

Amylase-Producing Fungi and Bacteria Associated with Some Food Processing Wastes

Okunwaye, T^{*1}, Uadia, P.O², Okogbenin, B.O¹, Okogbenin, E.A¹, Onyia, D.C¹ and Obibuzor, J.U¹

¹ Biochemistry Division, Nigerian institute for Oil Palm Research. PMB 1030 Benin City, Edo State, Nigeria

²University of Benin, Benin City, Edo State, Nigeria

Abstract

Amylases are enzymes that catalyze the hydrolysis of glycosidic bonds present in starch to release simple sugars. They are one of the most important enzymes in numerous commercial processes. In this investigation, fungal and bacterial strains from the following agro-industrial wastes were isolated and screened for amylolytic ability: soil from oil palm plantation, shea seed, date fruit, coconut meat, cassava effluent, cassava peel, cassava tubers, yam and potato tubers, starch medium, parboiled water from noodles and rice. The results revealed the presence of *Geotrichum*, *Aspergillus*, *Penicillium*, *Trichoderma*, *Rhizopus* and *Fusarium spp.* Five major genera of bacterial species namely *Corynebacterium*, *Pseudomonas*, *Lactobacillus*, *Micrococcus* and *Bacillus* were isolated and screened for amylase activity. Cassava soil had the highest heterotrophic bacterial count of 5.7×10^5 cfu/g and coconut meat waste had the lowest heterotrophic bacterial count of 1.3×10^5 cfu/g. All isolated microorganisms had the amylolytic ability. The fungal isolates had higher amylase activity when compared with the bacterial isolates. This investigation reveals organisms with high amylase activity.

Keywords: Amylase, Fungi, Bacteria

***Corresponding author:** +2347030505096, tonbra81@yahoo.com

Introduction

Enzymes are efficient biological catalysts that accelerate living cells' biochemical processes by increasing the rate of reactions. Amylases are a group of enzymes that break down glycosidic bonds found in starchy substrates to give end-products typical to the particular amylolytic enzymes used (Dhanya et al., 2009). They are commonly found in microbes, animals and plants (Pandey et al., 2000)

Microorganisms are major producers of enzymes that are of industrial significance. In various sectors, the use of amylases from microorganisms has exceeded synthetic sources (Pandey et al., 2000). Amylolytic enzymes are widely distributed in bacteria and fungi.

Microorganisms can easily be engineered to produce enzymes of desired attributes and the economic bulk production ability of microbes can be greatly enhanced. (Ramesh and Lonsane, 1990). Fungi and bacteria which are well-known producers of extracellular proteins have been extensively utilized in producing various enzymes as well as amylases (Kazunari et al., 2011). The choice of suitable microorganism is a major determining factor in the production of enzymes of interest (Jinu, 2017). The use of cheap and easily available wastes, such as agro-industrial waste, as an alternate substrate for amylase isolation and production for industrial purpose is a continuous process that helps in solving pollution challenges (Priya et al., 2011).

Screening of microorganisms with high amylase activities could enhance the discovery of novel amylases needed in commercial processes (Okunwaye et al., 2021). In this study, the isolation and screening of microbial amylases from some oilseeds and food processing waste are reported.

Materials and Methods

Sample collection: The following agro-industrial wastes and by-products including spoilt date fruit, shea seed waste, spoilt coconut meat, soil from oil palm plantation, water pressed from grated cassava, cassava peel, cassava tuber waste and the soil from the cassava grinding mill were collected from dump sites located at Nigerian Institute for Oil Palm Research (NIFOR) stations and housing estates: Wastewater from parboiled rice and noodles were obtained from a local fast food eatery in Benin City, Nigeria; Potato (Sweet and Irish) and yam tuber wastes were obtained from Uselu market, Benin City, Nigeria.

All chemicals, reagents, standard proteins and solvents used in this experiment were of analytical grades.

Isolation of Amylase Producing Microorganisms: Serial dilution was made on samples collected from the different food wastes and plated on nutrient agar (NA) and potato dextrose agar (PDA) according to the method described in deZaan Cocoa and Chocolate Manual, 2009. The pour plate method of inoculation technique was used in the isolation of the microorganisms associated with the samples. One millilitre each of the serially diluted samples was pipetted with the aid of a sterile syringe and then transferred into the corresponding labeled petri dishes. The prepared PDA medium was dispensed into Petri dishes and spread across with the aid of a glass spreader for fungi isolation. They were allowed to incubate at 30°C for 72 hours. In bacteria isolation, one millilitre of the diluted samples were spread on NA medium with 1 % w/v starch; these were allowed to incubate at room temperature for 24 hours. The various fungi and bacteria strains obtained were further sub-cultured to have pure strains.

