Research Article

pISSN: 2345-5357
eISSN: 2423-4214

OPEN ACCESS

APPLIED FOOD BIOTECHNOLOGY, 2022, 9 (4):9-19
Journal homepage: www.journals.sbmu.ac.ir/afb

Isolation, purification and biochemical characterization of alkaline α-amylase from *Bacillus subtilis* strain W3SFR5 isolated from kitchen Wastes

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Abstract

Background and Objective: Amylase is a hydrolytic enzyme that breaks starch into simple sugars. This enzyme includes uses in starch production, brewery, detergent formulation, paper production and pharmaceuticals as a digest aid. The aim of the present study was to isolate, identify and characterize an alkaline amylase from bacteria of food wastes.

Material and Methods: Bacteria were isolated using serial dilution, screened on agar plates and characterized through biochemical assessments and 16S rRNA sequencing. After optimizing the bacterial growth conditions using one factor at a time method, the alkaline amylase was extracted from the culture broth and partially purified using Sephadex G-75 chromatography. Enzyme activity generated by submerged fermentation was assessed using 3,5-dinitrosalicylic and recorded as the mean of three replicates.

Results and Conclusion: The bacterial isolate W3SFR5 showed high amylolytic activity in agar culture. Biochemical analysis and sequencing of the 16S rRNA verified the bacterial isolate as *Bacillus subtilis* (GenBank accession number: OM258620). *Bacillus subtilis* W3SFR5 was propagated within 30–50 ℃and pH 6-9. The partially purified *Bacillus subtilis* W3SFR5 amylase included a molecular weight of 65 kDa and demonstrated a maximum specific activity of 216.02U mg^{-1}. The optimum temperature for the enzyme was 60 °C and the pH was 9. The W3SFR5 amylase was actively stable under temperatures of 50–70 °C and pH of 7-9. Furthermore, 5 mM Fe^{2+} increased W3SFR5 amylase activity. The enzyme was more resistant to organic solvents, surfactants, inhibitors and oxidizing agents than that most amylases were. Additionally, results showed that W3SFR5 amylase was compatible with most commercial detergents, indicating that it could be used as a detergent additive.

Conflict of interest: The authors declare no conflict of interest.

How to cite this article

Niyomukiza S, Owino W, Mathara JM, Maina N. Isolation, purification and biochemical characterization of alkaline α-amylase from Bacillus subtilis strain W3SFR5 isolated from kitchen. .Appl Food Biotechnol. 2023; 10 (1): 9-19.
http://dx.doi.org/10.22037/afb.v10i1.39495

Article Information

Article history:
- Received 19 Sep 2022
- Revised 13 Oct 2022
- Accepted 24 Nov 2022

Keywords:
- *Bacillus subtilis*
- W3SFR5-amylase
- Biochemical characterization
- Detergent compatibility

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1. Introduction

Significant developments in the biochemical, agricultural and biotechnological industries have increased search for novel microorganisms that include potential uses in scientific and industrial sectors. Scientists are continuously investigating regions, where bacteria can thrive in favorable conditions. In the region adapted to cold, soil and water springs have been excellent habitats for researching novel bacteria [1-3]. In contrast, food wastes can be sources of microorganisms with specific characteristics. Naturally, microorganisms, animals and plants can produce amylases

(EC3.2.1.1) [4]. However, microorganisms include advantages over other sources because their low-cost products include fast production rates, are less harmful and are stable in extreme conditions. Amylase breaks starch into simple sugars (e.g., maltose, maltotriose, glucose and dextrin) with uses in starch industries for starch liquefaction, as well as in paper, food, detergent, pharmaceutical, brewing, textile and baking industries [5,6]. Amylase accounts for 30% of the output worldwide and 25% of the enzyme market [7-9]. Specificity, stability, temperature and pH dependence are parameters that must be detailed to each end use. It has been shown that interactions between these parameters signify-cantly affect enzyme synthesis [10]. Therefore, screening microorganisms and amylase activity may help identifying novel amylases for various uses. Submerged fermentation has been the primary method of producing this enzyme due to its simplicity; with which, the bacterial growth media are sterilized and the greater degree of control that can be engineered into the production process [11].

Optimizing various parameters and manipulating media components are the most important techniques used to produce enzymes in large quantities to meet industrial needs [12]. To decrease production costs, gelatinization and liquefaction processes have increasingly become dependent on thermostable enzymes (amylases) [13,14]. There is a gap between utilizing food wastes as bioresources and their utilization as substrates in submerged fermentation for amylase production. In this study, *Bacillus subtilis* strain W3SFR5 was isolated from starchy food wastes and its amylolytic activity was assessed *in vitro*. Growth of the isolated bacteria was optimized using one factor at a time (OFAT) method under submerged fermentation. Then, biochemical characteristics of the isolated bacteria were assessed. In addition, the isolated amylase was purified and characterized for its specific characteristics, suggesting that it might be helpful as a biological catalyst in various industries, specifically detergent industries.

