *Aspergillus tubingensis*-SCA$_2$ showed visible amylolytic activities. The amylolytic mould strains isolated from the soil samples could be source of amylases to solve the problem of enzyme needs of the local industries.
Keywords: Amylase; enzymes; moulds; soil samples; starch

INTRODUCTION

Enzymes are proteinous, biological catalysts that speed up the rate of chemical reactions. Enzymes are specific in action and their roles have been acknowledged for quite a whole. Since ages, enzymes are known to play vital roles in various production processes such bakery, brewing, and cheese production. Nowadays as a result of industrial revolution, enzymes are used in large quantity in textile, detergent, foods, paper and leather industries (Mukunda, Onkarappa, & Prashith, 2012).

Enzymes are obtained from plants, animals, bacteria, moulds and yeasts. Over the years enzymes were predominantly obtained from plants and animals, of recent attention has been shifted to microorganisms for biologically active enzymes for various industrial purposes. Microbial enzymes are easily obtained and cost effective, due to the fast growing nature of microorganisms under laboratory controlled environment. Different types of hydrolytic enzymes are used in industries for various purposes; these enzymes include lipases, proteases, cellulases, pectinases, amylases etc. Amylases hydrolysis starch, and there are two types of amylase; α-amylases (endoamylases) and β-amylases (exoamylases). The α-amylase randomly hydrolysis starch α-1, 4-glycosidic bonds to yield amylose and amylopectin molecules. The α-amylases are two categories; saccharifying amylase and liquefying amylase. The saccharifying amylase hydrolyses about 50 – 60% of starch α-1, 4-glycosidic bonds while the liquefying α-amylase breaks down 30 – 40% of starch α-1, 4-glycosidic linkages. The β-amylases solely breaks down α-1, 4-glycosidic linkages or both α-1, 4-glycosidic and β-1, 6-glycosidic linkages.to produce glucose or maltose (Tiwari *et al*., 2015). Importance of amylases in food, brewing, textile, and detergent industries has been well appreciated (Adeniran & Abiose, 2009). They are utilized for starch liquefaction (Adeniran & Abiose, 2009), improve

cleaning effect of detergent and removal of excess starch in textile industry (Aiyer, 2005).

In Nigeria high foreign exchange is expended annually to import enzymes of industrial and other purposes. Majority of these enzymes are of microbial origin and there is still high demand for more enzymes. Moulds as ubiquitous microorganisms, are abundant in soil; these organisms are well appreciated for their abilities to secrete a variety of novel enzymes of industrial importance. Apart from food materials, enzyme producing strains of moulds also abound in soil. Different species of mould reside in the soil, especially near the surface where aerobic conditions are available. Moulds strains from agricultural waste and soil has been reported by Adeniran and Abiose (2009) and Saranraj and Stella (2013), for their enzymes production potentials. The cost of enzymes importation by indigenous industries has skyrocketed due to increasing rates of foreign exchange, therefore, it is necessary that local production of these enzymes be considered This study is aimed at evaluating amylolytic abilities of moulds isolated from soil samples obtained from cassava and sugar cane plantations.

MATERIALS AND METHODS

Collection of Samples

A total of eight (8) soil samples were collected from different location within Federal Polytechnic, Ilaro and Papa-Lantoro; all in Ogun State. Three soil samples were collected from cassava plantation farms within the Federal Polytechnic, Ilaro while the others from sugarcane farms at Papa-Lantoro. The samples were collected from 5 to 10 inches depth using sterile trowels and polyethylene bags. The samples were transported to Microbiology Laboratory, Department of Science Laboratory Technology, Federal Polytechnic, Ilaro for immediate analyses.

Isolation of Moulds

Moulds associated with soil samples were isolated using spread plate technique ((Mukunda *et al*., 2012). A ten-fold serial dilution of the samples was carried out and the diluent/diluted suspension (0.2 ml) was plated on potato dextrose agar (PDA) augmented with streptomycin. The inoculum was spread on the entire surface of the plate using sterile spreading rod. The plates were incubated using thermostatically controlled incubator at 27±2^{o}C for 72 hours. The isolates were sub-cultured on same medium (PDA) to obtain pure cultures using spot plate technique (Sana, Anjum, Nawaz, Ahmad, & Rabani, 2017). The pure isolates obtained were sustained on PDA agar slants and maintained at refrigerated temperature (4^{o}C) until further analysis.

Identification of Mould Isolates

The pure isolates were identified using conventional method (morphological method). The Identification of the isolates was based of their cultural features and microscopic characteristics. The cultural features (colony texture, shape, margin and diffusible pigment with colour) were observed macroscopically. The microscopic characteristics (shape of hypha, presence of rhizoid, presence of sporangium, presence and absence of cross-walls, and type of spores) were observed under compound microscope using lactophenol cotton-blue stain technique. Two drops of the dye were introduced into clean glass slide and little aerial mycelia from the reproductive culture was picked and place into the dye. The mycelia were spread within the dye using sterile inoculating needles. Cover slip was lowered into the stained mycelia, the slide was observed under compound microscope

using X10 and X40 objectives (Ogbonna, Okpokwu, Okafor, & Onyia, 2014). Mycological identification keys, taxonomic identification description and fungal atlas were used to identify the isolates (Adeniran & Abiose, 2009).

