



Molecular Identification of Amylase-Producing Bacteria Isolated from Cassava Soil Sample at Abuja, Northern Nigeria

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Abstract

Background: Amylases, which are essential enzymes responsible for the breakdown of starch into glucose, serve pivotal functions across various industrial sectors, such as the food and pharmaceutical industries. This study focused on identifying amylase-producing bacteria from a soil sample collected at a cassava production site. **Methods:** Soil samples were subjected to serial dilution and cultured on starch agar plates to isolate potential amylase-producing bacteria. Nutrient agar was used as the basal medium, and plates were incubated at 37°C for optimal bacterial growth. Isolates demonstrating amylase activity were identified by the formation of clear zones around the colonies upon exposure to a 1% iodine solution, indicating starch hydrolysis. Enzymatic activity was further quantified using the dinitrosalicylic acid (DNSA) reagent method to assess reducing sugar release. For molecular identification, genomic DNA was extracted from the two isolates exhibiting the highest enzyme activity. The 16S rRNA gene was amplified via polymerase chain reaction (PCR) using universal bacterial primers 27F and 1492R. PCR products were sequenced, and the resulting nucleotide sequences were analyzed using BLAST for species-level identification. **Results:** A total of five bacterial isolates, designated B1 through B5, demonstrated amylase-producing potential as evidenced by clear zone formation on starch agar. Among them, isolates B1 and B3 exhibited the highest levels of enzymatic activity as determined by the DNSA assay. Subsequent molecular identification based on 16S rRNA gene sequencing revealed that isolate B1 shared 99.41% sequence similarity with *Bacillus cereus*, while isolate B3 exhibited 99.88% similarity to *Staphylococcus saprophyticus*. These findings confirm the identity of two high-performing amylase-producing strains iso-

lated from the soil sample. **Conclusion:** The results of this study provide insight into potential sources of amylase which could be exploited for industrial purposes.

Subject Areas

Microbiology

Keywords

Amylase-Producing Bacteria, Molecular Identification, *Bacillus Cereus*

1. Introduction

Enzymes are biological catalysts that accelerate specific biochemical reactions within living organisms by lowering the activation energy required. Their catalytic specificity arises from the unique three-dimensional structure of their active sites, which allows selective binding to their corresponding substrates. Amylase is an enzyme that catalyzes the hydrolysis of starch into smaller carbohydrates such as dextrans and simple sugars, including glucose. Amylase plays a critical catalytic role in the degradation of starch by breaking down its glycosidic bonds, ultimately yielding glucose as the monomeric end product [1]. Amylases represent a group of extracellular enzymes, which are α -amylase, β -amylase, and glucoamylase, that act on starch or oligosaccharide molecules randomly and hydrolyse starchy products into progressively smaller polymers composed of glucose units. Amylases are one of the most important enzymes and account for about 30% of the world's enzyme production [2]. Amylases break down starch into fermentable sugars and have a wide range of applications across various industries, including food, fermentation, textiles, paper, detergents, and sugar production [3]. They also play an important role in biotechnology by helping to remove environmental pollutants, converting starch into desired substrates with the aid of various microorganisms, and processing waste that contains starch [4]. Amylases have been reported to occur in microorganisms (fungi, yeast, bacteria, and actinomycetes), although they are found in plants and animals [5]. The soil harbours various microbes which have advantages over other sources for industrial use [6] [7]. Soil is a rich reservoir of diverse microbial communities, with commonly isolated bacterial genera including *Bacillus*, *Pseudomonas*, *Enterobacter*, *Staphylococcus*, and *Klebsiella*, among others [8]. Cassava production sites, in particular, have been reported to harbor a variety of microorganisms, some of which possess industrially relevant metabolic capabilities [9]. The present study aimed to identify amylase-producing bacteria from soil samples collected at cassava cultivation sites using molecular techniques.

2. Materials and Methods

Soil Collection and Isolation of Bacteria

Random soil samples were collected from the cassava production site (Gwagwalada area). Samples were placed in a clean polythene bag, transported to Sheda Science and Technology, Complex Abuja, and stored in the refrigerator for further analysis. One gram of the soil sample was suspended in 9 mL of sterile distilled water and thoroughly mixed in a test tube. Serial dilutions of the suspension were then prepared up to 10^{-8} and 10^{-5} , following the method described by Bhatt and Singh [10]. In this method, 1 mL of the soil suspension is transferred into a new tube containing 9 mL of sterile diluent, achieving a 10-fold (1:10) dilution. This process was repeated sequentially across multiple tubes to obtain progressively lower concentrations of the original sample, facilitating the isolation of individual microbial colonies. One millilitre (1 ml) of the sample was poured plated separately on a starch medium prepared by using 1% starch slurry in a Nutrient agar as described by Kanimozhi *et al.* [11]. It was then incubated at 37°C for 24 h for the isolation of bacteria. The bacterial isolates on the starch medium were further purified to obtain discrete representative colonies on a new plate containing the starch medium. The plates were incubated at 37°C for 24 hours to obtain pure cultures, which were transferred to a slant and stored in a refrigerator at 4°C [12].