Screening for Alpha-Amylase Producing Fungi (starch iodine test): The culture plate technique was used to detect and screen the fungal isolates

for α -amylase activity. The basis of detection and screening of fungi with alpha amylase activity using the culture plate method is the absence of a deep blue colour starch-iodine complex zone in the presence of Gram's iodine solution, i.e. in the zone of degradation no blue colour forms (Toye, 2009). Isolated colonies from each plate containing pure cultures grown on starch as the carbon source, for 72 hours at 30°C were submerged in Gram's iodine solution and a dark blue-coloured starch-iodine compound was observed. Pure isolates of fungi producing alpha-amylase which showed zones of degradation were preserved on potato dextrose agar slants containing 1 % starch, to grow spores and stored in the refrigerator until needed.

Screening for Alpha-Amylase Producing Bacteria: The amylolytic activity of the bacteria was ascertained using the starch agar medium (Toye, 2009). The bacterial colonies which formed clear zones around them were recorded and the strains showing high amylolytic potential (depending upon the zone diameter) were selected and screened further for efficient amylase production.

Macroscopic and Microscopic Analyses: Fungi strains isolated from all the samples were analyzed for morphological and cultural characteristics such as the colony top and reverse colours, margins, elevation.

Cultural, Morphological and Biochemical Characterization of Bacterial Isolates: The bacterial cultures were identified using the analytical profile index kit (API 20A system). This was performed according to the method of Murray (1985).

Amylase Extract from Bacteria: The bacterial amylase medium contained bacteriological peptone (6%), magnesium sulphate (0.5%), potassium chloride (0.5%) and starch (1%) in distilled water, pH 7.0 (Toye, 2009). The medium was mixed, distributed into 40 mL volumes in 100mL Erlenmeyer flasks and sterilized by autoclaving at 121°C for 15 minutes. A loop full of bacterial culture was added to the amylase production medium, to separate the crude extract, the bacterial culture was centrifuged at a speed rate of 5000 revolutions per minute (rpm) for 20 minutes using a refrigerated centrifuge.

The precipitate was discarded and the supernatant used as the crude amylase extract.

Amylase Extract from Fungi: The various isolated fungal species were grown in a potato dextrose medium with a pH of 7.0. Ten percent of the fungi growth medium was dispensed into 1000 mL of mineral salts medium (2.75g/L K₂HPO₄, 2.225g/L KH₂PO₄, 1.0g/L (NH₄)₂NO₃, 10.0g/L MgCl₂.6H₂O, 0.1g/L KCl, 0.01g/L FeSO₄.6H₂O and 0.02g/L CaCl₂) pH 7.0 and 1% w/v starch added (Toye, 2009). The medium was incubated at 30°C under a sterile condition on a shaker at 200 rpm, fungi growth monitored at 600 nm. Crude enzyme extract was prepared by filtering through a pre-weighed Whatman filter paper, the filtrate obtained was centrifuged at 4°C for 15 minutes at 5000 rpm. The supernatant was used as crude enzyme extract which was further dialyzed in distilled water for 24 hours to remove residual sugars.

Determination of Alpha Amylase-Activity: The alpha-amylase activity in the cultured filtrates of the isolated microorganisms was determined by the dinitrosalicylic acid (DNS) method (Toye, 2009).

Protein Determination: Protein concentrations were determined according to Lowry's method (Lowry et al., 1951) using BSA (1mg/ml) as the standard protein.

Results and Discussion

Isolation of amylolytic fungi: Table 1 reveals the presence of *Geotrichum*, *Aspergillus*, *Penicillium*,

