*Biotechnology Journal International*

***19(1): 1-14, 2017; Article no.BJI.33985***
***ISSN: 2456-7051***
*(Past name: British Biotechnology Journal, Past ISSN: 2231–2927, NLM ID: 101616695)*

# Properties of Alpha-amylase of *Lactobacillus plantarum* Isolated from Cassava Waste Samples

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author AAO designed managed the analyses and supervised the study. Author GSA wrote the protocol, wrote the first draft of the manuscript and carried out the laboratory analysis. Authors CE and GSA managed the literature searches, assisted in sample collection. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/BJI/2017/33985
Editor(s):
(1) Mahalingam Govindaraj, ICRISAT, Patancheru, India.
Reviewers:
(1) Ashabil Aygan, Kahramanmaras Sutcu Imam University, Turkey.
(2) Elif Demirkan, Uludag University, Turkey.
(3) Abigail I. Ogbonna, University of Jos, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/20261

***Original Research Article***

***Received 8th May 2017***
***Accepted 20th June 2017***
***Published 28th July 2017***

## ABSTRACT

The search for various industrial enzymes including starch degrading enzymes has received a great deal of attention for their perceived technological and economic benefits. Alpha-amylase has been produced from large varieties of organisms including pathogenic ones which are not Generally Regarded as Safe (GRAS). In this study, a total of Thirty nine (39) lactic acid bacteria were isolated from the different cassava waste samples (peels, cassava waste water, fibre and soil). The isolates were screened for their ability to produce α-amylase on starch agar. The amylolytic activity of the selected isolates was determined using starch-iodine complex while the enzymes were characterized based on parameters such as pH, temperature, substrate concentration, and metal ions. Upon starch hydrolysis, 29.9% of the LAB isolates demonstrated amylolytic properties with *L. plantarum* having the highest occurrence. The selected amylase producer was identified both phenotypically and molecularly as *L. plantarum* S7. Enzyme production was found to be highest (10.573U/ml) at 30 hours of incubation. Optimum temperature and pH for the enzyme activity was found to be 40°C and 7 respectively. The α-amylase retained more than 50% of it residual activity at pH 7 and 8 after preincubation for 90mins. Calcium chloride

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($CaCl_2$) and Ammonium disulphate ($NH_4$ $(SO_4)_2$) enhanced amylase activity however, the activity was inhibited at 5mM molar concentration by KCl, $BaCl_2$, $CuSO_4$, NaCl, EDTA (10%) and Urea (10%). The genomic level confirmation was done with 16s rDNA and the sequence showed 90% sequence similarity with *L. plantarum*.

*Keywords: Lactobacillus plantarum; Cassava wastes; amylase; amylolytic lactic acid bacteria.*

## 1. INTRODUCTION

Wastes are found to harbor heavy load of microorganisms and recent advances in industrial biotechnology have contributed to an improved and economic production of various industrial enzymes with starch degrading enzymes receiving great deal of attention. Several processes have been developed that utilize agro-industrial residue including cassava wastes for the production of bulk chemicals and value-added fine products such as ethanol, Single cell protein, enzymes, organic acids, amino acids and biologically active secondary metabolites [1].

Cassava is a perennial woody shrub cultivated for its starchy storage and remains an Africa's most important staple food crop. Africa produces more than half of the global supply of cassava with the annual production from Nigeria alone (about 45 metric tons) representing more than a third of the total African output [2]. In Nigeria, cassava is usually processed into gari, fufu, tapioca, cassava chips, cassava flour and starch which serve as the major source of carbohydrate for more than 60% of the population. In the course of cassava processing, large amount of wastes are generated. These wastes including cassava peels, fibre, and the wastes water are usually discharged indiscriminately thereby constituting nuisance in the environment. Cassava peel, which is a rich source of fermentable sugars [3] harbors heavy loads of microorganisms as a result of its essential elements and chemical composition which undoubtedly favour the growth and proliferation of microbial cells. These microorganisms and the waste stream rich in starch content and organic nutrient can be very useful for the production of valuable byproducts including amylase.

Amylase is an important and biotechnologically relevant enzyme, constituting a class of industrial enzymes having about 25% of the world enzyme market. Amylases are a group of enzymes that have been found in several organisms including bacteria and fungi. These groups of enzymes have found applications in various industries and this has stimulated interest in the exploration of extracellular enzymatic activity in several microorganisms [4]. Recent work by Avwioroko et al. [5] has revealed that amylase-degraded cassava peels may be good substitutes for maize in poultry feed formulation due to its cost-effectiveness and the rich content of starch, protein and some minerals in cassava peels.

The demand for amylase of microbial origin is ever increasing owing to their application in various industries such as pharmaceutical, detergent, food, textile, baking and starch conversion industries. Strains of lactic acid bacteria with starch degrading activities have been said to be very rare and only three genera (*Lactobacillus*, *Lactococcus* and *Streptococcus*) of Lactic Acid Bacteria (LAB) are reported to produce lactic acid from starch as carbon source [6]. Amylolytic LAB are basically distributed in fermented starchy products including fermented cassava, sorghum and maize. They have been implicated in several cereal beverages such as maize pazol [7], sorghum beer, beer malt and beer wort. Report by Sanni et al. [8] also revealed the presence of amylolytic *L. plantarum* and *L. fermentum* in Nigerian Ogi and mawe. Although ALAB have been isolated majorly from fermented food product, much work is still required to isolate new ALAB of industrial importance from other sources including cassava wastes.