2. Materials and Methods

2.1. Bacterial isolation and identification

The *Bacillus (B.) subtilis* W3SFR5 was isolated from starchy food wastes (maize meal and potato peel wastes) collected from two restaurants and one hotel in Juja Town, Kiambu County, Kenya. Serial dilution and nutrient agar were used to isolate and purify the bacterial colonies. To screen amylase-producing bacteria, 1% soluble starch was added to nutrient agar and the bacteria were identified by adding iodine solution to record the clearance zone [15].

2.2. Biochemical assays and identification of the isolate using 16S rRNA

Biochemical assays used to characterize bacterial strain included indole test, oxidase test, catalase test, gram staining, starch, lipid and protein hydrolyses and methyl red-Voges Proskauer (MR-VP) test. The DNA sample of the bacterial isolate W3SFR5 was used as a template to amplify a fragment using polymerase chain reaction (PCR). Universal primers and PCR conditions were reported in a previous study by the authors [15].

2.3. Inoculum preparation and submerged fermentation

One bacterial colony was picked and inoculated into Luria-Bertani broth, containing 10 g l^{-1} sodium chloride, 5 g l^{-1} yeast extract and 10 g l^{-1} tryptone). The bacterial broth was then agitated using rotary shaker with temperature and rotation speed set at 37 ℃ and 150 rpm, respectively. The overnight bacterial culture was harvested and centrifuged at 5000× g for 10 min. The bacterial cell pellet was suspended in normal saline and then measured to achieve one OD_{600nm} unit. For amylase production, 250-ml conical flasks were used with 100 ml of the fermentation media, containing 1% of starch, 1.5% of KH_2P0_4, 0.25% of Na_2HPO_4, 0.2% of tryptone, 0.005% of $CaCl_2$ and 0.05% of $MgSO_4$ [16]. Cultures were centrifuged at 10000× g for 30 min at 4 °C and the supernatant was used for the amylase activity assessment [17].

2.4. Amylase activity analysis

The 3,5-DNS was used to investigate release of glucose from starch at 40 °C for 10 min in 50-mM Tris-HCl buffer (pH 8.0) [14]. Absorbance was measured at 540 nm using double-beam spectrophotometer. One unit of amylase activity was equal to the quantity of enzyme that could produce 1 µm equivalent of glucose per minute under specified conditions, expressed in U ml^{-1} [5]

2.5. Optimization of the culture conditions

The *B. subtilis* W3SFR5 was inoculated under various temperatures of 20, 30, 40, 55 and 65 ºC; pH values of 3, 4, 6, 7, 8, 9, 10 and 12; inoculum volumes of 0.5, 1, 2, 3 and 4%; rotation speeds of 50, 100, 150, 200 and 250 rpm; and nitrogen sources (tryptone, urea, peptone and yeast extract as organic and sodium nitrate, ammonium sulfate and ammonium nitrate as inorganic sources). Incubation time was used as the production time of the enzyme of 12, 24, 36, 48, 60 and 72 h.

2.6. Amylase purification

Amylase purification was carried out in three steps of the process, including precipitation with 70% saturated ammonium sulfate and dialysis (dialysis membrane of 100 kDa cut off) followed by size exclusion chromatography with Sephadex G-75. The precipitated sample was dialyzed overnight against 50-Mm phosphate buffer (pH 7.4) and purified to homogeneity using Chromatography-G75. Gel filtration was carried out in a pre-packed column using fine-powder resin or Sephadex G-75 (Beijing Solarbio Science and Technology, China). Moreover, 0.01-M Tris-HCl buffer with pH 8.0 was used to equilibrate the Sephadex column. Pre-dialyzed enzyme samples were loaded individually into

Sephadex G-75 columns using identical buffers and eluted. Sample was collected in fractions (2 ml min^{-1}). The eluted solution optical density with the targeted protein was checked at 280 nm using spectrophotometer. Fractions showing high enzyme activity were collected and stored at -20 ℃ until use.

2.7. Assessment of molecular weight of W3SFR5 α-amylase using sodium dodecyl sulphate-polyacrylamide gel electrophoresis

In this study, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to assess the purified enzyme molecular weight (M W). Based on the method of Laemmli, SDS-PAGE was carried out on 5% stacking and 12% resolving gels. The active band was visualized by staining with 1% Coomassie brilliant blue G250 and destained by methanol:acetic acid:water solvents (20:5:40) [18,19].

2.8. Characterization of partially purified α-amylase

Size exclusion chromatography was used to separate the enzyme fractions. The assessed factors included pH, temperature, metal ions, organic solvents, surfactants and inhibitors. Every experiment was carried out with three replicates and results were presented as the mean of three replicates [20].

2.9. Effects of temperature and pH on W3SFR5 amylase from Bacillus subtilis W3SFR

The pH was assessed using several buffers, including 50-mM sodium acetate buffer (pH 3-6); 50-mM phosphate buffer (pH 7), 50-mM Tris-HCl (pH 8.0-10.0) and 50-mM glycine-NaOH buffer (pH 10.0-13). The optimum temperature was analyzed in 50-mM Tris-HCl buffer (pH 9.0) at 50, 60, 70, 80 and 90 ℃ using standard reaction mixtures and conditions. To assess thermostability of amylase, the enzyme was incubated at various temperatures (50, 60, 70, 80, 90 and 100 °C) for 1 h.

