Amylase-Producing Fungi from Cassava Peels: Isolation, Morphological Identification, and Hydrolytic Capability

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Abstract: Amylases are enzymes of considerable economic importance, extensively employed in the food, fermentation, textile, and biofuel sectors. Utilizing agro-industrial leftovers, like cassava peels, as substrates for enzyme-producing fungi is a sustainable and economical method for discovering microbial biocatalysts. This research sought to extract and evaluate fungal strains from degraded cassava peels for extracellular amylase production. Fungi were isolated via the pour plate method on potato dextrose agar and assessed for amylase production on starch agar medium, with hydrolysis zones detected through iodine staining. Of the four isolates obtained, two (AMYP 1 and AMYP 2) had positive amylase activity, with zones of clearance measuring 3.0 mm and 2.9 mm, respectively. Morphological characterization, utilizing colony morphology and microscopic attributes, classified AMYP 1 as *Aspergillus flavus* and AMYP 2 as *Aspergillus niger*. The results indicate that cassava peels serve as an effective substrate for the isolation of promising amylase-producing fungi. These isolates possess potential use in industrial starch degradation processes and facilitate waste valorization activities in accordance with circular bioeconomic principles.
Key word: *A. flavus*, *A. niger*, cassava peels, starch agar, hydrolytic capability

INTRODUCTION

The worldwide demand for industrial enzymes has been consistently rising due to their many applications in areas such food processing, textiles, pharmaceuticals, detergents, and biofuels (Shukla *et al.,* 2023). Amylases are notably important among these enzymes, constituting roughly 25–30% of the global enzyme market due to their essential function in catalyzing the hydrolysis of starch into simpler sugars like glucose and maltose (Samanta, 2022). However, bacterial strains like *Bacillus subtilis* have traditionally been significant sources of commercial amylases, but recent focus has transitioned to filamentous fungi owing to their enhanced capacity for extracellular enzyme secretion and their ability to thrive on cost-effective, renewable substrates (Patil *et al*., 2021).

Filamentous fungus, particularly from the genera *Aspergillus* and *Penicillium*, have exceptional efficacy in amylase production in both solid-state and submerged fermentation conditions (Patil *et al*., 2021). These fungi flourish on intricate organic substances typically sourced from agro-industrial byproducts, rendering them exceptional agents for biomass valorization. Fungal amylases are favoured in various industrial applications because of their stability over wide pH and temperature ranges, together with their established safety for food-grade uses (Yadav *et al*., 2024). Moreover, the economic and ecological advantages of filamentous fungi stem from their capacity to convert agricultural waste into valuable bioproducts, hence facilitating sustainable enzyme production (El-Gendi *et al*., 2021; El Sheikha *et al*., 2023).

Recent advancements in sustainable bioprocessing have catalyzed a transition from costly synthetic media to economical, renewable substrates. Agro-wastes, including cassava peels, have surfaced as viable possibilities (Mohamed *et al*., 2021). Cassava (*Manihot esculenta* Crantz) is extensively cultivated globally, particularly in sub-Saharan Africa, where its processing generates substantial quantities of peels, constituting up to 15% of tuber mass (Mwangi, 2023). The improper disposal of these peels presents environmental issues. Nonetheless, their abundant carbohydrate composition and fibrous characteristics foster an optimal milieu for microbial colonization and enzyme production (Mwangi, 2023). Multiple investigations have established that cassava peels are an abundant, economical substrate for isolating amylase-producing fungus, with strains like *Aspergillus niger* exhibiting significant enzymatic activity, occasionally surpassing

commercial alternatives (Okon *et al*., 2024). This study aims to isolate fungal strains from naturally decayed cassava peels and evaluate their extracellular amylase activity using starch agar media. The is with the purpose of identifying resilient fungal isolates with effective starch-hydrolyzing abilities that can be further improved as sustainable, economical sources of industrial amylase.

MATERIALS AND METHODS

Sample collection and preparation: Cassava peels were obtained from a native garri processing business situated in Asa Dam area, of Ilorin, Kwara State, Nigeria. Recently discarded peels were aseptically enclosed in sterile plastic bags and conveyed to the microbiological laboratory for subsequent investigation. The samples were maintained at room temperature (28 ± 2 °C) for 5–7 days to facilitate spontaneous microbial colonization and degradation.

