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Activity of β-Amylase in Some Fungi Strains Isolated from Forest Soil in South-Western Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author MME designed the study, author OAO performed the statistical analysis, wrote the protocol and the first draft of the manuscript, managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The aim of this study was to isolate fungi species from Omo natural forest soil in Ogun State of Nigeria and study the amylases from the fungi species which are digestive enzymes that hydrolyze glycosidic bonds in starch to glucose, maltose, maltotriose and dextrin; and in particular determine the activities of β-amylase from the forest soil.
Study Design: Nine different species of fungi were isolated from the Omo natural forest reserve soil *(Gonatobotrrys simplex*, *Aspergillus niger, Spiromyces minutus, Aspergillus flavus, Articulospora inflata, Botrytis cenera, Penicillium italicum, Aspergillus fumigatus* and *Aspergillus flavus).* Four species of the fungi (*Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus* and *Penicillum italicum*) exhibited amylolytic activities maximally were obtained and screened for the production of beta-amylase (1,4-α-D-glucan maltohydrolase) for five days in liquid medium using 2% starch as carbon source. All the strains of fungi produced β-amylase optimally within the first 24 hours with progressive decreased production as the days gone by.
Place and Duration of Study: Department of Biochemistry, Federal University of Technology, Akure, Ondo State, Nigeria, between February 2010 and March 2011.
Methodology: We isolated many fungi species from forest reserve soil, four species

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(*Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigantus* and *Penicillum itallicum*) were identified and assayed for β-amylase activity.
Results: All the organisms produced β-amylase activity favorably at 40ºC; all were observed to be thermally stable at between 30ºC and 40ºC with optimal pH in alkaline medium between pH 7.00 and 9.00.
Conclusion: The results obtained in this study however showed that all the fungi strains are promising sources of β-amylase for potential applications in food and pharmaceutical industries and for biotechnological and industrial applications.

Keywords: Forest-soil; fungi-strains; beta-amylase; optimum-activity; industry-application.

1. INTRODUCTION

The living organisms in the soil in terms of animals and plants are responsible for humus formation which is the product of degradation and synthesis in the soil. These organisms engineer myriad of biochemical changes as decay takes place [1], they also physically churn the soil and help stabilize soil structure. A vast number of organisms live in the soil [2]; so great are micro floral numbers that they dominate the biomass in spite of the minute size of each organism [3]. The influence of fungi is by no means entirely understood, but it is known that they play a very important part in the transformation of the soil constituents [4,5,6]. Over 690 species have been identified, representing 170 genera.

In the ability to decompose organic residues, fungi are the most versatile and perhaps the most persistent of any other group. Cellulose, starch, gums, lignin, as well as the most easily affected proteins and sugars, readily succumb to their attack [4]. In affecting the processes of humus formation and aggregate stabilization, molds are more important than bacteria. They are especially active in acid forest soils but play a more significant role than is generally recognized in all soils. Moreover, fungi function more economically than bacteria in that they transform into their tissues a larger proportion of the carbon and nitrogen of the compounds attacked and give off as by-products less carbon dioxide and ammonium. Nevertheless, soil fertility depends in no small degree on molds since they keep the decomposition process going after bacteria and actinomycetes have essentially ceased to function [4].

Enzymes responsible for the breakdown of starch are widely distributed in nature. Among these are the amylases, which act on starch, glycogen and derived polysaccharides to hydrolyze the α - 1, 4- glycosidic linkages. The amylase may thus be divided into three groups: the α - amylases (endoamylases), β-amylases (exo-amylases) and glucoamylases. The substrate and culture media component greatly influence the nature of amylase enzyme produced [7]. In starch processing industries, immobilized cells were used to optimally exploit the amylase producing machinery of the cells of which the β - amylase-producing cells are employed for bioconversion of starch to maltose [8]. Nowadays amylase from these sources is vastly used in amylase production under extreme conditions of pH and temperature. Amylases are of great importance in biochemical processes involving starch hydrolysis. The spectrum of application has gone beyond foods, beverages and pharmaceuticals to other fields such as medical and analytical chemistry [9]. In the present day biotechnology, approximately 25% of the enzyme market is dominated by amylases [10]. Therefore the present investigation therefore dealt with isolation and characterization of fungi species from soil samples collected from a forest reserve using physiological and

biochemical features and hence determination of amylolytic activity and some kinetics of the β-amylase from these fungi.