*L. plantarum* is generally found in environment with high level of carbohydrate and has been used extensively in various fermentation processes since they belong to the group of Generally Regarded as Safe (GRAS) organisms. Alpha-amylase has been produced from a large variety of organisms some of which are not regarded as safe, only a few reports is available on amylase production by *L. plantarum*. Amylase that will find application in food industries is expected to be produced from organism that has no potential to produce toxic substance along with metabolite of interest. Since *L. plantarum* falls into the GRAS status, amylase production

from such organism would not only reduce cost incur from toxicity and other test carried out during production but will also gain acceptance for use in food and other industries. Therefore, this research work is aimed at isolation of amylolytic LAB from cassava wastes and to evaluate the amylase activity of the LAB.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

Four cassava waste samples (waste water, fibre, peels and soil) were collected from different locations in Ibadan, Oyo State. The samples were collected in clean sterile bottles and transported to the Microbiology Laboratory of the University of Ibadan, for analysis.

#### 2.1.1 Isolation of lactic acid bacteria

For the waste water sample, 1 ml was aseptically introduced into 9 ml of sterile distilled water and mixed properly. For other solid wastes (cassava fibers, peels and soil), 1 g of each samples was weighed and introduced into10 ml sterile distilled water [9]. They were serially diluted to appropriate dilution factors ($10^4$-$10^6$) and 0.1 ml of the diluents was pour-plated using de Man Rogosa Sharpe (MRS) medium. The plates were incubated anaerobically in an anaerobic jar at 37°C for 48 hours. Representative discrete colonies developing on the plates were picked and purified by repeated sub culturing. Pure cultures were maintained on appropriate slants which were kept as stock culture under refrigeration temperature (4°C).

#### 2.1.2 Characterization and identification of isolates

Isolates were characterized based on basic microbiological techniques. The morphological characters examined include appearance of colony, Gram's reaction and motility test. The biochemical tests carried out include catalase test, methyl red test, Voges Proskauer, starch hydrolysis, sugar fermentation, Homo/ Hetero-fermentative Test, Growth in 4% and 6.5% sodium chloride broth, Arginine test, oxygen relation, Simmon's citrate slant test, urease production, casein hydrolysis, indole production, oxidase test as well as a test to determine oxidative or fermentative metabolism [10,11]. Further identification was done using molecular technique.

#### 2.1.3 Molecular characterization based on 16S rRNA sequence

The DNA sample was run on an agarose gel and the $3 \leq x \leq 5$ kb amplification product was extracted from agarose gel (Fig. 7). Single band was visualized when observed under the Gel, which confirmed the purity of sample, as the bands of DNA were single, distinct and no traces of contaminants were observed. Then, sequencing of the 16S rRNAgene of bacterium was done and the same was amplified by Taq DNA polymerase along with the DNA marker. This was then subjected to agarose gel electrophoresis. The sequence obtained was then blasted in NCBI database.

#### 2.1.4 Phlyogenetic tree analysis

Nucleotide sequence was compared to those in the Gene Bank database and the tree was generated by the neighbour joining [12] algorithm implemented in phydit.

#### 2.1.5 Inoculum size determination

One ml of the seed culture after 24 hours of incubation was stained with few drops of Lactophenol blue. Neubaurer haemocytometer was used to count the number of cells per milliliter.

### 2.2 Screening for α-amylase Production

The ability of isolates to hydrolyzed starch was observed using modified de Man Rogosa Sharpe (MRS) Agar. MRS starch composition used include (g/1): soluble starch, 20; bactopeptone, 10; beef extract., 10; yeast extract (Difco), 5; diammonium hydrogenocitrate, 2; anhydrous sodium acetate, 5; $K_2HPO_4$, 2; $MgSO_4.7H_2O$, 0.20; $MnSO_4.4H_2O$, 0.2 and Agar, 15. After incubation for 72 hours, the plates were covered with Gram's iodine solution and observed for the clear zone surrounding the isolates [7]

#### 2.2.1 Alpha-amylase production and extraction

The amylase was produced using modified method of Oyeleke and Oduwole [13]. Submerge fermentation was used for the production of α-amylase. Modified MRS containing (g/l) soluble starch, 20; bactopeptone, 10; yeast extract (Difco), 5; diammonium hydrogenocitrate, 2; anhydrous sodium acetate, 5; $K_2HPO_4$, 2; $MgSO_4.7H_2O$, 0.20; $MnSO_4.4H_2O$, 0.2 was used

for enzyme production. The media were sterilized using autoclave at 121°C for 20 minutes. The fermenting medium was inoculated with 5% (v/v) culture containing $2.6x10^6$ cfu/ml bacteria precultured on MRS agar for 24 hours and incubated for 24 hours at 37°C in a rotatory shaker. The cells were removed by centrifugation at 15000 rpm using Hettich Zentrifugen (MIKRO 220R) for 10 minutes at 4°C. The resulting cell-free supernatant was used as the crude enzyme.

