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Isolation, Identification, and Screening of Bacteria from Yam Waste for Amylase Production Ability

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ABSTRACT

This study aimed to isolate, identify, and screen bacteria from yam waste for their potential to produce amylase, a crucial enzyme involved in starch breakdown. Yam waste, often discarded as a by-product of yam processing, was explored as a source of microbial enzymatic activity, with a focus on amylase for industrial applications. Five samples of yam waste were collected from different locations in Ilorin, Kwara State, Nigeria, and bacteria were isolated, characterized, and identified using standard microbiological techniques. The isolates were screened for amylase production on starch-nutrient agar plates. The total viable count ranged from 9.2 x 10^8 to 16.8 x 10^8 cfu/g, with *Bacillus* sp*., Corynebacterium* sp., *Micrococcus* sp., *Serratia* sp., *Klebsiella* sp*.,* and *Clostridium sp*. identified as potential organisms. Four of the six isolated bacteria exhibited amylase production ability, with an index of amylolytic activity ranging from 0.40 mm to 0.14 mm. The findings suggest that *Bacillus* sp. shows promise for future industrial applications in amylase production. This study highlights the potential of utilizing yam waste as a valuable source of microbial enzymes, particularly for industries requiring amylase for various processes.

Keywords: Amylase, Bacteria, Starch hydrolysis, Yam waste

1. INTRODUCTION

Amylase is a group of enzymes that are responsible for catalyzing the breakdown of starch into various products, such as dextrins and smaller glucose polymers, through hydrolysis [1]. Enzymes that break down starch, such as amylase, have gained considerable attention due to their perceived technological importance and economic advantages [2]. Amylases (alpha amylase, beta amylase, and glucoamylase) are essential enzymes in modern biotechnology, playing a key role in various biotechnological processes such as starch degradation, detergent production, food manufacturing, pharmaceuticals, textiles, and paper production. These enzymes are responsible for nearly a quarter of total enzyme sales [3]. The origins of amylases date back to 1811, when the initial starch-

degrading enzyme was identified in wheat by Kirchhoff, marking the inception of research on amylase. In 1925, Kuhn named α-amylases based on the alpha configuration of their breakdown products, while in 1930, Ohlsson discovered another amylase that produced β-mannose and named it β-amylase [4]. Alpha amylases are enzymes that rely on calcium for their function and are capable of acting at any point on the substrate, while beta amylases work specifically on the non-reducing end by breaking the second α-1,4 glycosidic bond through hydrolysis. This action leads to the breakdown of starch into maltose, contributing to the development of the sweet taste observed in ripe fruits as they ripen [5]. Amylase can be found in the saliva of humans, initiating the chemical process of digestion. As a result, starchy foods like rice and potatoes, which have low sugar content but high starch content, acquire a mild sweetness when chewed. This transformation occurs due to the action of amylase, which converts a portion of the starch into sugar within the oral cavity [6].

Amylases are enzymes that can be obtained from various sources, including plants, animals, and microbes [7]. However, the microbial origin of amylase is often favored due to its widespread accessibility [8]. Amylase is a widely utilized enzyme in various industrial sectors, and it has been obtained from numerous sources, such as fungi, yeasts, bacteria, and actinomycetes. However, it is noteworthy that enzymes derived from fungal and bacterial origins have predominantly been favored and employed in industrial applications [9]. Utilizing microorganisms for the synthesis of amylases offers significant benefits, such as the ability to economically produce enzymes in large quantities and the ease of manipulating these microorganisms to yield enzymes with specific desired traits [10]. Bacterial amylases are employed in industrial manufacturing owing to their cost-effectiveness, uniformity, reduced production time, and space requirements [11]. Microorganisms can be genetically modified to enhance the production of effective amylases that exhibit stability under high temperatures and harsh environmental conditions [12].

Yam is a staple food in Nigeria and many other countries, with a significant portion of the yam being consumed and the peel typically discarded as waste. The disposal of yam waste presents environmental challenges and the potential for pollution. Therefore, there is a pressing need to explore innovative approaches for the effective utilization of this waste. In this study, we aim to isolate, identify, and screen bacteria from deteriorating yam waste for amylase production ability, which can be of great advantage for industrial amylase production as there is a growing demand for amylase enzyme, which causes a demand for new organisms for its production.

The findings of this study could have significant implications for waste management practices, enzyme production processes, and the utilization of agricultural by-products. Identifying bacteria with amylase production potential from yam waste could lead to the development of novel enzyme sources with potential applications in various industries and could also offer a sustainable and cost-effective solution for enzyme production.