2. MATERIALS AND METHODS

2.1 Isolation of Fungi

Amylolytic fungi used in this study were isolated from soil of Omo natural forest reserve in Ogun State of Nigeria using the method reported by Kader and Omar [5]. 1.0g of the freshly collected soil was mixed with 9.0mL of sterile distilled water in sterile test tube. The samples were serially diluted. 0.5mL of 10^{-3} diluents was pipetted, poured and dispersed by swirling on potato dextrose agar (PDA) and incubated at 30ºC for five days. The isolates were sub cultured until single pure isolate was obtained.

2.2 Identification of Fungi Species

Characterization method employed for the fungal isolates were made by both the inspection of colonial features, cellular characteristics at x 100 and x 40 microscopic magnification. Identification was done by employing the method of [11] and conventional techniques of isolating individual microorganisms and allowing them to grow and produce colonies.

2.3 Preparation of Enzyme Solution

With the aid of a sterile cork borer, a 5mm disk from the advancing edge of a 4 day old fungal isolates were separately inoculated into the cultivation medium (1.0 K_2HPO_4; 0.5 $MgSO_4.7H_2O$; 2.0 $NaNO_3$; 0.001 $FeSO_4.7H_2O$; 0.5 KCl) g/L). The culture medium was adjusted to pH 7.0 before sterilization, 2% (w/v) pure soluble starch as carbon source was used for β amylases. Incubation was carried out at 30^{o}C for 5 days on a shaker incubator operated at 150rpm.

2.4 Microbial Enzymes Assays

Enzyme solution (0.5mL) was added to 0.5ml of substrate (1% soluble starch was prepared in 0.016M sodium acetate of pH 4.8) and incubated at 25ºC for 3 minutes. 1mL of 3, 5-DNSA (color reagent) was added. The mixture was then heated in water bath set at 100^{0}C for 5 minutes after which, the mixture was cooled and 10mL of water was added. The color so formed was read in a colorimeter at 540nm against a blank containing buffer only. A calibration curve was made with maltose (0.3 – 3.0μmoles) to convert colorimeter readings to unit of activity [12].

One unit of β- amylase activity produces or release one micromole of β- maltose from soluble starch per min at 25ºC and pH 4.8 in a reaction system containing 0.5mL of 1% soluble starch in 0.016M sodium acetate buffer of pH 4.8 and 0.5mL of crude enzyme solution.

3. RESULTS AND DISSCUSSION

Nine different species of fungi were isolated from the soil of the Omo natural forest reserve as seen from Table 1, Four species (*Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillus flavus)* exhibited amylolytic activities maximally, therefore, β-amylase

activity was monitored for five days in cultivating medium and some kinetic parameters studied.

Table 1. Strains of fungi isolated from the soil

Soil description	Fungi isolates
Omo natural forest soil	*Gonatobotrrys simplex*, *Aspergillus niger* *Spiromyces minutus,* *Aspergillus flavus* *Articulospora inflata* *Botrytis cenera* *Penicillium italicum* *Aspergillus fumigatus* Aspergillus flavus

The β-amylase activity of the fungal isolates was monitored for five days as shown in Fig. 1. The results revealed that all the organisms produced the enzyme with highest activities at first