## 2.3 Assay for α-Amylase Activity

Extracellular α-amylase activity was assayed in the supernatant fluid of samples collected during starch fermentation, by measurement of the iodine-complexing ability of starch at pH 6.0 and 35°C using modified method previously described by Giraud et al. [14]. 0.1 millilitre of the crude enzyme was incubated with 0.8 ml of solution containing 0.03% soluble starch dissolved in phosphate buffer for 30 minutes. The reaction was stopped with 0.1 ml of 0.1 M $H_2SO_4$. 2.4 ml of iodine mixture was added to the reaction mixture and absorbance was measured with Lamda 25 UV Spectrophotometer at 620 nm wavelengths. One unit of enzyme activity is defined as the amount of enzyme that permits the hydrolysis of 10 g of starch in 30 minutes under the specified condition.

## 2.4 Characterization of the Crude Enzymes

Characterization of the enzyme was done using the modified method of Renu et al. [15]

### 2.4.1 Optimum pH determination

The pH optimum of crude α-amylase was determined by measuring its activity at pH values ranging from 3-10 using 100 mM citrate buffer (3-5), 100 mM sodium phosphate buffer (6-8), and 100 mM carbonate buffer (9-10). The effective pH value of the reaction mixture was measured after incubating the enzyme solution with respective buffers.

### 2.4.2 pH stability

The pH stability of the enzyme was determined by mixing equal volume of crude enzyme with buffer at different pH values (3-10). The mixture was preincubated for 45, 60 and 90 mins at 40°C after which the residual amylase activities were determined according to the standard assay condition.

### 2.4.3 Optimum temperature determination

The temperature optimum of the enzyme was determined using the method in which the assay mixtures were incubated for 30 minutes at different temperature ranging from 30°C-90°C in 100 mM phosphate buffer at pH 7.

### 2.4.4 Thermal stability

Thermal stability of the enzyme was determined by incubating the enzyme at 60°C for 2 hours. Samples were taken at 10 minutes interval and the residual enzyme activity determined.

### 2.4.5 Effects of metal ions and other reagents on α-amylase

The effect of metal ions and other reagents on α-amylase activity was determined by assaying for residual activity after incubating equal volume of the crude enzyme and each metal ion for 30 minutes at 40°C. The metal ions and reagents used include; $K^+$, $Na^+$, $Ca^{2+}$, $Fe^{3+}$, $Cu^{2+}$, $Mg^{2+}$, $NH_4^{2+}$, $Ba^{2+}$, EDTA and Urea at 5mM concentration [15].

# 3. RESULTS

## 3.1 Morphological and Biochemical Characteristics

A total number of 39 lactic acid bacteria were obtained from different cassava waste samples. Soil sample had the highest occurrence of LAB followed by cassava waste water, the least LAB occur in cassava peels as shown in Fig. 1. Upon screening (starch hydrolysis), 11 (28.9%) of the LAB isolates demonstrated amylolytic properties. The amylolytic lactic acid bacteria (ALAB) showed differences in colony colour, physiological characteristics (pH and temperature of growth). The ALAB *Lactobacillus plantarum* was identified based on the morphological, cultural, physiological and biochemical characteristics. The cells were Gram-positive, non-motile, catalase-negative and non-spore forming. They occurred in short rods, singly and in pairs. The isolate grew at 15°C but no growth was observer at 45°C. The isolate ferments trehalose, Salicin, Malobiose, Raffinose, Sorbitol, Sucrose, Lactose, Galactose, Glucose, Arabinose and mannitol. They do not ferment Sorbose, Xylose as described in Table 1.

**Table 1. Biochemical characteristics of the lactic acid bacteria isolates**

| isolates | Gram reaction | Catalase | Gas from Glucose | Arginine | Oxidase | Growth at 4.5% NaCl | Growth at 6.5% NaCl | Growth at 45$^0$C | Methyl Red test | Spore staining | Arabinose | Xylose | Sorbose | mannitol | Glucose | Galactose | Lactose | Sucrose | Sorbitol | Raffinose | Malobiose | Salicin | Trehalose | Probable isolates |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P4 | + rod | - | + | + | - | + | + | - | + | - | + | + | + | + | + | + | + | + | - | - | - | - | + | *Lactobacillus* sp. |
| F11 | +roe | - | - | + | - | + | - | - | + | - | + | - | - | + | + | + | + | + | + | + | - | - | + | *Lactobacillus* sp. |
| S1 | + rod | - | - | - | - | - | + | + | + | - | + | - | - | + | + | + | + | + | + | - | + | + | - | *Lactobacillus* sp. |
| F4 | + rod | - | - | - | - | - | + | - | + | - | + | - | - | + | + | + | + | + | - | + | + | + | - | *L. plantarum* |
| W6 | + rod | - | + | + | - | + | - | - | + | - | + | - | | + | + | + | + | + | + | + | + | + | + | *Lactobacillus amylovorus* |
| S2 | + rod | - | + | + | - | + | _ | + | + | - | + | - | | - | + | + | + | + | - | + | + | - | + | *L. fermentum* |
| W3 | + rod | - | + | - | - | - | - | + | + | - | + | + | + | + | + | + | + | + | - | - | + | + | - | *Lactobacillus* sp. |
| S5 | + rod | - | + | + | - | - | - | + | + | - | + | - | + | + | + | + | + | + | - | + | + | - | - | *L. fermentum* |
| F6 | + rod | - | - | + | - | - | + | - | + | - | | - | | - | + | + | + | + | - | + | + | - | + | *L. manihotivorans* |
| S7 | + rod | - | - | - | - | - | - | - | + | - | + | - | - | + | + | + | + | + | + | + | + | + | + | *L. plantarum* |
| P2 | +rod | - | - | - | - | - | - | - | + | - | + | - | - | + | + | + | + | + | + | + | - | + | + | *L. plantarum* |
| W8 | + rod | - | + | + | - | + | + | - | + | - | + | - | | - | + | + | + | | - | + | + | - | + | *L. fermentum* |