2.10. Effects of organic solvents on amylase activity

Several organic solvents miscible with water (isopropanol, methanol, ethanol and DMSO) and those water immiscible (toluene, chloroform and diethyl ether) were assessed for their effects on α-amylase activity. Additionally, 500 µl of solvent at concentration of 16% v v^{-1} were added to the reaction mixture and incubated for 1 h.

2.11. Effects of surfactants, inhibitors and metal ions on the activity of amylase

Based on their effects on amylase activity, metal ions such as $Ca^{2+,}$ Fe^{2+}, Cu^{2+}, Zn^{2+}, Mg^{2+}, Mn^{2+}, Ba^{2+} and K^{+} and chemical substances such as EDTA, PMSF, β-mercaptoethanol, SDS, Triton X-100, Tween 20, Tween 80 and H_2O_2 were investigated [21].

2.12. The W3SFR5 amylase and commercial detergent compatibility

Compatibility of W3SFR5 amylase was prepared by mixing 500 µl of 5 mg ml^{-1} enzyme-free commercial detergent with 500 µl of the diluted enzyme. Enzyme activity was assessed using starch as substrate under optimum conditions [22,23].

2.13. Statistical analysis

Cell density was analyzed by checking optical density (OD600nm) using spectrophotometer. Hydrolytic enzyme activity of the isolated strain was assessed in triplicates. Results were reported as mean ±SD (standard deviation) of triplicate samples.

3. Results and Discussion

3.1. Isolation, screening and identification of Bacillus subtilis W3SFR5

Bacterial strains were isolated and purified from food wastes. The bacterial isolates were assessed for extracellular α-amylase production. The initial screening revealed that ten bacteria produced amylase significantly on culture plates. Of these isolates, W3SFR5 isolate showed an excellent zone of clearance on starch agar (Figure. 1A). The strain W3SFR5 was rod-shaped, gray-white, catalase positive, oxidase variable, indole negative, methyl red negative and gram positive. The strain was able to hydrolyze starch, casein and lipids. Furthermore, verification of the bacterial strain was carried out genotypically using 16S rRNA. The bacterial rDNA was sequenced and results indicated that the isolate was closely linked to *B. subtilis* with 98% similarity to *B. subtilis* strain UYY. Sequences were submitted to GenBank database (accession number: OM258620.1). The bacterial strain phylogenetic relationships were presented in MEGA Software v.11 using neighbor-joining method [24] (Figure. 1B).

3.2. Cultural condition optimization of various parameters using OFAT

In this experiment, *B. subtilis* W3SFR5 was used to investigate the optimal culture conditions for the microbial growth and amylase production. Results clearly showed that *B. subtilis* W3RF5 produced amylase and grew more effectively at alkaline pH and temperatures of 20-50 ℃ (Figure 2. A,B). The optimal temperature and pH were 40 ℃ and 9, respectively. Based on the findings, synthesis of amylase by *B. subtilis* W3SFR5 strain depended proportionally to growth of the cell within all the factors. The acidic and neutral pH values resulted in W3SFR5 amylase having considerably lower levels of stability. Activity was optimum at alkaline conditions (pH 9) [25]. Temperature of 40 ℃ was optimum for the strain growth and amylase production.