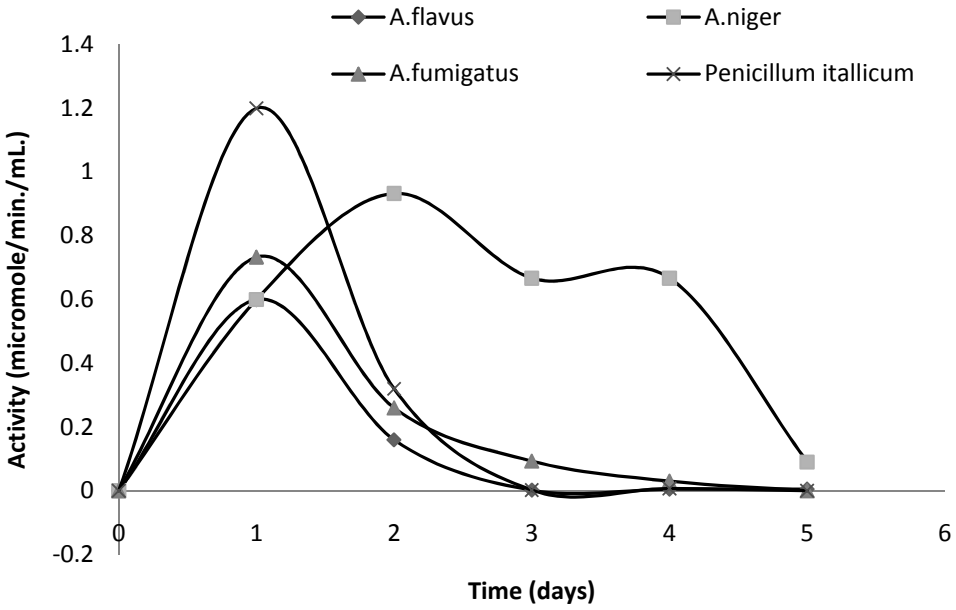

Fig. 1. β-amylase activity of different fungi isolates on daily basis

Day of culture showing *A. flavus,* with 0.6µmol./min/mL, *A. fumigatus* (0.733µmol./min/mL) and *P. italicum* 1.2µmol./min/mL, with exception of *A. niger* showing highest activity on second day of culture with 0.933µmol./min/mL. The results obtained in this work were in consonance with earlier observations of [13] that screened different species of *A. niger* for the synthesis of amylolytic enzymes using the submerged fermentation. Similarly [14] also obtained *A. flavus* with highest ability for amylolytic enzyme production among the isolated filamentous fungi from cereals, while [15] isolated a fungal strain, *Aspergillus tamari* from the soil having the ability to produce amylolytic enzymes, which formed both alpha amylase and

glucoamylase in the mineral medium with 1.0% starch and maltose as carbon source respectively. The observation in this study also substantiate the earlier work of [16] where some fungi hydrolytic enzyme activities among which β-amylase were investigated from *Aspergillus niger* and *Penicillium italicum* isolated from natural forest and *Gmellina* plantation soils in Ondo State of Nigeria.

Similarly the 2 days maximum amylase production for *A. niger* in this study was in consonance with the observations of [17] for *A. ochraceus* and [18] where incubation period of between 1 and 2 days was observed to be the optimum time for all the fungi studied as increased incubation period decreased amylase activity. The reduced and subsequent diminished activity in the later phase of growth might be as a result of catabolite repression by glucose released from starch hydrolysis as earlier observed by [19] for *Humicola grisea,* [11] for *H. brevis,* but different from [20,21] for *Papulasporia thermophilia* in which the maximum amylase activity was recorded during the period of fungus autolysis.

The optimum temperature of 40^0C was observed for all the studied organisms with highest production of beta-amylase activity of *A. flavus, (*0.4µmol./min/mL), *A. niger (*0.667 µmol./min/mL), *A. fumigatus (*0.75µmol./min/mL) and *Penicillum itallicum (*0.883µmol./min/mL) as obtained from Fig. 2.