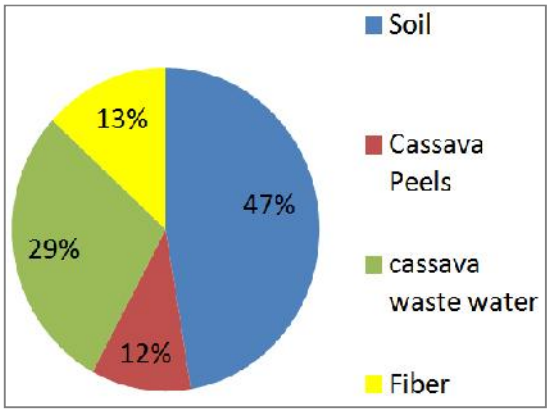


**Fig. 1. Frequency of occurrence of lactic acid bacteria from cassava waste samples**

*Physiological characteristics of α-amylase produced by L. plantarum S7*

### 3.2 Molecular Characterization of the Isolate

Based on the 16s rRNA sequences, the bacterium was confirmed as *L. plantarum.* The 16srDNA sequence exhibiting a percentage of similarity of 90% was considered for species authentication (Fig. 8).

### 3.3 Effect of pH and Temperature on Amylase Activity

Fig. 2 showed the pattern of amylase activity at different pH. The relative activity experienced a gradual increase from pH of 3.0 to reach a peak of 100% at pH 7. Thereafter, a gradual drop in relative activity was observed until it reached a relative activity less than 50% at pH of 10.0. Fig. 3 shows the effect of temperature on α-amylase activity and relative activity of α-amylase experienced a gradual increase as the temperature increases to reached optimum at 40°C. Further increase in temperature resulted in gradual decrease in relative activity to about 10% at 70°C.

### 3.4 pH and Thermal Stability of the Enzyme

Fig. 4 shows the pH stability of the amylase at different pH levels after preincubation for 45, 60 and 90 mins at 40°C. The enzyme retained about 95.66% of its activity at pH 7 after preincubation for 60 mins. The enzyme retained above 50% of its activity at pH 7 and 8 whereas the lowest residual activity was observed at pH 3 retaining about 2.27% residual activity after preincubation of 90 mins. Fig. 5 shows the thermal stability of the amylase pre-incubated at 60°C for 1 hour. It was observed that as the incubation period increases residual activity decreases.

### 3.5 Effect of Metal Ions and Some inhibitors on the Activity of α-amylase Activity

The amylase activity was enhanced by $CaCl_2$ and $NH_4$ $(SO_4)_2$ however, the activity was inhibited at 5mM molar concentration by KCl, $BaCl_2$, $CuSO_4$, NaCl, EDTA (10%) and Urea (10%).