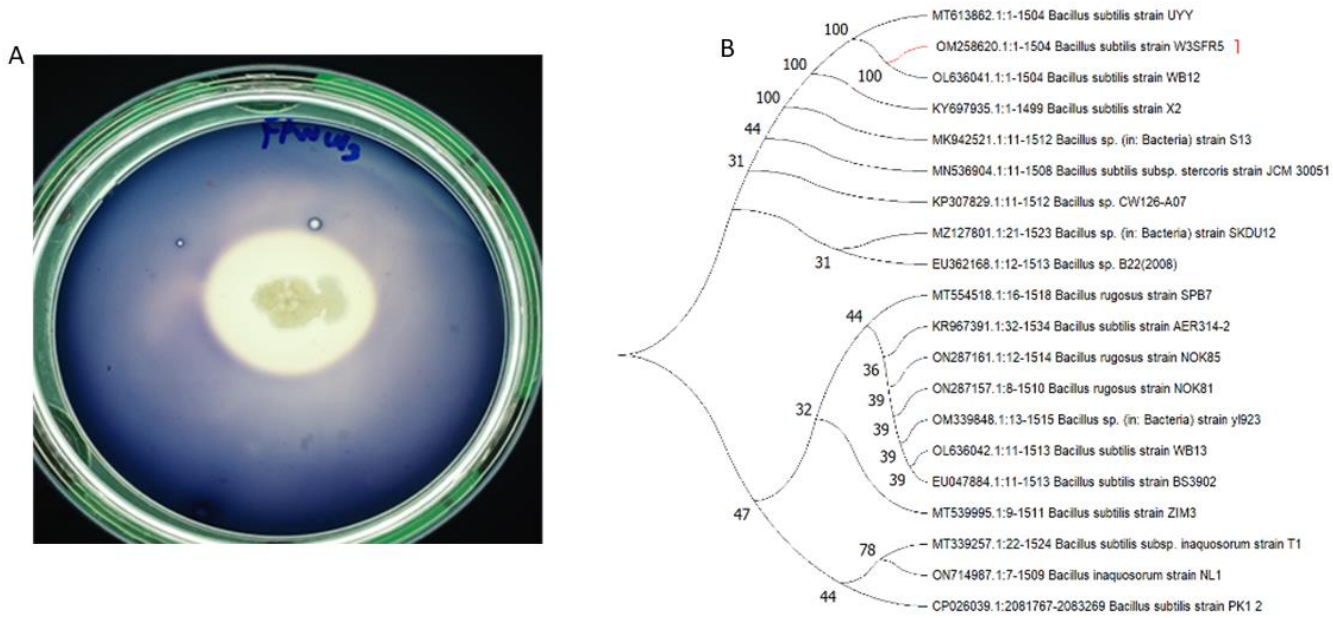

Figure.1: a) Plate assay showing clearance zone for starch hydrolysis b) Phylogenetic tree. There were 20 nucleotide sequences in this investigation. The Neighbor-Joining approach was used to infer the evolutionary history. The evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per site.

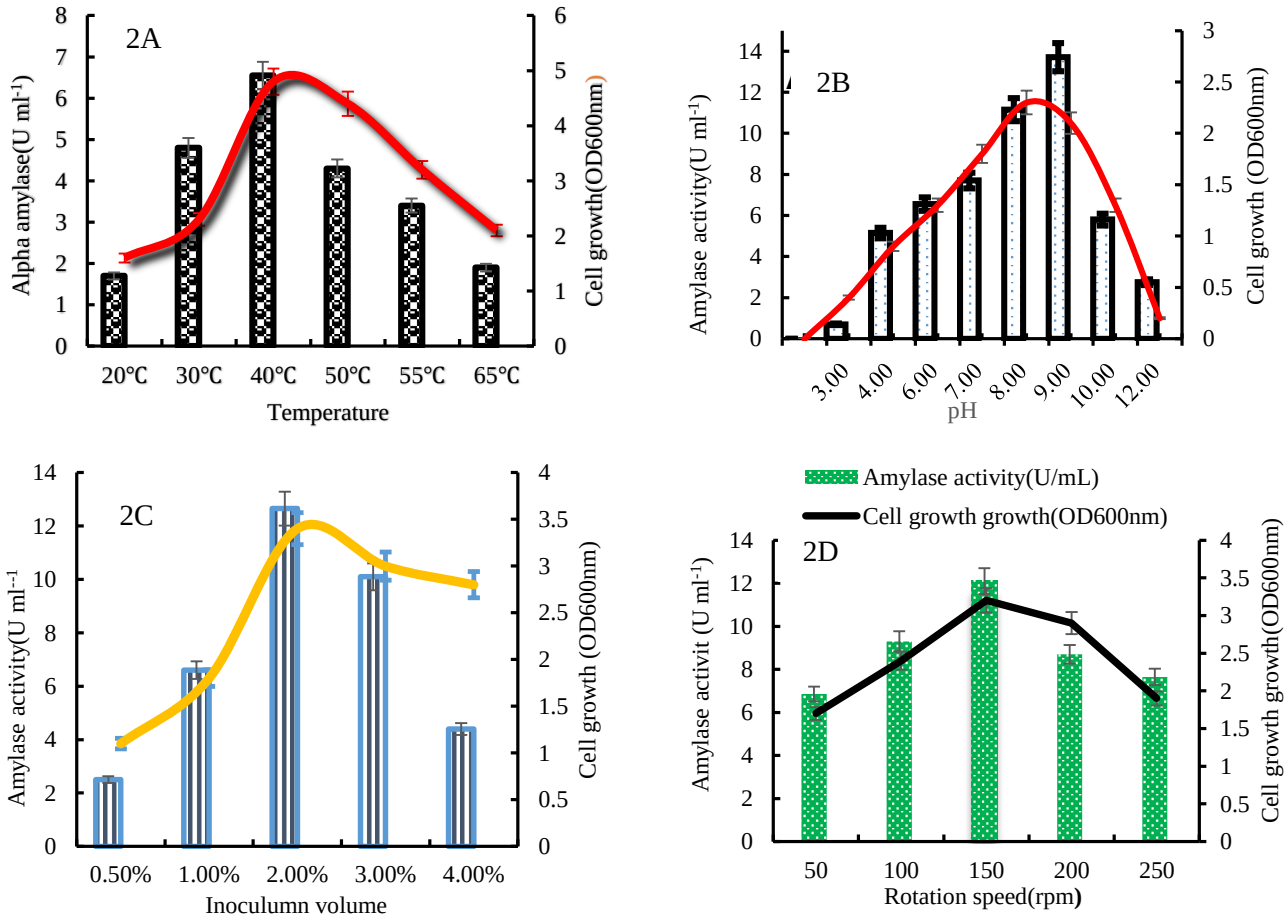

Figure 2. Culture condition optimization: a) Effects of temperature on amylase activity and growth of *Bacillus subtilis* W3SFR5, b) effects of pH on amylase activity and growth of *Bacillus subtilis* W3SFR5, c) effects of inoculum volume amylase activity and growth of *Bacillus subtilis* W3SFR5, and d) effects of rotation speed on amylase activity and growth of *Bacillus subtilis* W3SFR5