Isolation of fungi: After observable decomposition, 10 g of cassava peels were homogenized in 90 ml of sterile distilled water and serially diluted to 10^{-5}. Aliquots of 1 ml from the 10^{-3} to 10^{-5} dilutions were aseptically placed into sterile Petri dishes, after which molten Potato Dextrose Agar (PDA), cooled to approximately 45 °C and treated with streptomycin (50 µg/ml) to suppress bacterial proliferation. The plates were gently agitated to facilitate mixing, allowed to harden, and thereafter incubated at 28 ± 2 °C for 72 hours. Emerging fungal colonies exhibiting diverse morphologies were sub-cultured onto fresh PDA plates in order to obtain pure isolates. The pure cultures were maintained on PDA slants and refrigerated at 4 °C for future screening and identification.

Screening for amylase-producing fungi: The methodology outlined by Ekedegba *et al.* (2022) was utilized with little alterations. The amylolytic activity of fungal isolates was assessed utilizing a starch agar medium consisting of the following components (g/l): soluble starch 10.0, KCl 0.2, KH_2PO_4 0.7, $MgSO_4$ 0.2, $FeSO_4$ 0.5, and agar 20.0. The medium was sterilized at 121 °C for 15 minutes and thereafter transferred into sterile Petri dishes. Subsequent to solidification, the plates were inoculated with 6 mm mycelial plugs extracted from the actively growing fungal cultures. Inoculated plates were incubated at 28 ± 2 °C for 48 hours. Subsequent to incubation, the plates were inundated with Lugol's iodine solution to elucidate starch breakdown. The emergence of a distinct halo zone surrounding the fungal colony signified positive amylase activity. A control plate devoid of fungal inoculation was incorporated for comparative analysis. The diameter of the clear zones was quantified in millimeters and documented for each isolate.

Morphological identification of fungal isolates: Fungal isolates with amylolytic activity were characterized using macroscopic and microscopic features. Macroscopic parameters, including colony colour, form, and texture, were documented following 5 days of development on PDA. Microscopic identification was conducted via lactophenol cotton blue staining. A little sample of mycelium was placed on a clean glass slide, stained with a drop of lactophenol cotton blue, and covered with a coverslip. The slide was analyzed using a light microscope (×400 magnification) to evaluate characteristics like conidiophores, conidia, hyphal structures, and spore configurations for identification purposes.

RESULTS AND DISCUSSION

Screening of fungal isolates for amylase activity

Four unique fungal isolates were extracted from decomposed cassava peels. The isolates were evaluated for extracellular amylase activity utilizing starch agar plates. After incubation and staining, two isolates displayed significant clear zones surrounding their colonies, signifying active starch hydrolysis. The two amylolytic isolates were named AMYP 1 and AMYP 2. The recorded halo zones were 3.0 mm and 2.9 mm, respectively, but the colony

diameter remained consisted at 1.1 mm for both isolates (Table 1).

The distinct halo encircling the fungal growth is a consequence of enzymatic hydrolysis of starch in the agar medium, which becomes colourless upon reaction with iodine, in contrast to unhydrolyzed starch that exhibits a blue-black colouration. This qualitative assay is a straightforward and effective approach for initial amylase screening in filamentous fungus. The control plate, devoid of fungal inoculation, had a consistent blue-black hue without distinct zones, affirming that halo formation resulted from enzymatic activity rather than autogenous starch degradation (Figure 1).

The existence of active amylase-producing fungal isolates from cassava peels emphasizes the potential of agro-industrial waste as a source of industrially important microorganisms. Prior research has indicated that decomposing agricultural by-products, including banana peels, orange rinds, and potato residues, include varied fungal communities with hydrolytic abilities (Chaturvedi et al., 2019; Matei et al., 2021). The elevated carbohydrate levels in cassava peels, encompassing residual starch, may create a selective environment conducive to the growth of amylolytic fungus.