Screening of Isolates for Amylase Activity Using Lugol's Iodine Method

The Lugol's iodine method is used as a qualitative assay to detect the activity of amylase-producing bacteria. Bacterial isolates were cultured on starch agar plates and incubated under optimal conditions. After incubation, the plates were flooded with Lugol's iodine solution. Clear zones surrounding the bacterial colony indicate starch hydrolysis due to amylase activity [13].

Crude Amylase Assay

The purified bacterial isolates were inoculated into starch broth and incubated for 24 hours at 37°C. Following incubation, cultures were centrifuged at 4000 rpm for 20 minutes to obtain cell-free supernatants for enzyme analysis. Amylase activity was quantified using the dinitrosalicylic acid (DNSA) assay, as described by Sundarapandiyam and Jayalakshmi [14] and Al-khafaji *et al.* [15]. The reaction mixture consisted of 1 mL of 1% soluble starch solution, 1 mL of potassium phosphate buffer (pH 6.9), and 0.1 mL of the enzyme-containing supernatant. The mixture was incubated at room temperature for 15 minutes. To terminate the reaction, 2 mL of DNSA reagent was added, and the tubes were placed in a boiling water bath (100°C) for 10 minutes. Reducing sugar released during the reaction was measured spectrophotometrically, indicating the amylase activity. One unit of enzyme activity was defined as the amount of enzyme that hydrolyses 1mg of starch per minute under assay conditions. The reducing sugar liberated was estimated using the dinitrosalicylic acid method (DNSA). One unit of amylase was defined as the amount of 1µmol glucose equivalent per minute under the assay conditions [1].

Molecular Identification of Amylase-Producing Bacteria

Genomic DNA extraction from the two bacterial isolates with the highest am-

ylase activity was performed using the DNA extraction kit following the protocol provided by Zymo Research USA, the manufacturer. Further, DNA was amplified by Polymerase Chain Reaction (PCR) using universal bacterial primers, Forward primer (27 F-AGAGTTTGATCCTGGCTCAG) and Reverse primer (1492r GGTACCTTGTTACGACTT). The PCR was carried out at an initial denaturation step at 94°C for 2 min, followed by 30 cycles at 94°C for 30 sec, 52°C for 30 sec and 72°C for 2 min, and a final extension step at 72°C for 5 min. The RT-PCR products were examined by electrophoresis on a 1% agarose gel [8] [4] [16].

DNA Sequencing and Analysis

The genomic DNA of the bacterial isolates exhibiting the highest amylase activity was subjected to sequencing. The obtained nucleotide sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) to perform similarity searches against the GenBank nucleotide database maintained by the National Center for Biotechnology Information (NCBI). Taxonomic identification was based on the highest sequence similarity with reference strains in the database [17].

3. Result

Description of Study Area

Soil samples were collected from a cassava-producing site in the Gwagwalada Area of Abuja. Gwagwalada, Abuja, Northern Nigeria. Abuja experiences a tropical savanna climate with two distinct seasons, which are the rainy season and the dry season. The rainy season lasts from April to October, with the peak rainfall occurring from July to September, while the dry season occurs from November to March, and it is usually accompanied by Harmattan winds from December to February [18]. The Temperatures in this area range from 30°C to 37°C during the day and from 18°C to 24°C at night, with the hottest period from March to May. Gwagwalada Area of Abuja lies at an altitude of approximately 150 to 300 meters above sea level and has an average annual rainfall of about 1100 to 1600 millimetres.

Isolation of Amylase-Producing Bacteria

The Soil samples were serially diluted to isolate bacteria, spread on a starch agar medium, and incubated at 37°C for 24 hours. A dense, thick population of mixed bacterial colonies originating from a dilution factor of 10^{-5} was observed on the starch agar plates. A pure culture of 5 bacterial isolates (B1 to B5) was obtained as shown in **Figure 1**, which were randomly picked up from the mass bacterial population based on their colony morphology.