Optimization, the inoculum volume of a bacterial cell, is the critical stage during microbial enzyme production. Enzyme production and cell growth are both affected by this effect. When volume of the culture increased further, significant decreases occurred in enzyme production; possibly because the bacteria experienced excessive growth at high inoculum levels and the nutrients present in the media were insufficient to prevent the bacteria from proliferating. Simultaneously, the inoculum size was adjusted from 0.5 to 4%. The 2% volume of inoculum culture was optimum. (Figure 2C). based on the current findings, amylase production by *B. subtilis* strain was growth dependent. Effects of agitation speeds, ranging from 50 to 250 rpm, on the cell growth and amylase production were assessed. Results showed that increasing the agitation speed up to 150 rpm led to increased cell growth as well as production of an enzyme (Figure 2D). However, the two values decreased when the agitation speed increased to higher than 150 rpm, likely due to the bacterial cell death by the high rotation speed [25].

3.3. Effects of nitrogen sources and incubation time on amylase activity

Briefly, *B. subtilis* W3SFR5 was cultured in presence of various nitrogen sources (organic and inorganic) to replace the total nitrogen sources in production media with equivalent quantities of nitrogen from these alternative sourc es. From the organic nitrogen sources, yeast extract and sodium nitrate significantly affected extracellular amylase production and cell growth (Figure. 3A).

The enzyme activity increased as biomass of the cell increased. Sodium nitrate and yeast extract affected bacterial growth and increased production of amylase activity. This was due to the content of nitrogen and sodium metal ions, while yeast extract provided protein and vitamin necessary for the bacterial growth and metabolites production. For example, another nitrogen source of urea produced inhibitory effects on production of amylase by *B. subtilis* W3SFR5. Decreases of activity in presence of urea could be attributed to changes in conformational structure of the enzyme, which resulted in significant decreases in enzyme activity [20]. A similar finding that urea decreased the amylase activity of *B. tequilensis* RG-01 was reported by Tewari [11]

This study optimized the incubation time for the cell growth and amylase production. After 48 h of incubation, enzyme activity decreased due to catabolite suppression by glucose as a byproduct of starch hydrolysis and α-amylase degradation by proteases. It could be due to interactions of the produced enzymes with other components in the media or decreases in cell death. Optimization of the incubation time was critical for maximizing amylase production [18]. The current study showed that W3SFR5 amylase production peaked at 21.3 U ml^{-1}, 48 h after rapid decreasing (Figure 3B). The maximum enzyme production at 48 h could be attributed to the bacterial exponential phase. When nutrients were used, they eventually reached their stationary phase and abundant metabolites were produced; thus, resulting in a lower yield of enzymes and decreased cell growth [26].

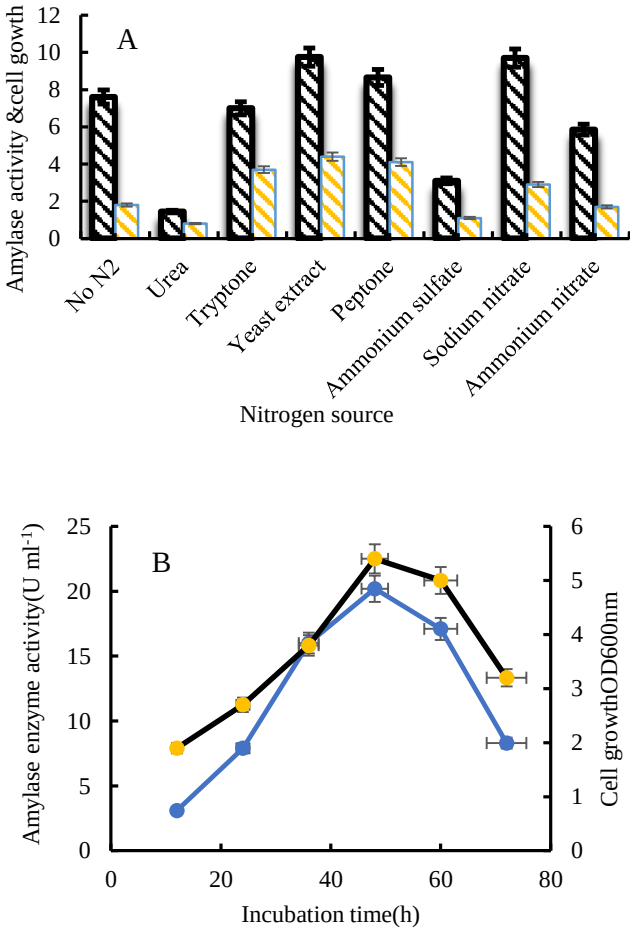

Figure 3. a) Effects of organic and inorganic nitrogen source on amylase activity and growth of the cell, and b) effects of incubation time on amylase activity and growth of *Bacillus subtilis* W3SFR5. Fermentation was carried out under optimum temperature of 40 ℃, pH 9, rotation speed of 150 rpm and inoculum volume of 2%