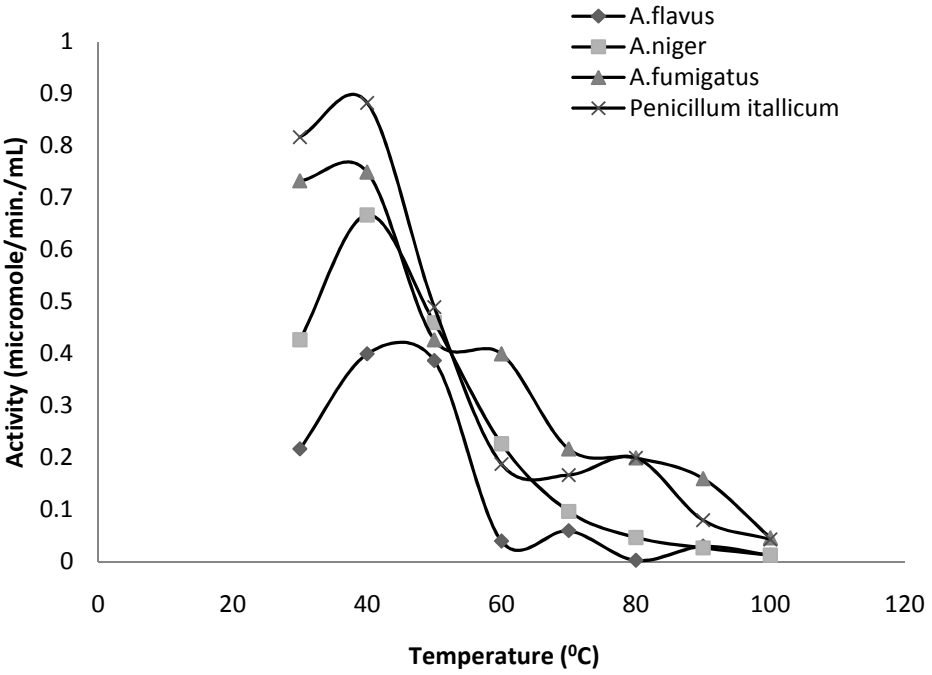

Fig. 2. Effect of temperature on β-amylase activity of different fungi isolates.

This observation however agreed with the works of [22,23,10,24] for studied fungi, but fail to agree with [25] who reported an optimum temperature of 55^0C for a fungus *Humicola grisea var. thermoidea* from Brazillian soil sample. Similarly the results of this study corroborates [26] in his investigation on production of microbial protease from selected soil fungal isolates

where the optimum temperature was observed to fall between 30ºC and 60ºC but totally lost activities at between 80ºC and 100ºC for different fungi species.

The results obtained from this study also revealed that all the organisms were thermally stable and produced beta-amylase even at 80ºC but lost activity completely at between 90ºC and 100ºC as seen from Fig. 3. However, this observation agreed with earlier work of [26] that microbial enzymes are thermally stable up till 80ºC.

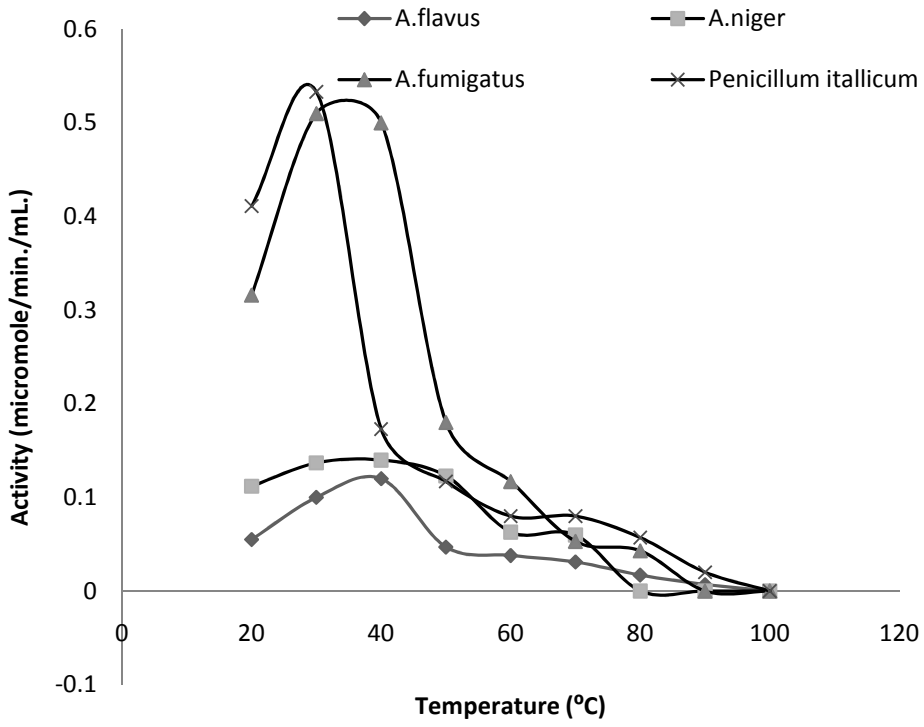

Fig. 3. Effect of Temperature on thermal stability of β- amylase activity of fungi isolates

The results of the study also showed that the organisms produced beta-amylase at both alkaline and acid range but the maximum pH recorded for the production of beta-amylase by all the organisms was at 8.5 as seen from Fig. 4, this observation agreed with [26,27] who obtained an optimum pH of between 8 and 9 for microbial enzymes activity from various fungal isolates though the activity of these enzymes were relatively high at pH between 3.0 and 9.0.