2. METHODOLOGY

2.1. Materials, Equipment, and Apparatus

Materials, equipment, and apparatus used include a glove, nose mask, sellotape, cotton wool, aluminum foil, inoculating loop, forceps, glass rod, spreader, McCartney bottle, Bunsen burner, microscope slide, slide cover clip, oil immersion, polythene bag, petri dish, test tubes, test tube rack, conical flask, measuring cylinder, Pasteur pipette, autoclave, incubator, hot air oven, microscope,

refrigerator, inoculation chamber, digital balance, and shaker.

2.2. Media and Reagents

Media such as nutrient agar, starch nutrient agar, urea agar, citrate agar, glucose phosphate broth (MRVP broth), and peptone water were used. Reagents used include 70% ethanol, normal saline, crystal violet dye, Lugol's iodine, acetone, safranin, hydrogen peroxide, oxidase reagent, kovac reagent, methyl red indicator, alpha-naphthol, and potassium hydroxide.

2.3. Washing and Sterilizing of Glassware and Laboratory Bench

Prior to culture media preparation, all glassware was thoroughly washed and sterilized to ensure aseptic conditions for the experiments. Glassware, including test tubes, conical flasks, and measuring cylinders, was first rinsed with distilled water to remove debris. Then, it was soaked in a detergent solution for 30 minutes and scrubbed with a bottle brush to dislodge any adherent material. After rinsing with tap water, the glassware was submerged in a 10% bleach solution for 1 hour to sterilize it. Similarly, laboratory benches were cleaned with a disinfectant solution and wiped down with 70% ethanol to eliminate any microbial contamination. This procedure was repeated before and after each experiment to maintain a sterile working environment.

2.4. Sample Collection

The yam samples were collected in five different places in Ilorin, Kwara State, Nigeria (Sango, Eleko, Oyun, Yakuba, and Okesuna), transferred into a sterile polyethylene bag, and transported to the laboratory for microbial analysis. The samples were coded as Y1–Y5 respectively.

2.5. Preparation of Cultural Media

Nutrient agar was prepared according to the manufacturer's specification (28 g of nutrient agar for 1000 ml of distilled water) and sterilized using the autoclave at 121 °C for 15 minutes, and starch nutrient agar was prepared by mending nutrient agar with 2% soluble starch. 250 ml of nutrient agar amended with 2% of soluble starch was prepared by first calculating the amount of nutrient agar needed based on the manufacturer's specification, which indicated 28 g of nutrient agar for 1000 ml; hence, 7 g of nutrient agar will be required to prepare 250 ml. Since 250 ml represents 100% of the total volume, to obtain 2%, we multiply 250 by 2 and divide by 100, giving us 5 ml. Then, to match the state of soluble starch, the 5 ml was converted to 5 g. Therefore, 7 g of nutrient agar and 5 g of soluble starch were weighed and added to 250 ml of distilled water to prepare nutrient agar amended with 2% of soluble starch (starch nutrient agar). The mixture was heated and stirred until the agar was completely dissolved, and then sterilized using the autoclave at 121 °C for 15 minutes.

2.6. Isolation of Bacteria

Ten (10) grams of grounded yam waste samples were mixed with 90 ml of sterile distilled water and stirred. Six-fold serial dilutions were prepared with 1 ml of aliquot portion and labeled as 10^{-1} to 10^{-6}, respectively. Zero point one (0.1) milliliter from dilution 10^{-6} was pipetted into a nutrient agar plate, then incubated at 37 °C for 24 hours for the isolation of bacteria. After incubation, colonies were counted using an illuminated colony counter, and bacterial isolates were streaked on newly prepared nutrient agar plates and incubated at 37 °C for 24 hours in order to obtain pure cultures [8].

2.7. Morphological Characterization and Gram Staining of Isolates

The colonial characteristics of all isolates, including shape, size, color, surface texture, elevation, and margin on nutrient media, were observed and recorded. Gram staining was conducted to check for the gram's reaction (positive and negative) under a microscope. Crystal violet was added to the fixed smear on the slide for 1 minute. Then it was washed with distilled water. Lugol`s iodine was then added for 1 minute, then decolorized with acetone-alcohol for 10 seconds after washing with distilled water. Safranin was added for 30 seconds and washed with distilled water. The slide was then examined under a microscope with an oil immersion objective lens. Gram-positive bacteria appeared purple, while Gram-negative bacteria appeared red [13].