3.4. Extraction, purification and characterization of W3SFR5 α-amylase

After optimization of the culture conditions using physical and biochemical parameters, the extracellular enzyme collected via centrifugation and the supernatant was subjected to ammonium sulfate precipitation followed by dialysis via membranes. This was followed to purify W3SFR5 α-amylase by *B. subtilis* and yields are summarized in Table 1.

3.5. Characterization of α-amylase from *Bacillus subtilis* W3SFR5

3.5.1. SDS-PAGE analysis

The SDS-PAGE profile revealed a single protein band (65 kDa) of extracellular α-amylase after purification of the enzyme from the culture supernatant. This verified that the w3SFR5 amylase was partially purified (Figure 4).

Table 1. An overview of the purification procedures of α-amylase produced by *Bacillus subtilis* W3SFR5

Steps	Amylase Activity U ml^{-1}	Protein content (µg ml^{-1})	Amylase-specific activity (U mg^{-1})	Purification fold
Crude enzyme	21.4	0.23	84.3	1
70% Ammonium sulfate+ Dialysis membrane	14.8	0.105	140.95	1.7
Chromatography Sephadex-G75	10.6	0.0495	216.3	2.6

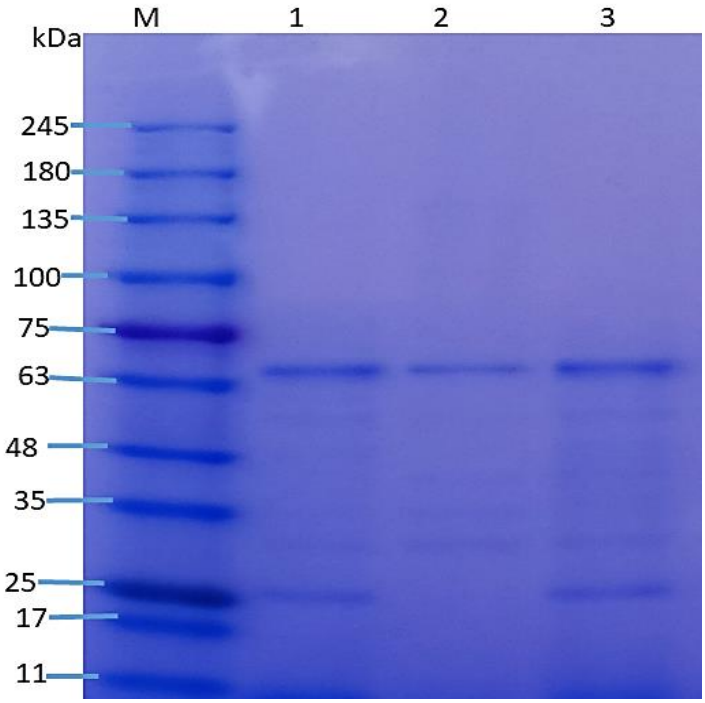

Figure 4. SDS-PAGE analysis of Lane M, protein ladder (5-245 kDa); Lane 1, ammonium sulfate precipitation and dialysis; Lane 2, purified enzyme using Sephadex G-75; and Lane 3, the crude enzyme

After purification of the product from optimizing production conditions, the enzyme was characterized. The partially purified enzyme was subjected to SDS-PAGE to investigate its molecular weight. The molecular weight was detected between 75 and 63 kDa of the protein marker. This MW was estimated as 65 kDa, similar to the MW of α-amylase isolated from *B. tequilensis* RG-01 (67 kDa) [11] and *B. subtilis* KIBGE (68 kDa) [26]. It is lower than the MW of α-amylase from *B. licheniformis* AT70 of 85 kDa [21]. The MW of W3SFR5 amylase was relatively higher than that of α-amylase isolated from *B. subtilis* DM-03 (42.8 kDa) [27] and *Pseudomonas balearica* VITPS19 47 kDa [20].

3.5.2. Temperature stability for W3SFR5 amylase activity

The *B. subtilis* W3SFR5 showed enzyme activity within a broad temperature range, with 60 °C as the optimal temperature and gradual decreases in activity (Figure 5A). Decreases of activity at high temperature were seen. After pre-incubation at 50, 60, 70, 80, 90 and 100 °C for 60 min, enzyme original activity was preserved at 70, 100, 80, 69, 56 and 24.5%, respectively, allowing for thermal stability estimations (Figure 5B). The partially purified amylase was stable at a temperature range of 50-70 ℃ and high stability was achieved at 60 ℃.