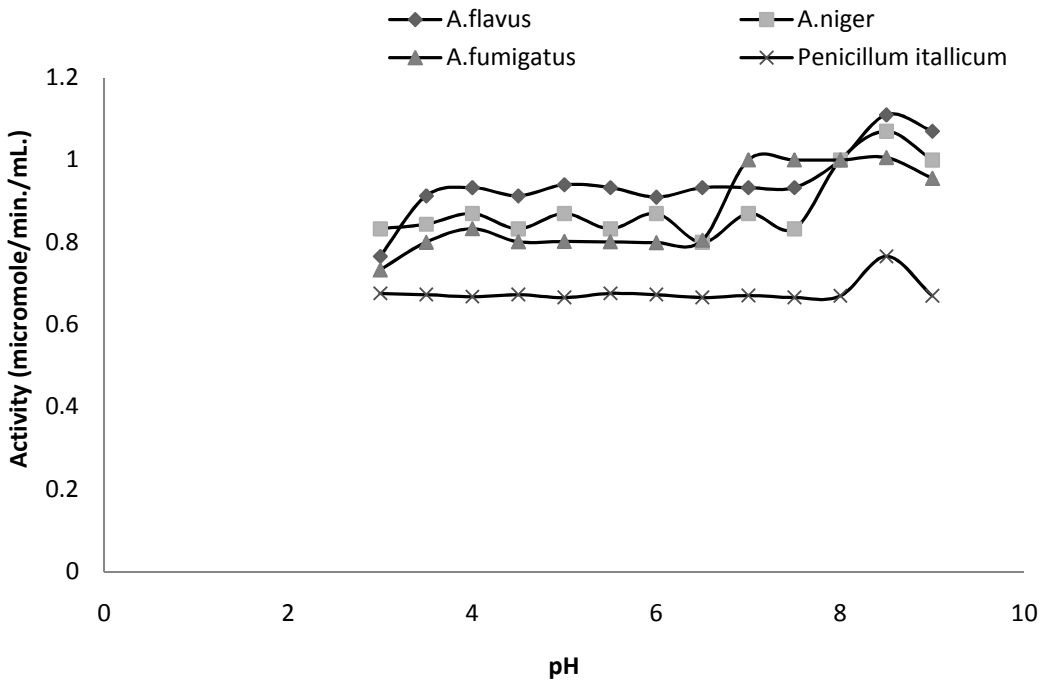

Fig. 4. Effect of pH on β-amylase activity of fungi isolates

However, various pH range has been reported for amylase production by different organisms; [28] reported pH range of 7.0-7.5 for maximum amylase production by *A. oryzae,* even though activity was observed at pH range of 5.0-10.0, while [29,30] reported a pH range of 6.0 and 8.0 for *A. fumigatus* as [31] reported a pH range of 3.0-7.0 for amylase production by *Aspergillus niger* though [32] reported amylase activity at pH range of 4.0-8.0, with maximum at pH 7.0 for *Talaromyces emersonii*. [17] also observed amylase production in the pH range of 4.0 to 6.0 for *Aspergillus ochraceus*. The differences observed however might be due to the source of isolation, the strains of the particular fungi and the type of culture medium used.

4. CONCLUSION

Conclusively, the results obtained in this investigation revealed that the studied fungi strains are promising sources of amylase for biotechnological and industrial applications, especially in medical and pharmaceutical uses, starch industry for ethanol production, production of high-fructose corn syrup, production of shorter chains of sugars called oligosaccharides and production of dishwashing and de-starching detergents and host of other useful products. Therefore efforts will be geared up in elucidating the molecular and protein sequence of β-Amylase from these microorganisms in this studied location.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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