Determination of Amylolytic Activities of the Isolates

Screening for amylolytic activity of the isolated moulds was carried out using modified starch agar medium of Balkan and Ertan (2005). The medium was supplemented with chloramphenicol. Mould disc (7mm) from previous plate was centrally inoculated on the starch agar plate. The plates were incubated at 27±2^{o}C for 4 days. Subsequent flooding of the plates with Iodine solution was carried out and halo zone examination round the colony was checked. The halo zone depicts starchy hydrolysis and amylolytic activity (Mukunda *et al*., 2012.). Those moulds that showed hydrolytic activity were noted and kept for further studies.

RESULTS AND DISCUSSION

Results

The moulds isolated were identified using conventional method (morphological method. The cultural and microscopic characteristics of the isolates are shown in Table 1. Only four (4) moulds genera; *Aspergillus, Mucor, Rhizopus* and *Penicillium* were isolated from the soil samples. *Penicillium glabrum*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus turbingensis*, *Rhizopus* species and *Mucor* species were the moulds isolated.

Table 2 shows the various moulds associated with soil samples collected from the different sampling sites. Aspergilli were the dominant the organisms recorded in all the samples. *Aspergillus* species were the only organisms isolated in samples SSC, SSD and SCB. *Mucor* species were isolated from samples SSA, SSB, SCC and SSE. *Penicillium glabrum* and *Rhizopus* species were isolated from samples SSA and SCB respectively.

The amylolytic activities of the isolates are as shown in Table 3. They showed varied response to hydrolysis of soluble starch in the starch agar medium. A total of twenty two (22) moulds were isolated, amylolytic activities of nineteen (19) isolates could only be determined. *Aspergillus tubingensis*-$SSC_{3,}$ *Aspergillus tubingensis*-$SSB_{4,}$ *Aspergillus tubingensis*-$SSE_{3,}$ *Aspergillus tubingensis*-SSB_1 and *Aspergillus tubingensis*-SCA_2 strains showed amylolytic potential. *Aspergillus tubingensis*-SSC_3, Most of the amylolytic strains, *Aspergillus tubingensis*-SSB_4, *Aspergillus tubingensis*-$SSE_{3,}$ and *Aspergillus tubingensis*-SSB_1 were isolated from sugar cane plantation soil samples.

Table 1: Identification of the Isolated Moulds

S/N	Cultural Features	Microscopic Characteristics	Isolate Identity
1	Dark brown with white margin (colour). Wrinkle light yellow (reverse colour)	They are large, globose, dark-brown conidial heads, which become radiate, tending to split into several loose colonies with age. Smooth-wall conidiophores, turning dark towards the vesicle. Biseriate conidia heads with the phialides, and septate and branched hyphae.	*Aspergillus niger*
2	Brown in colour but colorless in reverse, finely wrinkled texture	Smooth, long and narrow conidiophores, radiated vesicle head, biseriated phialides or conidia heads, globular spores.	*Aspergillus tubingensis*
3	Green-brown colour. But white colour in reverse	Uniseriated conidial heads, short and smooth conidiophore wall, conical shaped terminal vesicle, Conidia born on phiallides on the upper two-thirds of the vesicle.	*Aspergillus fumigatus*
4	Fast-growing colonies that look like white-to-green cotton candy, darkening with time. Reverse is light-coloured to white	Presence of long and non-septate branched sporangiophores, globular/round black sporangia, lacks rhizoid and stolon.	*Mucor* species
5	Fast-growing colony, whitish mycelia that is cotton wool-like in appearance and colour, fluffy growth, grey/brown reverse.	Aseptate hyphae with uptight aseptate sporangiophores linked by stolon, rhizoids beneath, unbranched sporangiophores, dark pear-shaped sporangium on hemispherical columella bears white to dark sporangiospores	*Rhizopus* species
6	Greenish white colony and slow growing organism	Septate hypha that showed much branches, the conidiospores arrangement show a brush-like structure.	*Penicillium glabrum*