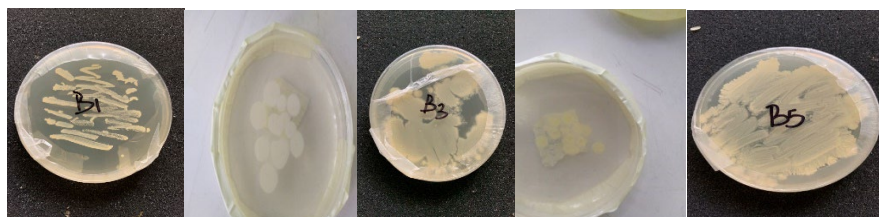


Figure 1. Bacterial isolates from the cassava production site.

Screening for Amylase Activity

The bacterial isolates were assessed for amylolytic activity using starch agar medium. A distinct, clear zone surrounding the bacterial colonies, as shown in **Figure 2**, indicated positive starch hydrolysis. This clear zone results from the enzymatic degradation of starch by amylase, producing a starch-free region. In contrast, the unhydrolyzed starch in the surrounding medium produced a characteristic dark blue to purple coloration upon iodine application. Over time, the diameter of the clear zones increased, suggesting continued amylase activity and progressive starch degradation by the bacterial isolates.



Figure 2. Screening for amylase-producing microbes using Lugol's iodine.

Enzyme Activity Determination of Amylase-Producing

The Enzyme activity was estimated using the dinitrosalicylic acid (DNSA) reagent method, and the results are presented in **Table 1**. Among the tested isolates, bacterial isolate B1 exhibited the highest amylase activity, indicating strong starch-degrading potential. Isolate B2 also demonstrated relatively high enzyme activity, though slightly lower than B1. In contrast, isolate B4 showed the lowest amylase activity, suggesting comparatively reduced amylolytic capacity among the evaluated samples.

$$\text{Amylase activity } (\mu\text{mol}/\text{min}/\text{ml}) = \frac{\text{Concentration of product of Rxn} \times \text{Total Volume}}{\text{Reaction time (min)} \times \text{Enzyme Volume (ml)}}$$

Table 1. Showing enzyme concentration in each sample of bacteria isolates (B1 - B5).

Samples	Enzyme concentration (μ/ml)
B1	10.89
B2	6.80
B3	9.75
B4	6.89
B5	8.90

Molecular Identification of the Amylase-producing bacteria the earlier NCBI database. The result revealed *B. cereus* 99.41 % and *S. saprophyticus* 99.88% nucleotide similarities.

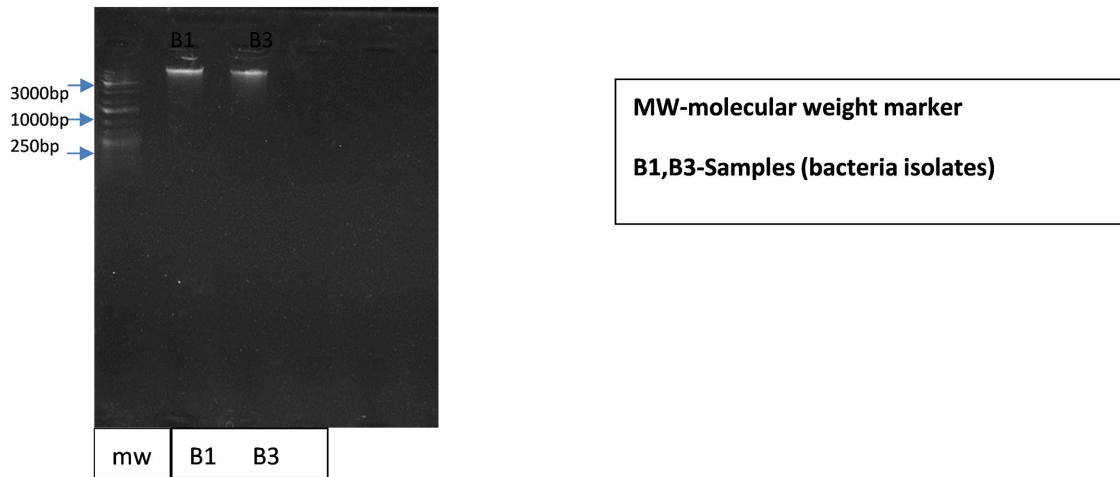


Figure 3. Agarose gel electrophoresis identifying the bacterial amplicons of amylase-producing bacteria.