Trichoderma, *Rhizopus* and *Fusarium spp.* Fungal strains are prolific producers of extracellular proteins that are widely exploited for the production of different enzymes including alpha-amylases (Kazunari et al., 2011). The most dominant fungi strain was *Penicillium* occurring in the wastes of parboiled water from noodles, parboiled water from rice, cassava peel, shea nut and yam, having percentage occurrences of 100, 60, 100, 100 and 100 respectively (Table 1). The genus *Penicillium* has been isolated from various terrestrial environments such as soil, food e.t.c. and is known to play a major role in the decomposition of biodegradable materials (Frisvad and Samson, 2004). *Penicillium sp.* has been observed to be tolerant to high osmotic compositions and harsh conditions. The species, *Rhizopus* belonging to the genera Mucorales, was the most occurring fungi found in the substrates of palm fruit and coconut fruit wastes with percentage occurrence of 100 in both. *Rhizopus* is a very-fast-growing spreading type of mold that exhibits complex metabolism and produces a variety of enzymes that enable them to utilize a wide range of nutrients (Bullerman, 2003; Lennartsson et al., 2014). The most diverse groups of fungi isolated and identified were *Aspergillus*, *Penicillium* and *Fusarium*. *Penicillium*, *Aspergillus* and *Trichoderma* species have been reported in the recent past as a microbial source for producing alpha-amylases (Erdal and Taskin, 2010; Abdulaal, 2018; Lemo et al., 2019).

Table 1: Fungi associated with different waste materials

Sample names	CFU	Macroscopic description Colony description on PDA	Microscopic description		Fungi Isolated	% Occurrence
			Texture	Conidia shape		
Parboiled water from noodles	2×10 ⁵	Grey mycelial growth	Rough	Globose	<i>Penicillium sp.</i>	70
					<i>Penicillium sp.</i>	30
Soil from cassava dumpsite	4×10 ⁵	White mycelial growth	Rough	Filiform	<i>Fusarium sp.</i>	25
		Army green mycelial growth	Smooth	Globose	<i>Trichoderma sp</i>	25
		Cream mycelial growth	Butyrous	Subglobose	<i>Geotricum sp</i>	25
		Black mycelial growth	Smooth	Globose	<i>Aspergillus sp.</i>	25

Spoilt date seed	1×10 ⁵	Black mycelial growth	Rough	Subglobose	<i>Aspergillus sp.</i>	100
Soil from oil palm plantation	1×10 ⁵	White mycelial growth	Rough	Filiform (2-3 septate)	<i>Fusarium sp.</i>	100
Parboiled rice water	2×10 ⁵	White mycelial growth	Rough	Filiform	<i>Fusarium sp.</i>	40
		Dark mycelial growth	Smooth	Globose	<i>Penicillium sp</i>	60
Palm fruit waste	1×10 ⁵	Dark fluffy mycelial growth	Smooth	Globose	<i>Rhizopus sp.</i>	100
Coconut meat waste	1×10 ⁵	Dark fluffy mycelial growth	Smooth	Globose	<i>Rhizopus sp.</i>	100
Cassava peel	1×10 ⁵	Brown dense mycelia growth	Smooth	Globose	<i>Penicillium sp.</i>	100
Shea nut waste	1×10 ⁵	Grey green mycelial growth	Rough	Globose	<i>Penicillium sp</i>	100
Irish potato waste	1×10 ⁵	White mycelial growth	Smooth	Globose	<i>Geotricum sp</i>	100
Soil from oil palm plantation	1×10 ⁵	White mycelial growth	Rough	Filiform (2-3 septate)	<i>Fusarium sp.</i>	100
Yam waste	2×10 ⁵	Brown dense mycelia growth	Smooth	Globose	<i>Penicillium sp</i>	50
		Brown dense mycelia growth	Smooth	Globose	<i>Penicillium sp</i>	50
Starch medium	1×10 ⁵	White mycelial growth	Smooth	Filiform	<i>Fusarium sp.</i>	100

Isolation of amylase producing bacteria: The bacteria species isolated (A-H) were identified to be *Corynebacterium*, *Pseudomonas*,

Lactobacillus, *Micrococcus* and *Bacillus* as shown in Table 2.

Table 2: Cultural, Morphological and Biochemical Characterization of Bacteria Isolates

Characterization	A	B	C	D	E	F	G	H
Cultural Characterization								
Shape	Irregular	Circular	Circular	Circular	Circular	Irregular	Circular	Circular
Colour	Creamy	Milky	Milky	Pink	Creamy	Creamy	Creamy	Creamy
Margin	Lobate	Entire	Entire	Crenate	Entire	Lobate	Entire	Entire
Opacity	Translucent	Opaque	Translucent	Translucent	Opaque	Opaque	Opaque	Opaque
Elevation	Flat	Flat	Flat	Flat	Flat	Flat	Flat	Flat
Wet/dry	Dry	Wet	Dry	Dry	Wet	Wet	Wet	Wet
Morphological Characterization								