3.5.3. Effects of pH on W3SFR5 amylase activity

The partially purified enzyme of *B. subtilis* W3SFR5 α-amylase was incubated at pH 7–13. The enzyme activity increased from neutral to alkaline with the optimum pH 9. Due to the protein denaturation, activity decreased when pH was less or greater than the optimum level. Figure 6A Stability of the enzyme under various pH values is critical for its use in industries. The enzyme optimum processing range was between pH 7 and 9 and the enzyme lost its activity after incubation at pH 13 for 1 h. Figure 6B

3.5.4. The W3SFR5 amylase stability under metal ions

The largest number of amylases include metal ion-dependent enzymes, needing divalent ions such as Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Fe^{2+}, Ba^{2+} and Cu^{2+}. Figure 7 illustrates various effects of metal ions on W3SFR5 amylase activity at a concentration of 5 mM.

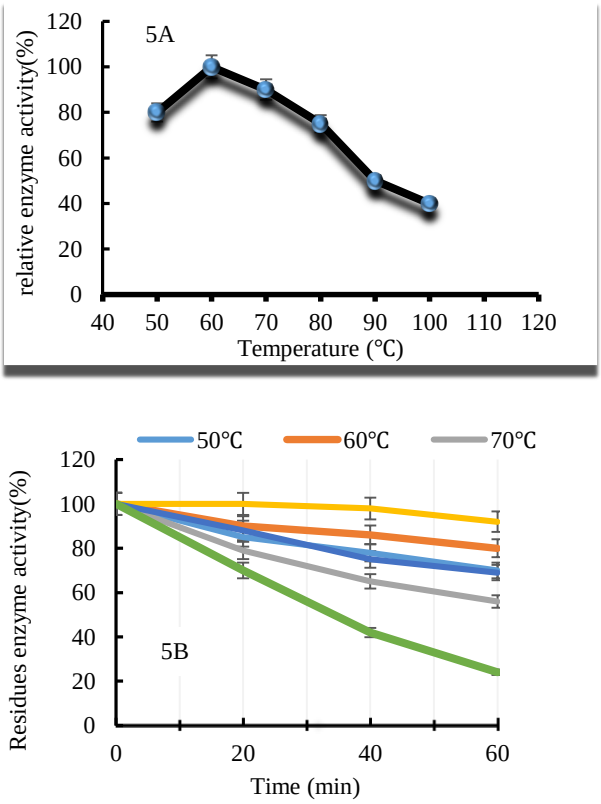

Figure 5. Effects of temperature and stability on *Bacillus subtilis* W3RF5 α-amylase activity

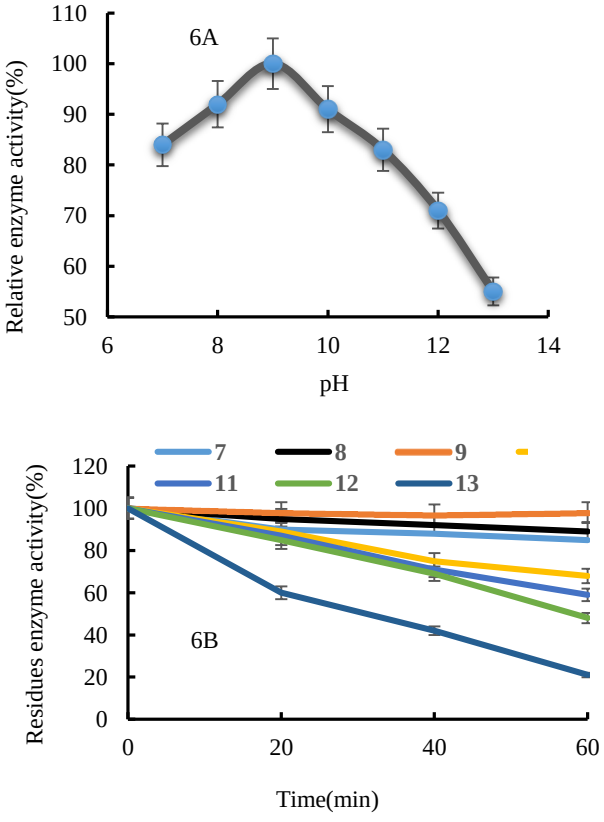

Figure 6. Effects of pH on stability of purified w3SFR5 α-amylase. a) Effects of pH on purified W3SFR5 amylase, and b) enzyme stability under various pH levels after incubation for 60 min

In fact, Fe^{2+} and Mg^{2+} increased activity of the partially purified enzyme by 116 and 107%, respectively, while Ba^{2+}, Cu^{2+}, Mn^{2+} and Zn^{2+} slightly decreased enzyme activity to various degrees, compared to the control. This is majorly occurred because these heavy metal ions reacted with the sulphur atom of cysteine amino acids, forming covalent bonds in the process. This caused most enzymes inactive to carry out their catalytic roles; thus, significantly slowing down. Promita et al. reported similar results that Ca^{2+}, Fe^{2+} and Mg^{2+} ions positively affected amylase from *B. amyloliquefaciens* P-001 [28]. In contrast, Zn^{2+} heavy metal affected enzyme activity of amylase produced by marine actinomycetes [18]. The significant characteristic could be linked to the amylase unique structure and the process needs additional investigations [29].