Sequences producing significant alignments Download Select columns Show 100

select all 100 sequences selected GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<input checked="" type="checkbox"/> Bacillus cereus strain NG-IB-S6 16S ribosomal RNA gene, partial sequence	Bacillus cereus	1522	1522	100%	0.0	99.41%	1524	PP819524.1
<input checked="" type="checkbox"/> Bacillus cereus strain AJWB 16S ribosomal RNA gene, partial sequence	Bacillus cereus	1517	1517	100%	0.0	99.29%	1514	PP938879.1
<input checked="" type="checkbox"/> Bacillus cereus strain IFN14 16S ribosomal RNA gene, partial sequence	Bacillus cereus	1517	1517	100%	0.0	99.29%	1515	PQ340327.1
<input checked="" type="checkbox"/> Bacillus cereus strain SB4 16S ribosomal RNA gene, partial sequence	Bacillus cereus	1513	1513	100%	0.0	99.17%	1521	PP239587.1
<input checked="" type="checkbox"/> Bacillus sp. (in: firmicutes) strain KCN24 16S ribosomal RNA gene, partial sequence	Bacillus sp. (in: firmicutes)	1513	1513	99%	0.0	99.40%	906	MH141430.1
<input checked="" type="checkbox"/> Bacillus thuringiensis strain CUAB-GB13 16S ribosomal RNA gene, partial sequence	Bacillus thuringiensis	1513	1513	100%	0.0	99.17%	895	QR114675.1
<input checked="" type="checkbox"/> Uncultured bacterium clone EGSB_200_5-12 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1511	1511	99%	0.0	99.28%	1515	KJ881344.1
<input checked="" type="checkbox"/> Bacterium Te30A 16S ribosomal RNA gene, partial sequence	bacterium Te30A	1511	1511	99%	0.0	99.28%	1512	AY587784.1
<input checked="" type="checkbox"/> Uncultured bacterium clone EGSB_200_5-5 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1511	1511	99%	0.0	99.28%	1514	KJ881337.1
<input checked="" type="checkbox"/> Bacillus sp. W-21 16S ribosomal RNA gene, partial sequence	Bacillus sp. W-21	1511	1511	100%	0.0	99.05%	1440	AF390088.1
<input checked="" type="checkbox"/> Bacillus cereus strain GXNN20201206-6 16S ribosomal RNA gene, partial sequence	Bacillus cereus	1511	1511	99%	0.0	99.28%	1510	MZ443931.1
<input checked="" type="checkbox"/> Bacillus cereus strain GXBC-3 16S ribosomal RNA gene, partial sequence	Bacillus cereus	1511	1511	99%	0.0	99.28%	1521	JX218990.1

(a)

<input checked="" type="checkbox"/> Staphylococcus saprophyticus subsp. bovis strain MM1-9 16S ribosomal RNA gene, partial sequence	Staphylococcus...	1528	1528	99%	0.0	99.88%	1440	JX144949.1
<input checked="" type="checkbox"/> Uncultured bacterium clone ncd2038h07c1 16S ribosomal RNA gene, partial sequence	uncultured bacte...	1528	1528	99%	0.0	99.88%	1372	JF168070.1
<input checked="" type="checkbox"/> Staphylococcus saprophyticus strain Colony294 chromosome	Staphylococcus...	1528	9125	99%	0.0	99.88%	2516640	CP075502.1
<input checked="" type="checkbox"/> Staphylococcus saprophyticus strain FC2981 16S ribosomal RNA gene, partial sequence	Staphylococcus...	1528	1528	99%	0.0	99.88%	1511	MG871251.1
<input checked="" type="checkbox"/> Staphylococcus saprophyticus strain GDY8P168P chromosome, complete genome	Staphylococcus...	1528	9142	99%	0.0	99.88%	2651289	CP065797.1
<input checked="" type="checkbox"/> Staphylococcus sp. KC1 16S ribosomal RNA gene, partial sequence	Staphylococcus...	1528	1528	99%	0.0	99.88%	1481	JQ433926.1
<input checked="" type="checkbox"/> Bacterium strain A53A1 16S ribosomal RNA gene, partial sequence	bacterium	1528	1528	99%	0.0	99.88%	850	MK506704.1
<input checked="" type="checkbox"/> Staphylococcus sp. strain G-MEP 16S ribosomal RNA gene, partial sequence	Staphylococcus...	1528	1528	99%	0.0	99.88%	1471	OQ540819.1
<input checked="" type="checkbox"/> Staphylococcus edaphicus strain ACU71 16S ribosomal RNA gene, partial sequence	Staphylococcus...	1528	1528	99%	0.0	99.88%	1031	OQ996812.1
<input checked="" type="checkbox"/> Staphylococcus saprophyticus strain RPWL12 16S ribosomal RNA gene, partial sequence	Staphylococcus...	1528	1528	99%	0.0	99.88%	1417	MF185372.1
<input checked="" type="checkbox"/> Staphylococcus edaphicus strain 0511MAR21U1 16S ribosomal RNA gene, partial sequence	Staphylococcus...	1528	1528	99%	0.0	99.88%	1031	MZ338977.1
<input checked="" type="checkbox"/> Staphylococcus sp. strain Atecer5C 16S ribosomal RNA gene, partial sequence	Staphylococcus...	1528	1528	99%	0.0	99.88%	1408	MT386153.1
<input checked="" type="checkbox"/> Staphylococcus saprophyticus strain AUCASVE3 16S ribosomal RNA gene, partial sequence	Staphylococcus...	1528	1528	99%	0.0	99.88%	1441	JQ043188.1