2.8. Biochemical Characterization of Isolates

Biochemical tests such as catalase, oxidase, indole, methyl red, voges proskaeur, citrate, and urease were conducted to identify the isolates. The steps are listed below.

2.8.1. Catalase test

This test was conducted to detect the catalase enzyme in bacteria. Using a sterile inoculating loop, a small amount of organism from pure culture was collected and placed onto the microscope slide. Using a Pasteur pipette (dropper), 1 drop of 3% H_2O_2 was dropped onto the organism on the microscope slide. The formation of bubbles indicates a positive result [14].

2.8.2. Oxidase test

This test was conducted to detect the cytochrome oxidase (indophenol oxidase) enzyme in bacteria. A strip of filter paper, which was soaked in a 1% solution of tetra-methyl-phenylene diamine dihydrochloride (oxidase reagent) and dried, was placed on a clean glass slide by forceps. A fresh, young test culture from nutrient agar was picked off with a sterile glass rod and rubbed on the filter paper strip. The development of blue-purple color within 5–10 seconds indicates a positive result, while the absence of color change indicates a negative result [15].

2.8.3. Indole test

This test was conducted to detect bacteria with the ability to degrade the amino acid tryptophan and produce indole. The test organism was cultured in peptone water, which contains tryptophan, and incubated at 37 °C for 24 hours. One milliliter of Kovac`s reagent, which contains 4-P-dimethylamine benzaldehyde, was run down the side of the test tube. The appearance of a pink color in the reagent layer within a minute indicated a positive reaction [16].

2.8.4. Methyl red test

This test was conducted to detect bacteria with the ability to ferment glucose and produce acidic end products (acetic acid). Tubes containing glucose-phosphate broth were inoculated with cultures and then incubated at 37 °C for 24 hours. Two drops of methyl red reagent were added, shaken well, and examined. The appearance of a bright red color indicated a positive reaction, whereas an orange-yellow color indicated a negative reaction [17].

2.8.5. Voges proskaeur test

This test was conducted to detect bacteria with the ability to ferment glucose and produce neutral end products (acetoin). A pure test culture was inoculated in a test tube containing glucose phosphate broth and incubated at 37 °C for 24 hours. 0.2 ml of 40% KOH and 1.6 ml of 5% alpha-naphthol solution were added to 1 ml of the culture broth, shaken, and the tubes were placed in a slope position and examined. The appearance of cherry red indicated a positive reaction,

whereas orange yellow indicated a negative reaction [17].

2.8.6. Citrate test

This test was conducted to identify bacteria that can utilize citrate as the sole source of carbon for metabolism. The surface of a citrate agar slant was streaked with a portion of a well-isolated colony and then incubated at 37 °C for 48 hours. A color change from green to blue is a positive result. The absence of any growth as well as no change in color indicates a negative reaction [18].

2.8.7. Urease Test

This test was conducted to identify bacteria that are capable of hydrolyzing urea to produce ammonia and carbon dioxide. The surface of a urea agar slant was streaked with a portion of a well-isolated colony and then incubated at 37 °C for 48 hours and then examined for the development of a pink color [19].

2.9. Screening of Isolates for Amylase Production Ability

The isolates were sub-cultured on a nutrient agar amended with 2% soluble starch (starch nutrient agar) and incubated for 24 hours to initiate growth. The plates were flooded with Gram's iodine for 10 minutes and observed for starch hydrolysis. The amylolytic ability of isolates with a clear zone or yellowish color was then measured [6]. The diameter of the colonies was measured before flooding, and the diameter of the zone of clearance was measured after flooding. Both results were used to calculate the index of amylolytic activity (diameter of clearance zone minus diameter of colony zone divided by diameter of colony zone).

3. RESULTS AND DISCUSSION

3.1. Total Viable Count

The total viable count of the bacterial isolates from the yam waste samples is presented in Table 1. Sample Y4 has the highest bacterial count (microbial load) of 16.8 x 10^8 cfu/g, while sample Y3 has the lowest microbial load of 9.2 x 10^8 cfu/g. The total viable count was found to be very high. These results indicate that yam waste provides a favorable environment for bacterial growth, likely due to its organic composition.