Morphological identification of fungal isolates

The two amylase-positive fungal isolates were further analyzed according to their observable colony characteristics and microscopic structures to facilitate their identification. The two active isolates, labeled AMYP 1 and AMYP 2, had unique colony morphologies that facilitated their identification. AMYP 1 exhibited a greenish colony characterized by a slightly rough texture and radially grooved surface, attributes that align with the morphology of *A. flavus*. Conversely, AMYP 2 yielded dark brown to black colonies characterized by a dense, powdery texture, consistent with the descriptions of *A. niger*. Both isolates exhibited rapid proliferation on potato dextrose agar, with colonies attaining their maximum diameter within five days of incubation at 28 °C.

Microscopic examinations, performed with lactophenol cotton blue staining, corroborated these identifications. AMYP 1 exhibited radial conidial heads with uniseriate phialides derived from globose vesicles, which are crucial diagnostic characteristics of *A. flavus*. Concurrently, AMYP 2 exhibited biseriate phialides organized around a globose vesicle, generating chains of smooth, darkly pigmented conidia, indicative of *A. niger*. Figure 2 illustrates the observed colony features and microscopic structures of AMYP 1 and AMYP 2. These characteristics align with the conventional characterization articulated by Rosa (2021) and are substantiated by more recent research on industrially significant *Aspergillus* species. Table 2 delineates the principal macroscopic and microscopic characteristics identified in both samples.

This study reliably identifies *A. flavus* and *A. niger* as amylolytic fungi, consistent with numerous publications emphasizing their ability to manufacture industrial enzymes, especially amylases, across various environmental and nutritional conditions (Ekedegba et al., 2022). Also, their structural characteristics, swift sporulation, and capacity to thrive on inexpensive substrates like cassava peels highlight their significance in enzyme biotechnology and agro-waste valorization.