Table 2: Moulds Associated with the Soil Samples

Sample Code	Sampling Site	Associated Moulds
SSA	Sugar cane farm A	*Aspergillus niger*-SSA_1 *Penicillium glabrum*-SSA_2 *Mucor* species-SSA_3
SSB	Sugar cane farm B	*Aspergillus tubingensis*-SSB_1 *Mucor* species-SSB_2 *Aspergillus niger*-SSB_3 *Aspergillus tubingensis*-SSB_4
SCA	Cassava farm A	*Mucor* species-SCA_1 *Aspergillus tubingensis*-SCA_2 *Aspergillus niger*-SCA_3 *Aspergillus tubingensis*-SCA_4
SCB	Cassava farm B	*Aspergillus fumigatus*-SCB_3 *Rhizopus* species SCB_2
SCC	Cassava farm C	*Mucor* species-SCC_1 *Aspergillus tubingensis*-SCC_3 *Aspergillus niger*-SCC_4
SSC	Sugar cane farm C	*Aspergillus niger*-SSC_2 *Aspergillus tubingensis*-SSC_3 *Aspergillus tubigensis*-SSC_4
SSD	Sugar cane farm D	*Aspergillus niger*-SSD_6
SSE	Sugar cane farm E	*Mucor* species-SSE_1 *Aspergillus tubingensis*-SSE_3

Key: SS= Sugar cane farm sample, SC= Cassava farm sample

Table 3: Determination of Amylolytic Activities of the Isolates

Isolates	Iodine Test	Amylolytic Potentials
Aspergillus tubingensis-SSC$_3$	+	Amylolytic
Aspergillus niger-SSC$_2$	-	Non-amylolytic
Aspergillus tubingensis-SSB$_4$	+	Amylolytic
Aspergillus tubingensis-SSE$_3$	+	Amylolytic
Aspergillus niger-SSD$_6$	-	Non-amylolytic
Mucor species-*SCC*$_2$	-	Non-amylolytic
Aspergillus tubingensis-SSB$_1$	+	Amylolytic
Aspergillus tubingensis-SCA$_2$	+	Amylolytic
Aspergillus niger-SSB$_3$	-	Non-amylolytic
Mucor species-SSA$_3$	-	Non-amylolytic
Aspergillus tubigensis-SSC$_4$	-	Non-amylolytic
Mucor species-*SCC*$_1$	-	Non-amylolytic
Aspergillus niger-SCC$_4$	-	Non-amylolytic
Aspergillus tubingensis-SCC$_3$	-	Non-amylolytic
Mucor species-*SSE*$_1$	-	Non-amylolytic

Key: + = Iodine test positive - = Iodine test negative

DISCUSSION

Moulds are ubiquitous in nature; their biodiversity presents potential for exploitation of commercially-viable metabolites including enzymes (Strobel, Daisy, Castillo, & Harper, 2004). Moulds are associated with spoilage of so many food materials, owing to their ability to produce extracellular enzymes (amylases) production abilities. The moulds genera; *Aspergillus*, *Mucor*, *Rhizopus* and *Penicillium* isolated from the soil samples in this study, conformed with the earlier report of Adeniran and Abiose (2009). This finding corroborates the result of Raja, Praveen and William (2017) on soil samples from Loyola Campus, Chennai, India. More so, the presence of *Aspergillus tubingensis* recorded in the soil samples has earlier been reported by Chenghua, Yanhong, Buofang, and Haibin (2008). The presence of *Aspergillus niger* in soil samples was in concurrence with the findings of Saranraj and Stella (2013). The study also coincided with the findings of Mukunda *et al*. (2012) that isolated moulds from soil samples from Western Ghats of Agumbe and Koppa, Karnatakra, India. Similar result was also reported by Ogbonna *et al*. (2014), which isolated moulds from garri processing sites. The presence of these organisms might be

due to availability of substrate (s) required for their growth and proliferation. Moulds are also known to be present everywhere due to their nutritional versatility. Microorganisms are generally known to be prevalent in soil due to presence of good amount of substrates for their utilization.

Four (4) strains of *Aspergillus tubingensis* showed good amylolytic activities with varying degrees. Their ability to hydrolyse the soluble starch in the starch agar medium could be due to secretion of extracellular amylase. *Aspergillus* species have been reported for their amylase producing potentials (Saranraj and Stella, 2013). This finding was in agreement with work of Ogbonna *et al*. (2014) that isolated amylase producing *Aspergillus niger* from garri processing soil samples. The isolation of amylolytic mould strains from the soil samples concurred with similar study carried out by Omemu, Akpan, Bankole, and Teniola (2004). The presence of amylolytic producing strains of *Aspergillus tubingensis* could be due to substrate available in the soil sample, since the soil samples were collected from sugar cane plantation. The organisms must have adapted to the substrate available in their environment. Production of halo-zone around *Aspergillus tubingensis* strains colonies on starch agar is an indication of extracellular amylases secretion by the organisms. Amylases are enzymes that hydrolysis soluble sugar into simple sugars. The α-amylase randomly hydrolysis starch α-1, 4-glycosidic bonds to yield amylose and amylopectin molecules.

CONCLUSION

Moulds as ubiquitous organisms, four (4) genera: *Aspergillus, Penicillium*, *Rhizopus* and *Mucor* were isolated from the soil samples. Four strains of *Aspergillus tubingensis* showed promising potential for amylase production, therefore the strains can be manipulated and optimized for industrial production of amylase. This will help in reducing foreign exchange expended yearly on amylases importation in the country.

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