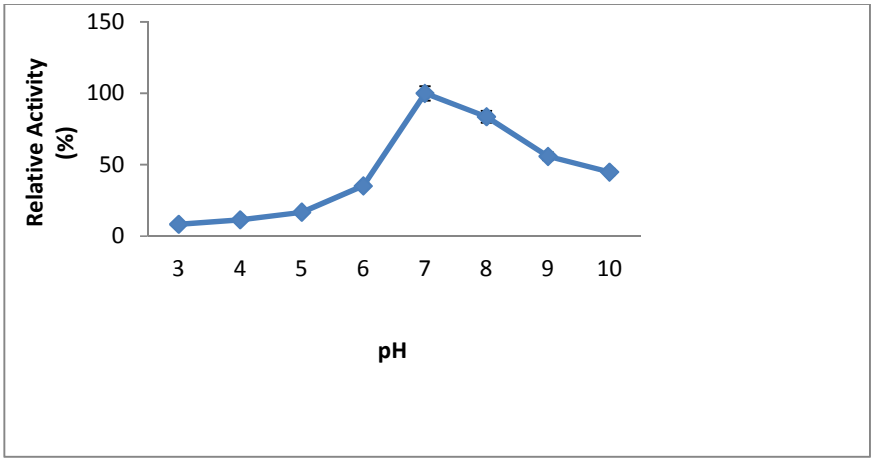


**Fig. 2. Effect of pH on α-amylase activity produced from *L. plantarum* S7**

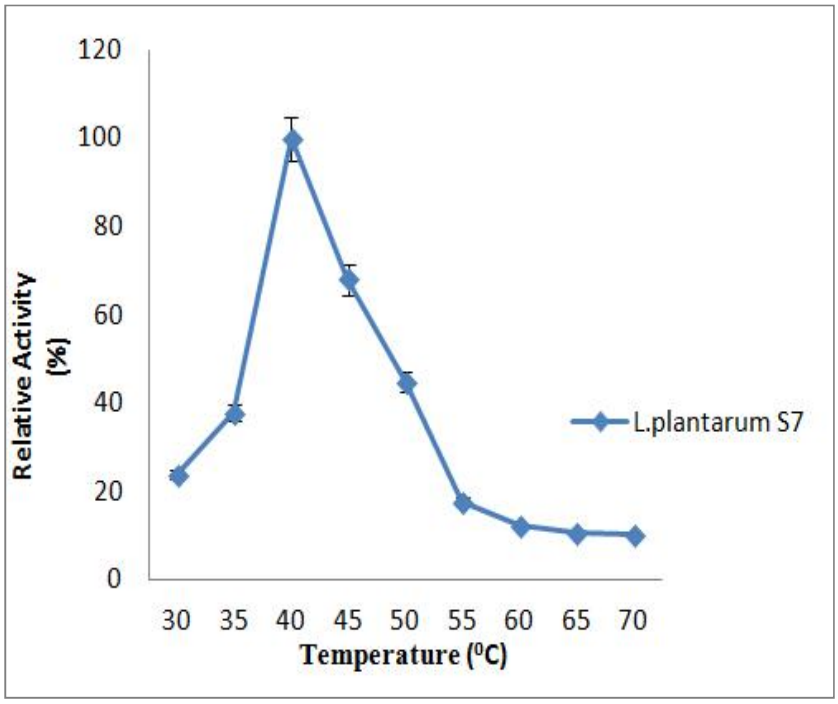


**Fig. 3. Effect of temperature on α-amylase activity**

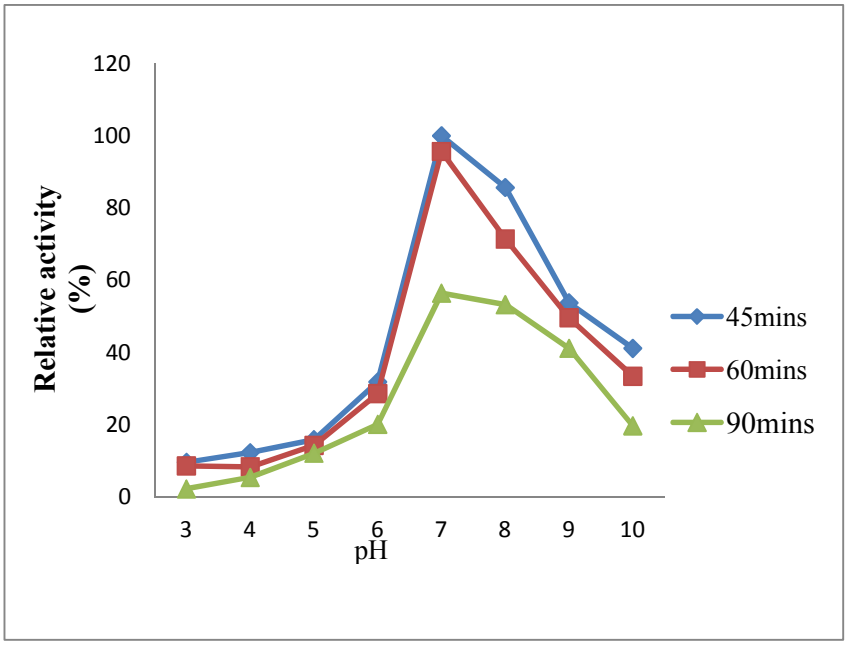


**Fig. 4. pH stability of α-amylase produced by *L. plantarum* S7**

## 4. DISCUSSION

The ecological flexibility of certain LAB species, such as *L. plantarum*, or their specificity to certain environments is due to their capacity to metabolize and/or transport different types of carbohydrates and/or to modulate their metabolic pathways [16]. Given that starch is a carbon and energy source, only a few LAB are able to use this substrate. Nonetheless, amylolytic LAB

(ALAB) are common members of the microbiota of amylaceous fermented foods [17]. Though the conditions of selected site for study is favorable for the growth of lactic acid bacteria and better niche for moderate isolation of amylolytic lactic acid bacteria but the results with screening of amylases production shows the variability and potentiality among isolated *LAB.* Partial starch hydrolysis by α-amylase to liquefy starch is a potentially useful characteristic that could be exploited to improve the energy density of gruels used for the complementary feeding of young children [18], or fermented to produce ethanol [19].