Table 1: Clear zones and colony diameters of amylolytic fungal isolates

Isolate code	Clear zone (mm)	Colony diameter (mm)
AMYP 1	3.0	1.1
AMYP 2	2.9	1.1

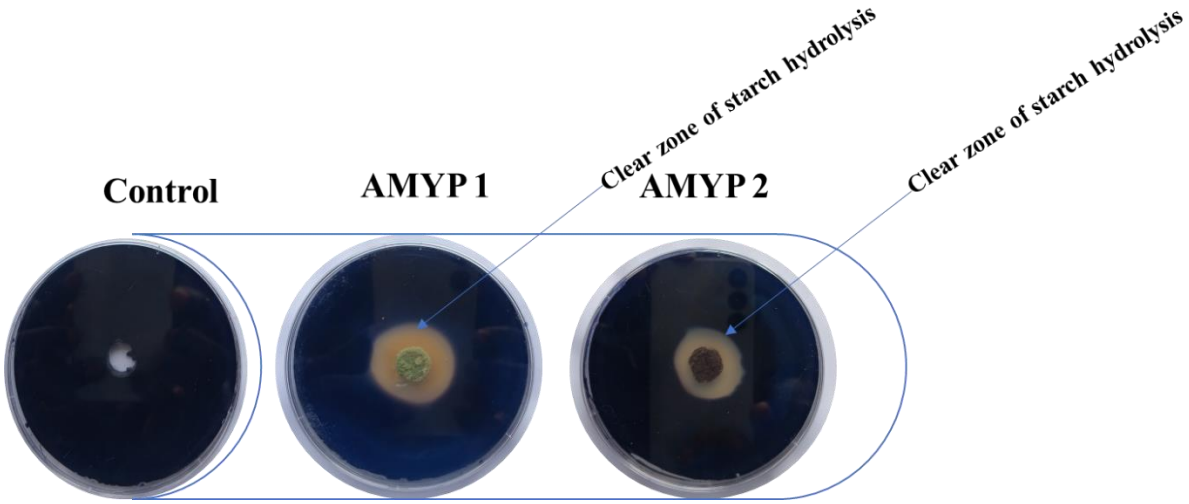

Figure 1: Clear zone of starch hydrolysis by fungal isolates

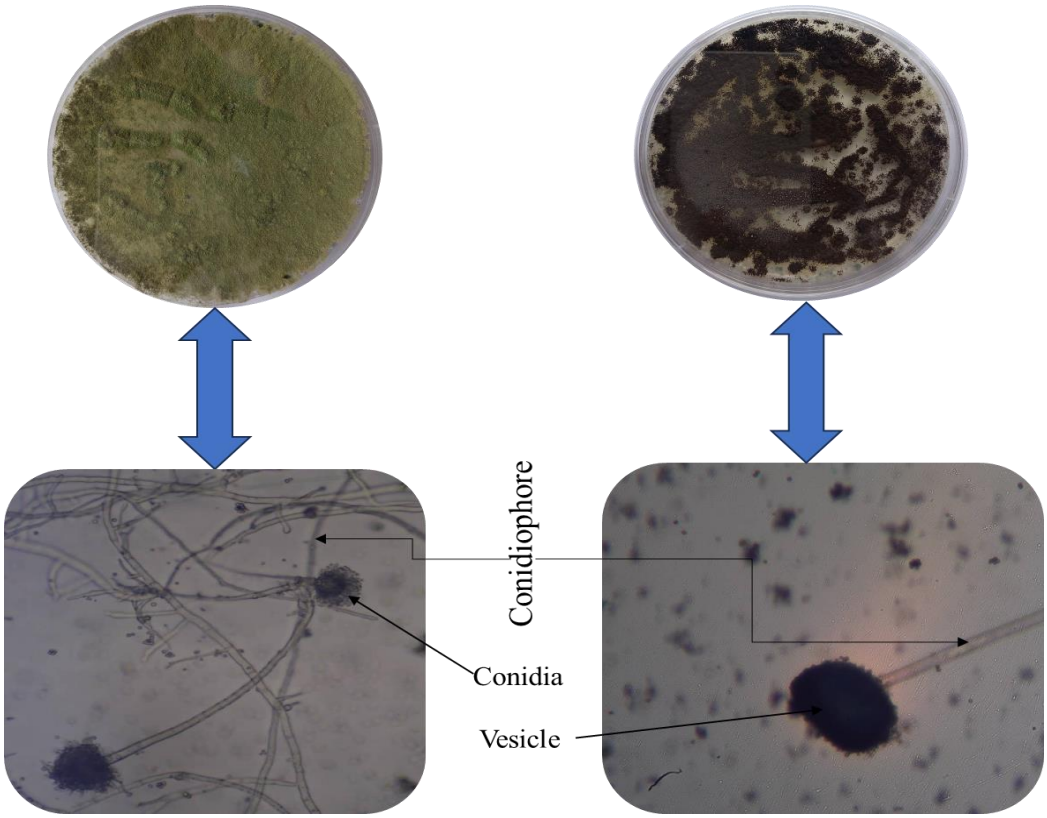

Figure 2: Macroscopic and microscopic features of amylase-positive fungal isolates

Table 2: Morphological characteristics of amylase-producing fungal isolates

Isolate Code	Colony Color	Surface texture	Microscopic features	Identified species
AMYP 1	Greenish	Rough, radially grooved	Radiate conidial heads; uniseriate phialides on globose vesicle	*Aspergillus flavus*
AMYP 2	Black to dark brown	Powdery, dense	Biseriate phialides; large globose vesicle; round, dark conidia in chains	*Aspergillus niger*

CONCLUSION

The effective isolation and screening of amylase-producing fungus from cassava peels underscore the significance of agro-industrial leftovers as feasible and sustainable resource for microbial enzyme discovery. The recognition of *Aspergillus flavus* and *Aspergillus niger* as effective amylolytic agents highlights their potential application in industrial starch degradation processes. Due to their halo formation and stable morphological characteristics, these isolates may be viable candidates for subsequent optimization in enzyme synthesis. In addition to their enzymatic function, utilizing cassava peel as a fermentation substrate illustrates a circular bioeconomic approach, transforming agricultural waste into valuable biocatalysts. Subsequent investigations encompassing molecular identification, enzyme purification, and activity profiling are advised to evaluate their industrial scalability and functional resilience.

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