Gram stain	+	-	+	+	+	+	+	+
Shape	Rod	Rod	Rod	Cocci	Rod	Rod	Rod	Rod
Arrangement	Tetrad	Single	Pair	Single	Pair	Single	Pair	Single
Spore	-	-	-	-	+	+	+	+
Motility	-	+	+	-	-	-	-	-
Biochemical Characterization								
Catalase	+	+	-	+	+	-	-	+
Oxidase	-	-	-	+	+	+	+	+
Indole	+	+	-	+	-	+	-	-
Urease	-	-	-	+	+	+	-	+
Citrate	+	-	+	-	-	+	-	+
VP/MR	+	+	+	+	+	-	-	+
Sugar Fermentation								
Lactose	+	+	-	+	-	+	+	+
Sucrose	+	-	+	-	+	+	+	-
Maltose	+	+	-	+	+	+	+	+
Sorbitol	-	-	+	-	-	+	+	+
Glucose	+	+	+	+	-	+	+	-
Mannitol	-	-	-	-	-	-	-	-
Probable isolates	<i>Corynebacterium sp.</i>	<i>Pseudomonas sp.</i>	<i>Lactobacillus sp.</i>	<i>Micrococcus sp.</i>	<i>Bacillus sp.</i>	<i>Bacillus sp.</i>	<i>Bacillus sp.</i>	<i>Bacillus sp.</i>

The result of the bacterial isolates associated with the different sources shown in Table 3 revealed that cassava soil had the highest heterotrophic bacterial count of 5.7×10^5 cfu/g and coconut meat waste with the lowest heterotrophic bacterial count of 1.3×10^5 cfu/g. *Bacillus* and *Pseudomonas* spp were found to be the most dominant bacteria in the wastes of cassava effluent, cassava soil, yam, parboiled rice, date

fruit and coconut meat. The genera, *Bacillus*, was found to be the most diverse group. Karnwal and Nigam (2013) reported the isolation of alpha-amylases from *bacillus* strains isolated from soil. Among bacteria, *Bacillus* species is the most widely used source for the production of amylases (Sundarram and Murthy, 2014; Jinu, 2017).

Table 3: Isolation and distribution of bacteria isolates

Samples	Total Heterotrophic Bacteria Count ($\times 10^5$ cfu/g)	Isolates distribution
Cassava effluent	4.3	<i>Corynebacterium sp.</i> , <i>Pseudomonas sp.</i> , <i>Lactobacillus sp.</i> , <i>Micrococcus sp.</i> , <i>Bacillus sp.</i>
Cassava soil	5.7	<i>Corynebacterium sp.</i> , <i>Pseudomonas sp.</i> , <i>Lactobacillus sp.</i> , <i>Micrococcus sp.</i> , <i>Bacillus sp.</i>
Yam waste	1.7	<i>Corynebacterium sp.</i> , <i>Pseudomonas sp.</i> , <i>Bacillus sp.</i>

Parboiled rice wastewater	3.9	<i>Corynebacterium sp., Pseudomonas sp., Bacillus sp.</i>
Date fruit	2.1	<i>Pseudomonas sp., Micrococcus sp., Bacillus sp.</i>
Coconut meat waste	1.3	<i>Pseudomonas sp., Lactobacillus sp., Bacillus sp.</i>

Screening for amylase producing fungi and bacteria (Starch Iodine Test): The result in Table 4 reveals 19 fungal strains isolated and all showed amylolytic activity by the zone of clearance displayed. The fungi, *Trichoderma* isolated from the soil of cassava dump site and *Rhizopus* from coconut meat waste had the highest zone of clearance of 9.0 cm diameter while *Fusarium* from the soil of oil palm plantation had the least zone of clearance of 2.0 cm diameter. *Rhizopus* sp. from coconut meat waste also showed the highest zone of clearance (9.0 cm) when compared with the *Rhizopus* sp (2.5 cm) from palm fruit waste. All isolated bacteria species had the amylolytic ability.

Table 4. The alpha- amylase activity of the different fungi isolates based on their zone of clearance