(b)

Figure 4. Genbank data for the Samples B1 and B3 showing nucleotide similarities (a) Gene bank data for B1 - *B. cereus* shows a 99.41% similarity score. (b) Gene bank data for B3 - *S. saprophyticus* shows a 99.88% similarity score.

4. Discussion

Amylases are essential industrial enzymes widely utilized in the hydrolysis of starch into glucose, with significant applications in the food, pharmaceutical, and fermentation industries. In the present study, five bacterial isolates (designated B1 - B5) were recovered from soil samples collected at a cassava production site using starch agar medium (Figure 3 & Figure 4). To isolate potential amylase producers, soil samples were subjected to serial dilution and plated on starch agar, with nutrient agar used as the basal growth medium. Plates were incubated at 37°C for 24 - 72 hours. Amylase activity was screened using a qualitative plate assay involving flooding with 1% Lugol's iodine solution. The formation of distinct, clear zones around bacterial colonies indicated starch hydrolysis, while unaffected areas stained dark blue, confirming the presence of residual starch. This approach is consistent with the method described by Hossain [13], who utilized Lugol's iodine flooding to identify amylase-producing bacteria from agricultural soils.

Quantitative estimation of enzymatic activity confirmed amylolytic potential in all isolates, with B1 and B3 exhibiting the highest levels of activity. 16S rRNA gene sequencing identified isolate B1 as *Bacillus cereus* with 99.41% nucleotide sequence similarity, while isolate B3 was identified as *Staphylococcus saprophyticus* with 99.88% similarity. The identification of *B. cereus* is consistent with previous studies highlighting the genus *Bacillus* as a prolific producer of industrially relevant enzymes [15] [16]. Semira *et al.* [19] reported the isolation of thermostable amylase-producing *Bacillus* species from soil, while Abd-Elhalim *et al.* [20] confirmed the high α -amylase activity of *B. cereus* from similar environments. [21] further demonstrated the beneficial role of *Bacillus thuringiensis* in improving seed germination and plant growth. Although *Staphylococcus saprophyticus* is less commonly reported as an amylase producer, its identification in this study as a high-performing isolate expands the diversity of microbial sources for amylase production and offers new opportunities for biotechnological exploitation. These findings emphasize the potential of cassava cultivation environments as reservoirs of valuable enzyme-producing microorganisms. Further research should focus on optimizing culture conditions for maximum enzyme yield, scaling up fermentation processes, and characterizing the biochemical properties of the amylases. Such efforts will support the development of efficient and sustainable starch bio-conversion strategies for industrial applications.

5. Conclusions

This study reveals the untapped potential of soil from cassava production environments as a rich reservoir of industrially relevant microorganisms, particularly amylase-producing bacteria. Among the isolates identified, *Bacillus cereus* and *Staphylococcus saprophyticus* exhibited the highest amylase activity, highlighting their strong potential for biotechnological exploitation. These strains represent promising candidates for the sustainable, large-scale production of amylase enzymes, which are integral to diverse industrial sectors including food processing,

pharmaceuticals, biofuels, textiles, and paper manufacturing. Additionally, their use in enzyme-based detergent formulations offers an environmentally friendly alternative to conventional chemical surfactants.

Given the increasing global demand for sustainable and cost-effective enzyme sources, the identification of *B. cereus* and *S. saprophyticus* as efficient amylase producers presents a valuable opportunity for commercial enzyme development. Future work should focus on optimizing culture conditions, employing genetic or metabolic engineering strategies, and scaling up fermentation processes to maximize enzyme yield and industrial applicability.

Authors' Contribution

All authors contributed to the concept and design of this work.

Conflicts of Interest

The authors declare no conflicts of interest.

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