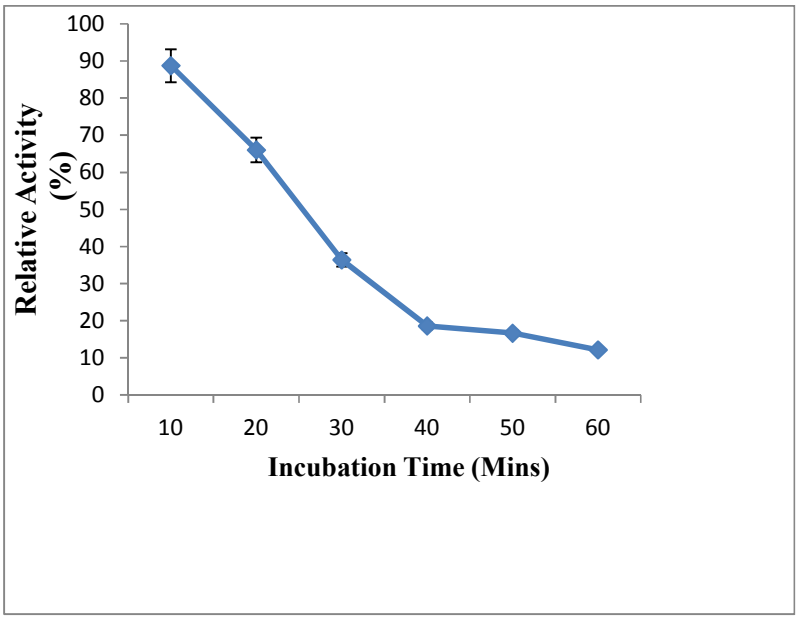


**Fig. 5. Thermal stability of α-amylase residual activity**

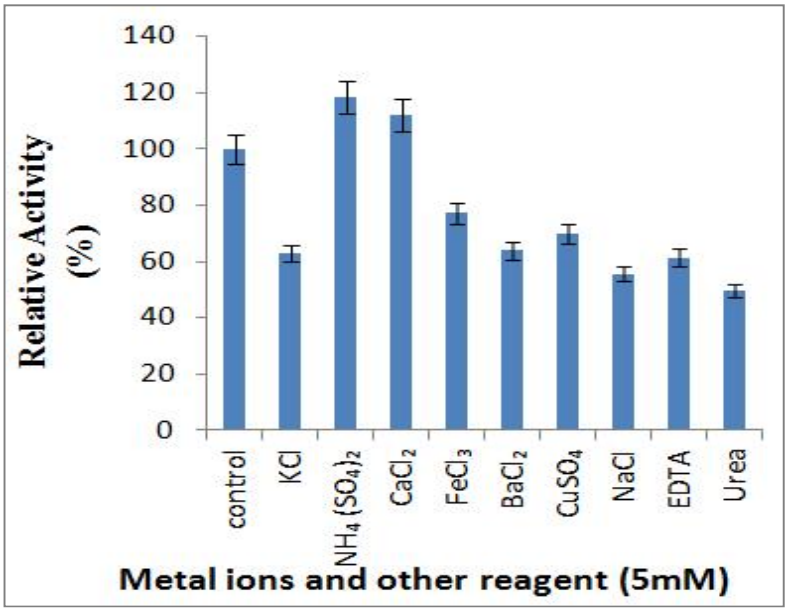


**Fig. 6. Effect of metal ions and other reagents on the activity of α-amylase produced from *L. plantarum* S7**

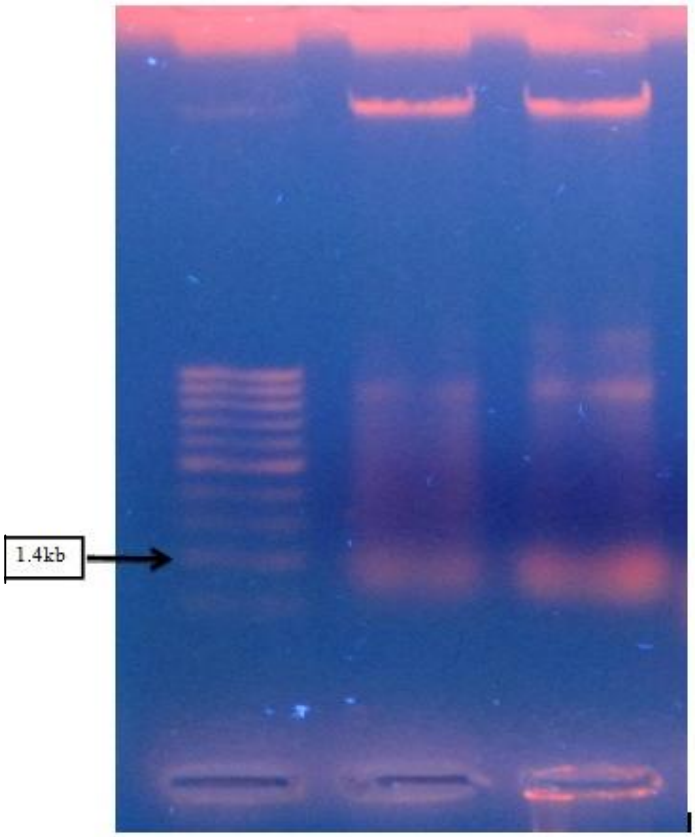


**Fig. 7. Agarose gel electrophoresis product of the isolates**

The isolation of amylolytic lactic acid bacteria from cassava based products has been reported by different workers [20]. Twelve amylolytic Gram-positive anaerobic bacteria isolated from different cassava waste samples were selected for their ability to hydrolyse soluble starch on agar plates. This is in agreement with the work of Songre-Ouattara et al. [21] ho preselected 30 isolates out of which 11 were amylolytic with *L. plantarum* having the highest hydrolysis.