Table 1. Total Viable Count of the Yam Waste Samples (cfu/g)

Samples	Total Viable Count (cfu/g)
Y1	15.2 × 108 cfu/g
Y2	10.8 × 108 cfu/g
Y3	9.2 × 108 cfu/g
Y4	16.8 × 108 cfu/g
Y5	9.6 × 108 cfu/g

Table 2. Morphological Characteristics and Gram's Reaction of Bacterial Isolates

Isolate	Shape	Size	Color	Texture	Elevation	Margin	CS	GR
1	Circular	Medium	Cream	Smooth	Convex	Entire	Bacilli	+
2	Irregular	Medium	Gray	Rough	Convex	Entire	Bacilli	+
3	Circular	Small	Yellow	Smooth	Convex	Entire	Cocci	+
4	Circular	Medium	Red	Smooth	Convex	Entire	Bacilli	-
5	Circular	Medium	White	Mucoid	Convex	Undulate	Bacilli	-
6	Filamentous	Large	Gray	Rough	Convex	Undulate	Bacilli	+

KEY: CS = cell's shape, GR = gram's reaction, + = positive, - = negative

3.2. Morphological Characteristics and Gram's Reaction of the Isolates

Table 2 summarizes the colonial characteristics, including color, shape, size, texture, elevation, and Gram's reaction of the isolates. Among the six isolates, four were identified as gram-positive bacteria, while the remaining two were gram-negative bacteria. The gram-positive bacteria retained the purple stain under the microscope, indicative of their thick peptidoglycan cell wall, while the gram-negative bacteria appeared pink, suggesting a thinner peptidoglycan layer. The presence of both Gram-positive and Gram-negative bacteria suggests a diverse microbial community within the yam waste.

3.3. Biochemical Characteristics and Identification of the Bacterial Isolates

Biochemical tests were performed to identify the isolated bacteria, and the results are presented in Table 3. The suspected organisms were identified as *Bacillus* sp., *Corynebacterium* sp., *Micrococcus* sp., *Serratia* sp., *Klebsiella* sp., and *Clostridium* sp. These organisms were identified based on their colonial characteristics (color, shape, size, texture, and elevation), Gram's reaction, and biochemical test results using Bergey's manual of systemic bacteriology and a standard bacteria identification chart. These organisms have been previously encountered in soil samples, suggesting their presence in yam waste as a contaminant from the soil. For instance, *Bacillus sp*. was isolated and identified from the soil samples in various studies [7, 8, 20, 21]. Similarly, the isolation of *Corynebacterium* sp. aligns with previous findings by Chen *et al.* [22], who identified *Corynebacterium* species in soil samples and Okunwaye *et al*. [23], who identified their own from yam waste. *Micrococcus* sp. was also supported by findings by Abdulkadir and Waliyu [24], and Kawo and Bacha [25], who identified Micrococcus species in soil samples. Furthermore, the isolation of *Serratia* sp. supports previous research by Giri *et al.* [26], and Sethi *et al.* [27], who found *Serratia* species in the soil. *Klebsiella* sp. is commonly found in the gastrointestinal tract of humans. However, their occurrence in yam waste is supported by the work of Shiriki *et al.* [28], who

Table 3. Biochemical Characteristics of Bacterial Isolates

Isolates	CAT	OX	IND	MR	VP	CIT	UR	Susected Organism
1	+	+	-	-	+	+	-	*Bacillus* sp.
2	+	-	-	+	-	-	-	*Corynebacterium* sp.
3	+	+	-	-	-	-	-	*Micrococcus* sp
4	+	-	+	-	+	+	+	*Serratia* sp
5	+	-	-	+	-	+	+	*Klebsiella* sp.
6	-	-	-	+	-	-	+	*Clostridium* sp.

KEY: CAT= catalase, OX= oxidase, IND= indole, MR= methyl red, VR= voges proskauer, CIT=citrate, UR=urease, + = positive, - = negative

Table 4. Amylolytic Ability of Bacterial Isolates

Isolates	Colony Diameter (mm)	Zone of Clearance (mm)	Index of Amylolytic activity (mm)
Bacillus sp.	25	35	0.4
Corynebacterium sp.	27	34	0.26
Micrococcus sp	23	28	0.22
Serratia sp	28	32	0.14
Klebsiella sp.	30	No Zone	NA
Clostridium sp.	24	No Zone	NA

reported the isolation of *Klebsiella oxytoca* from deteriorating yam waste. Finally, the isolation of Clostridium sp. corresponds with findings by Meng *et al.* [29], indicating the presence of *Clostridium* species in soil environments.