Sources	Fungi	Diameter of zone of clearance (cm)
Parboiled waste water from noodles	<i>Penicillium sp.</i>	2.2
	<i>Penicillium sp.</i>	2.8
Soil from cassava dumpsite	<i>Fusarium sp.</i>	4.0
	<i>Trichoderma sp.</i>	9.0
	<i>Geotricum sp.</i>	4.6
	<i>Aspergillus sp.</i>	5.0
Spoilt date seed	<i>Aspergillus sp.</i>	2.7
Soil from oil palm plantation	<i>Fusarium sp.</i>	2.0
Parboiled rice water waste	<i>Fusarium sp.</i>	3.2
	<i>Penicillium sp.</i>	3.8
Palm fruit waste	<i>Rhizopus sp.</i>	2.5
Coconut meat waste	<i>Rhizopus sp.</i>	9.0
Shea nut waste	<i>Penicillium sp.</i>	2.4
Irish potato waste	<i>Geotrichum sp.</i>	2.6
Soil from oil palm plantation	<i>Fusarium sp.</i>	4.5
Starch medium	<i>Fusarium sp.</i>	3.7
Yam waste	<i>Penicillium sp.</i>	6.0
	<i>Penicillium sp.</i>	5.5
Cassava peel	<i>Penicillium sp.</i>	8.9

Amylase activity and protein concentration of screened amyolytic fungi. In Fig.1, all fungi isolates with zones of clearance in the screening test had amylase activity with the highest activity seen in *Rhizopus sp.* from coconut meat waste followed by *Trichoderma sp.* isolated from the soil of cassava dumpsite. The fungi, *Geotricum* and

Penicillium spp had the least amyolytic activity. The fungi, *Rhizopus microsporus* and *Trichoderma viride* have the most active amylase enzymes because of their high amylase activity and moderately low protein content when compared to other organisms isolated.

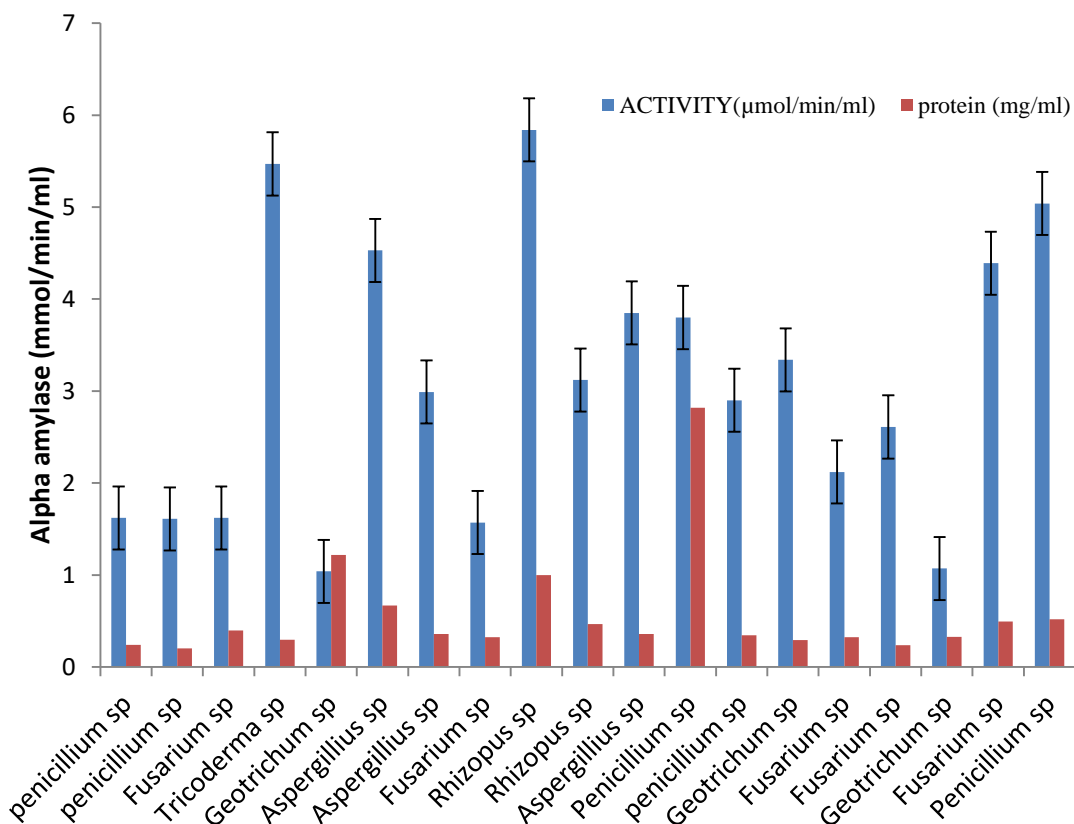


Fig. 1: Amylase activity and protein content of all fungal isolates

Conclusion

From this investigation, it can be concluded that the fungal and bacterial isolates obtained are good producers of alpha-amylase enzyme and the sourcing of microorganisms with high amylase activity from cheap, affordable, easily available sources should be recommended.

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