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SEM of *Theobroma cacao*

Isolation and Screening of Amylase-producing Fungi from Spoiled Fruits

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ABSTRACT

Amylase is the enzyme that is employed in the breakdown of starch into its simpler components. The research was aimed at isolating and screening *Aspergillus niger* from spoiled fruits for amylase enzyme production. The spoiled fruits utilized in this study were orange, tomato, banana, mango, hot pepper and pineapple, and were taken from fruit sellers in Kasuwar Mata within the Gombe metropolis. Six different fungi were isolated from spoiled fruits and were identified using cultural and morphological characteristics. They were screened on starch agar for amylase production. *A. niger* isolated from banana produced the biggest zone of hydrolysis (76 mm) on starch agar while *A. niger* from tomato produced the smallest zone of hydrolysis (38 mm). The result of this research indicates that *A. niger* from spoiled fruits are capable of producing amylase enzyme which can be utilized for different industrial processes.

INTRODUCTION

Fungi as *Aspergillus niger* degrade external polysaccharide of substances by secreting hydrolytic enzymes that breakdown polysaccharides into smaller molecules that can then be assimilated. Amylase and other organic acids are produced by *A. niger*, also Proteases, Lipases and other industrial enzymes can be produced by these fungi. *A. niger* is very important because these products are basic material in food transformation [1-2]. *A. niger* is considered as a fermentation organism that is needed in various industrial process because of their products, especially amylase that is widely used in baking, brewing, confectionary, sugar, paper coating, alcohol and syrup.

Aspergillus niger is the fungi that is commonly called the black mold. They are widely distributed and everywhere seems to have the spores of these organisms. The soil contains the spores of the *Aspergillus,* and these organisms have the capacity of utilizing different types of substrates for food because of the large number of enzymes they are capable of producing, and because of their great enzymatic activities. *Aspergillus* are used in several industrial processes for the commercial production of amylase enzyme [2]. The use of starch agar and iodine for detecting amylase (hydrolytic enzyme) producing microorganisms have been reported by Kalpana *et al*. [1] that starch hydrolysis can be detected on plates as a clear zone surrounding a colony.

MATERIALS AND METHODS

Collection of Samples

Spoiled fruits were collected from Kasuwar Mata within Gombe metropolis. They were collected in a clean polyethene bag.

Isolation of Fungi from Spoiled Fruits

Fungi was isolated from spoiled tomato, orange, mango, banana, pineapples and pepper by swapping their juices onto potato dextrose agar containing antibiotic and were then incubated for five (5) days at 28 °C.

Identification of the Isolates

The fungi were identified using cultural, morphological and lacto phenol cotton blue. The black colonies with yellow edges

produced by the organisms were observed on the plates and were then purified by repeated sub-culturing on separate PDA plates.

Screening of the Isolates for Amylase Production

The ability of the *A. niger* isolates to produce amylase enzyme was determined by the qualitative iodine clearing zone assay. It was done by inoculating *A. niger* on starch agar and incubating it for two days at 28 °C. The starch agar was then flooded with legol iodine. The production of amylase was viewed by appearance of clear zone surrounding the organisms [1].

RESULTS

Isolation and Identification of *Aspergillus niger* from Spoiled Fruits

Table 1 shows the result of isolation and identification of different species of *Aspergillus niger* from spoiled fruits. Six different species of fungi were isolated from different spoiled fruits and were confirmed as *Aspergillus niger* using cultural and morphological characteristics.

Table 1. Cultural and Morphological Characteristics of Fungi isolated from spoiled fruits.

S/N	Sources	Cultural features		Inference
1	Orange	Black and with yellow edges	Hyphae are septate, conidiospore terminate and a swollen vesicle	*A. niger*
2	Tomato	Black and with yellow edges	Hyphae are septate, conidiospore terminate and a swollen vesicle	*A. niger*
3	banana	Black and with yellow edges	Hyphae are septate, conidiospore terminate and a swollen vesicle	*A. niger*
4	Mangi	Black and with yellow edges	Hyphae are septate, conidiospore terminate and a swollen vesicle	*A. niger*
5	Hot pepper	Black and with yellow edges	Hyphae are septate, conidiospore terminate and a swollen vesicle	*A. niger*
6	pineapple	Black and with yellow edges	Hyphae are septate, conidiospore terminate and a swollen vesicle	*A. niger*

Screening of Isolates for Amylase Production on Starch Agar

Table 2 shows the result of screening of *A. niger* for amylase production on starch agar. The result of the screening indicated that *A. niger* isolated spoiled banana had the biggest zone of hydrolysis (76 mm), while *A. niger* Isolated from tomato had the smallest (38 mm). The clearance zone for all samples is shown in **Figs. 2** to **6.**

Table 2. Screening of different Isolates of *A. niger* for amylase production on starch agar.

S/N	Isolates	Sources	Clearance zone (mm)
1	*A. niger*	Orange	63
2	*A. niger*	Tomato	38
3	*A. niger*	banana	76
4	*A. niger*	Mangi	51
5	*A. niger*	Hot pepper	62
6	*A. niger*	pineapple	63

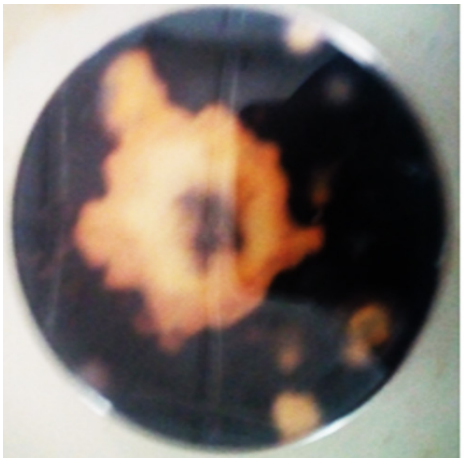

Fig. 1. Zone of clearance produced by *A. niger* isolated from mango.