Based on morphology, physiology and metabolic properties, the LAB isolates were characterized as *Lactobacillus*. Strains of amylolytic *L. plantarum* have been isolated from cereal based foods [8] and African cassava-based fermented products [22]. Only few reports are available on the isolation of ALAB from cassava wastes such as peels, waste water and fibrous tissue. Reports have shown that most of the amylolytic lactic acid bacteria isolated from starchy foods are homofermentative *Lactobacillus*. For instance, among 17 ALAB isolated from ogi, only one was heterofermentative (*Leuconostoc mesenteroides*) the others were *L. plantarum* [19].

Temperature and pH are the most important factors which markedly influence enzyme activity [23]. The properties of each α-amylase such as thermostability, pH profile, pH stability, and Ca-independency must be matched to its application. For example, α-amylases used in starch industry must be active and stable at low pH, but at high pH values in the detergent industry [19]. The properties of α-amylases secreted by this strain of *L. plantarum* shows its probable applicability in different industries. As it is important to evaluate the enzymatic properties of amylase for better exploitation in different industries, the effect of pH on amylase activity was investigated. The result indicates that the amylase is active in the pH range 3-10 with the optimum activity at 7. This suggests that the enzyme would be useful in processes that require wide range of pH from acidic range to neutral pH. For example the amylase may serve better in detergent and textile industries. It may also find application in the production of anomalously linked oligosaccharides mixture, removal of starch sizer from textile, treatment of starch processing waste water and

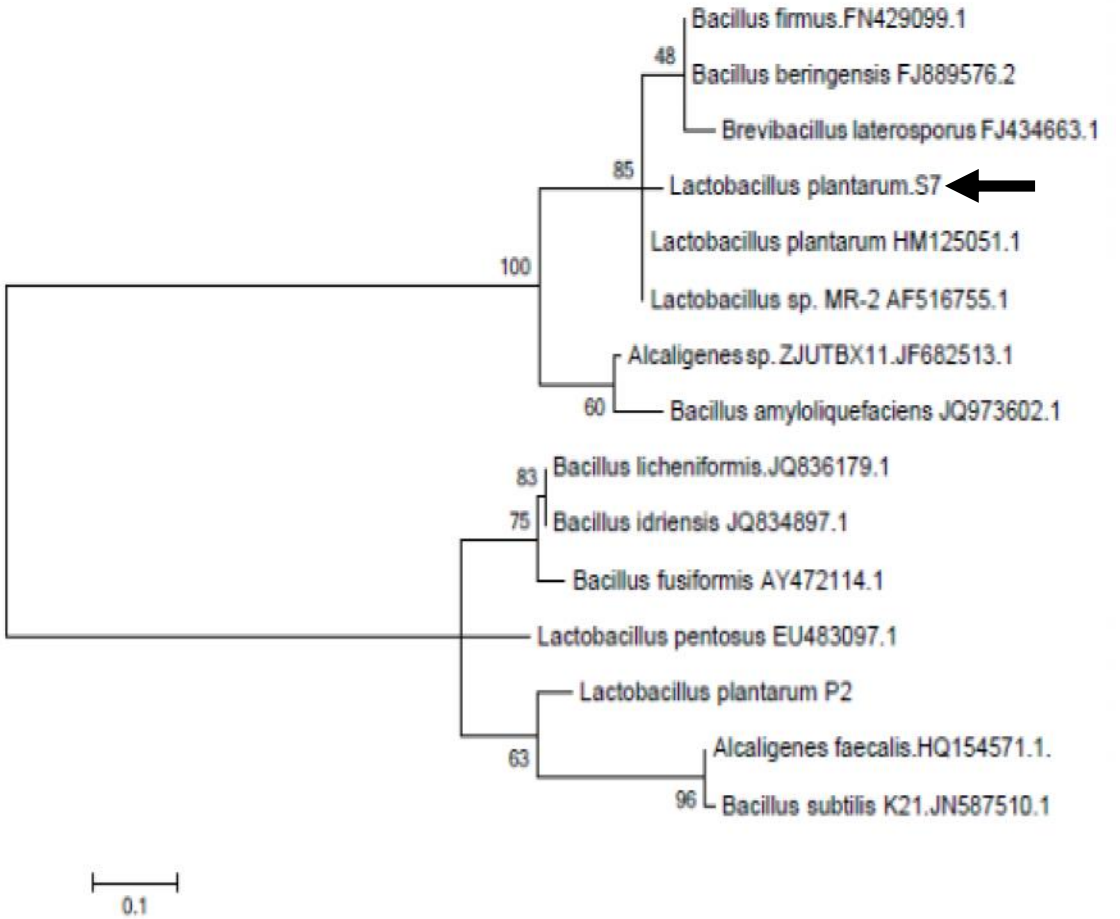


**Fig. 8. Phylogenetic tree analysis of *L. plantarum* strain drawn by multiple sequence alignment with neighbor joining method**

manufacturing of maltose, high fructose containing syrups, oligosaccharides mixture, maltotetraose syrup, and high molecular weight branched dextrins [24].