3.4. Amylolytic Ability of Bacterial Isolates

Table 4 displays the amylolytic ability of the bacterial isolates. Four isolates exhibited a clear zone of clearance around their colonies on the starch nutrient agar plates, indicating amylase production ability. *Bacillus* sp. displayed the highest hydrolysis with an index of amylolytic activity of 0.40 mm, while *Serratia* sp. showed the lowest hydrolysis with an index of amylolytic activity of 0.14 mm. The two isolates (*Klebsiella* sp*. and Clostridium* sp.) that did not show a zone of clearance on the starch nutrient agar plates indicate that they lack the ability to produce the amylase enzyme. The mechanism behind the zone of clearance of bacteria with the ability to produce amylase enzyme is that when such bacteria are introduced to culture media to initiate growth, they secrete amylase enzyme into their environment as part of their normal metabolic process. Since the media (starch nutrient agar) contains starch, the amylase enzymes secreted by the bacteria break down the starch molecules present in the agar into simple sugars like maltose and glucose. The iodine solution added to the plate after incubation reacts with starch to form a blue-black complex. However, if the starch has been hydrolyzed (broken down to simple sugar) by the amylase enzymes, the iodine will not turn blue-black in the area where starch has been broken into simple sugars. As a result, a clear zone appears around the bacteria's growth where iodine does not turn blue or black, indicating that the bacteria have produced amylase and hydrolyzed the starch. Likewise, the bacteria with no ability to produce amylase enzyme will not secrete enzyme to their environment that will break down starch when they are introduced to the media containing starch. Because of their inability to hydrolyze starch, when iodine solution is added to the agar plate, the iodine will react with intact starch molecules, turning the area around the bacteria's growth blue-black. As a result, no clear zone of clearance is observed around the bacterial growth, indicating that these bacteria have not produced amylase to break down starch. *Bacillus sp*. was identified as the organism with the most amylase production ability, and this is supported by previous research findings [7, 8, 20, 21]. Similarly, the identification of *Corynebacterium* sp. bacteria with amylase production ability is supported by the work of Okunwaye *et al.* [23], and Nsofor and Isaiah [30], who identified *Corynebacterium* sp. as amylase-producing bacteria. Micrococcus sp. has also been reported as a bacterial with amylase production ability by various findings [23, 30], and likewise *Serratia* sp. [27]. The absence of amylolytic activity in *Klebsiella* sp. supports the classification of these bacteria as non-amylase producers, as reported by Fasiku *et al.* [31]. The positive amylolytic activity observed in *Bacillus* sp., *Corynebacterium* sp*., Micrococcus* sp*., and Serratia* sp*.* indicates their potential for industrial applications in enzyme production and also suggests the potential for utilizing yam waste as a source for industrial enzyme production. These bacteria could be further studied for their amylase production efficiency and stability under various conditions. On the other hand, the absence of amylolytic activity in *Klebsiella* sp. and *Clostridium* sp. suggests that these bacteria may not be suitable candidates for enzyme production.

4. CONCLUSION

This study investigated the amylase production potential of bacteria isolated from yam waste. The results revealed a high microbial load in the waste

samples, with diverse bacterial genera identified, including *Bacillus, Corynebacterium, Micrococcus, Serratia, Klebsiella,* and *Clostridium*. These bacteria likely originated in the soil environment. Furthermore, four out of the six identified bacterial isolates exhibited positive amylolytic activity, indicating their potential for industrial enzyme production. *Bacillus* sp*., Micrococcus* sp., *Serratia* sp., *and Corynebacterium* sp. demonstrated significant starch hydrolysis ability, making them promising candidates for further study in enzyme optimization and application. The findings of this study have important implications for waste management practices and biotechnological applications. Utilizing yam waste as a source of microbial enzymes could offer a sustainable and cost-effective solution for enzyme production while simultaneously addressing environmental challenges associated with waste disposal. Future research could focus on optimizing amylase production from the identified bacterial isolates and exploring their potential applications in various industries.

5. RECOMMENDATION

It could be recommended from this study that further research is warranted to explore the diversity of microbial communities in yam waste and their potential for enzyme production. By expanding the scope of microbial isolation and screening efforts, a broader range of enzyme-producing organisms could be identified, leading to the discovery of novel enzymes with unique properties and applications. Additionally, optimization of the fermentation process for enzyme production should be pursued. Fine-tuning parameters such as pH, temperature, agitation, and substrate concentration could enhance enzyme yields and productivity, thereby improving the efficiency of enzyme production from yam waste.

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7. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

8. SOURCE/S OF FUNDING

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