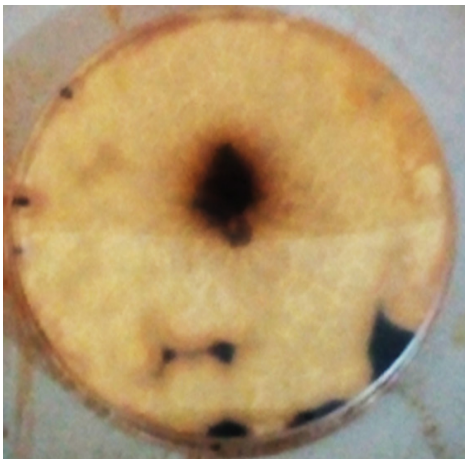

Fig. 2. Zone of clearance produced by *A. niger* isolated from banana.

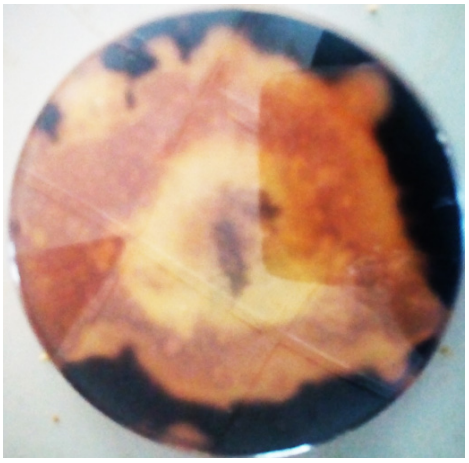

Fig. 3. zone of clearance produced by *A. niger* isolated from orange.

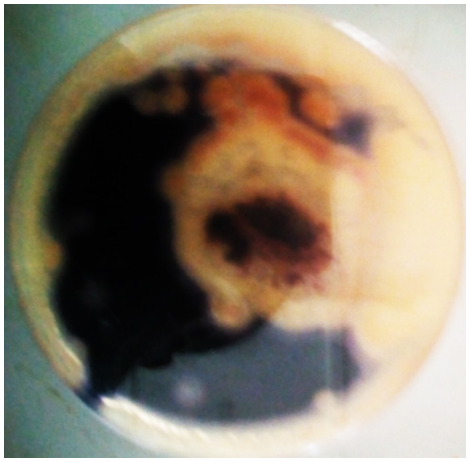

Fig. 4. Zone of clearance produced by *A. niger* isolated from pineapple.

Fig 5. Zone of clearance produced by *A. niger* isolated from hot pepper.

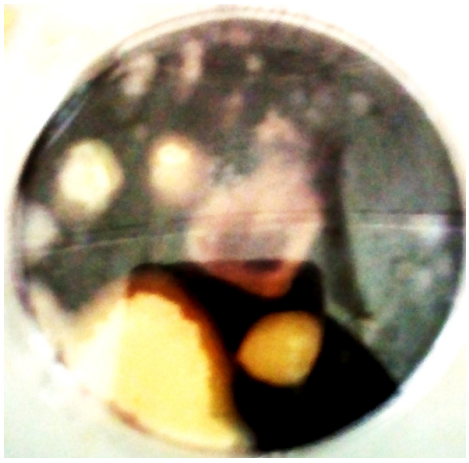

Fig. 6. Zone of clearance produced by *A. niger* isolated from tomato.

DISCUSSION

Aspergillus niger was isolated from spoiled fruits such as spoiled mango, pineapple, oranges and banana because fruits have high content of sugar and nutrient elements and their low pH make them desirable for fungal growth [3]. The isolation of *A. niger* from fruits such as tomato has indicated that fruits such as tomatoes are good source of *A. niger* with the ability of producing cell wall degrading enzymes such as amylase that can breaks down large polysaccharide into simple sugar which are used for growth and multiplication [4]. It was reported A. niger can be isolated from fruits especially tomato [5] and also Aspergillus sp. as fungi associated with spoilage of tomato fruits [6]. Sujeeta et al. [7] reported the isolation of fungi from different sources such as fruits and vegetables.

All the six isolates screened were found to be positive for amylase production. The biggest zone of hydrolysis (76 mm) was produced by A. niger isolated from banana. The biggest zone of hydrolysis produced by *A. niger* isolated from banana has shown that it has ability to produce amylase enzyme in substantial quantity on the starch agar and thereby having higher potential for the production of amylase while, *Aspergillus niger isolated* from tomato has the least potential for the production of amylase enzyme since it produced smaller amount of the enzyme on the starch agar [8].

The use of starch agar and iodine for detecting amylase production in amylase producing microorganisms have been reported that starch hydrolysis can be detected on plates as a clear zone surrounding a colony [9-12]. This is in line with the result obtained during screening of the different species of *A. niger* isolated from different fruits for amylase production.

CONCLUSION

Six different species of *A. niger* were isolated from different spo iled fruits and were identified using cultural and morphological characteristics. *A. niger* isolated from banana and *A. niger* from tomato were observed to have the biggest and smallest zones of hydrolysis of 76 and 38 mm on starch agar respectively. This has shown that *A. niger* from fruits are good candidates for amylase enzyme production. It is recommended that further research should be conducted to identify the isolates to their specie level. Crude amylase should be produced using cheap substances such as agricultural waste.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest between them.

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