With regards to the characteristics of the lactic acid bacterial α-amylases described in some literatures, the properties of the α-amylase synthesized by *L. plantarum* S7 is different. The majority of ALAB, including *L.plantarum*, has an optimum pH of 4-5 [25,26,27,28,29].

*L. plantarum* S7 was found to be active at pH range of 6-9 with highest activity at pH 7. This agrees with the work of Anteneh and prapulla [30] who reported an optimum amylase activity at neutral pH (7.0) for *L. plantarum*. Because most of the ALAB that have been reported are usually from fermented foods, α-amylase from such LAB appears to be adapted to the fermentation conditions.

Extremes in temperature can disrupt hydrogen bonds that hold enzymes in its three dimensional structure leading to denaturation of the protein [31]. Meanwhile, biological reactions are catalysed by proteineous enzymes, and each enzyme has a temperature above which is distrupted by heat. Therefore biological reactions occur faster with increasing temperature up to the point of enzyme activity and the rate of the reaction decreases sharply [32]. Nevertheless, it is necessary to adapt assay conditions (mainly temperature) to determine amylase activity. With regard to temperature optimum, α-amylase produced by this strain had temperature optimum at 40°C. This agrees with the work of Lindgren and Refai [33] and Sen and Chakrabarty [34] who reported 40°C for amylase activity produced by *Leuconostoc* spp. and temperature optimum of 40-50°C for amylase produced by *L. celobiosus* respectively. The data suggest a low sensitivity to temperature and this property might

limit industrial applications of the enzyme at high temperatures, but favors its application in processes that require complete inactivation of the enzyme, such as the baking industry [35]. A modern trend among consumers is to use colder temperatures for laundry or dishwashing [36]. At lower temperatures, the removal of starch from cloth and porcelain becomes more problematic. Detergents with α-amylases working optimally at low or moderate temperatures can overcome this problem [37].

Investigation on the thermal stability of amylase produced by this strain revealed that the enzyme was relatively stable. Against the report of Aguilar et al. [28] relative to complete loss of activity of α-amylase produced by *L. manihotivorans* at 55°C for 1hr, α-amylase produced from *L. plantarum* S7 retained about 50% of its activity at temperature of 60°C for 30 mins above which the activity decreases. Loss of activity above this temperature may be due to the fact that, as the temperature rises, a progressive inactivation of enzyme occurs, due to thermal inactivation of proteins in their structure, or it may also be due to incorrect conformation of protein, hydrolysis of the peptide chain, destruction of amino acid, or aggregation [38].

Most of the amylases are known to be metal ion-dependent enzymes. The amylase was activated by $Ca^{2+}$ unlike some reported by other workers [39,14,28] except for *L. amylovorus* amylase [39] and inhibited by $K^{+}$, $Ba^{2+}$, $Na^{+}$, $Cu^{2+}$, Urea and EDTA. Similar to α-amylase produced from *L. amylophilus* [39], $Fe^{3+}$, $Cu^{2}$+ partially inhibited the enzyme activity unlike the *L. plantarum* A6 and *L. amylovorus* α-amylases [14,39].

Microorganism morphology is a poor descriptive attribute that can vary widely depending upon nutritional conditions. Employing molecular biological methods provide an alternative approach for the detection of microorganisms that are difficult to identify by conventional culture techniques or microscopy especially lactic acid bacteria [40]. The analysis of 16S rRNA genes, aided by using PCR to amplify target sequences in environmental samples, has enabled microbial ecologists to identify and characterize microorganisms in a natural community, without prior cultivation [41]. The taxonomic position of an organism can be determined by comparing the sequence with those of other bacteria [42]. Molecular methods as mentioned above provide microbiologists with the tools to study the ecology and population dynamics of these LAB in waste samples including cassava wastes. The use of molecular techniques in the characterization and identification of bacteria has many advantages [43]. Molecular techniques are major tools for the analysis of microorganisms from various sources. Molecular techniques are effective and fast technology for identification of microbial diversity in different environments [44].

The DNA was used as template to amplify by polymerase chain reaction by designed two primers that is forward and reverse primer with reaction mixtures at appropriate conditions [45]. The PCR product was run in agrose gel electrophroses showed 788bp for strain S7. The DNA sequence of the strain S7 having Gene Bank ACC.NO. JN039347.1 when compared to those in Gene bank data bases with BLAST were found 90% similarites with *L. plantarum*. This strain named as *L. plantarum* S7 (Gene bank ACC. No. JN039347.1).The pholygenetic tree showed resemblance with *L. plantarum*.

## 5. CONCLUSION

Though the production of α-amylase-enzyme has been improved significantly by the utilization of hyper-producing strains of bacteria, the emergence of ALAB and the use of such LAB especially *Lactobacillus plantarum* to produce amylase for industrial use is an added advantage compared to amylase of other bacteria origin. In addition, the enzyme is from organisms that are generally regarded as safe (GRAS), hence α-amylase from *L. plantarum* got potential to be used in food and other industrial sectors where utilization of starch is concerned.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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