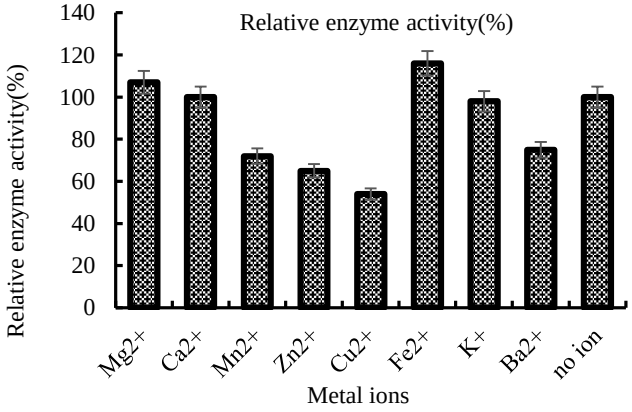

Figure 7. Effects of metal ions at 5 mM on W3SFR5 amylase activity. Control included the reaction mixture with no metal ions

3.5.5. Effects of various solvents on W3SFR5 amylase activity

The partially purified enzyme was incubated in various types of solvents. The enzyme activity was assessed under optimum conditions. No effects were reported on amylase activity under DMSO treatment. Simultaneously, activity was slightly affected in presence of butanol (91.45%), methanol (78%) and ethanol (88.9%). However, chloroform (53.1%) significantly affected amylase activity and diethyl ether (12.11%). Formaldehyde completely inhibited W3SFR5 amylase activity. Furthermore, W3SFR5 amylase was affected by toluene (119.3%) and DMSO (113%)as shown in (Table 2). Because pH of laundry detergents is typically 9.0-12.0 [30], high activity of W3SFR5 amylase in high pH solutions is a critical characteristic of ultimate use as laundry detergent additives. Hydrolytic enzymes become popular due to their numerous industrial uses such as serving as catalysts in protein synthesis in presence of organic solvents. The present study showed effects of various water-miscible and non-miscible organic solvents on amylase activity. Stability in solvents known for most synthetic reactions such as ethanol, methanol and DMSO reveals that W3FR5 α-amylase is an ideal candidate, compared with other amylases [20].

Table 2. Effects of water-miscible and immiscible solvents on amylase activity

Solvents	The concentration	Relative activity (%)
Control	None	100
Methanol	16%	70.4
Toluene	16%	110.3
Chloroform	16%	52.47
Diethyl ether	16%	12.11
Ethanol	16%	88.92
DMSO	16%	112
Isopropanol	16%	100.68
Formol	16%	0.000

3.5.6. Effects of surfactants and other chemicals on amylase activity

Effects of surfactants and other chemicals on amylase activity was studied. Enzyme showed compatibility with utilized surfactants such as Tween 80, Tween 20 and Triton X100. Stability of the enzyme in Tween 20 and Tween 80 was excellent and high increases (26 and 4.9%) in the activity was detected, respectively (Table 3). The SDS (5 mM) affected the amylase activity by 42.2%. The enzyme stability decreased to 19.5% in presence of EDTA. Standard enzyme assays were used to assess residual activity of the W3SFR5 crude enzyme after 1 h of incubation in various enzyme inhibitors. Enzyme activity of the sample incubated with no additional chemicals was reported as 100%. Regarding effects of inhibitors and other chemicals used in molecular and synthetic biologies, PMSF did not include inhibitory effects on W3FR5 amylase activity (95.4%). In fact, β-mercaptoethanol inhibited W3SFR5 amylase activity, possibly due to its effects on interruption of disulfide bridges in the structure of the produced enzyme. These results were in contrast to those published by Hmidet et al. that amylase activity produced by *B. lichneformis* increased using β-mercaptoethanol [23]. This result was similar to that reporting SDS and EDTA inhibited amylase from *P. balearica* VITPS19. The W3SFR5 enzyme activity was delayed in presence of EDTA and SDS, indicating that purified W3SFR5 amylase was a metal-dependent enzyme. This result was similar to the result of previous reports, demonstrating that chelating reagents often inhibited amylases [20].

Table 3. Effects of surfactants and other chemicals on α-amylase activity

Effectors	Concentration	Relative enzyme activity (%)
Control	None	100
SDS	5mM	42.28
H_2O_2	0.3%	94.5
PMSF	5mM	95.4
Tween 20	0.3%	104.9
Mercaptoethanol	0.3%	3.82
Tween 80	0.3%	126
Triton X100	0.3%	75.9
EDTA	1mM	19.5

3.5.7. Compatibility of W3SFR5 amylase with commercial detergents

The purified enzyme was investigated to show if it was compatible with commercially available laundry detergents. The W3SFR5 amylase showed excellent stability to all the commercial liquid detergents and preserved more than 90% of its activity on four detergents (Sunlight, Ariel, Campeira and Tide) from overall six detergents (Figure 8) after 30 min of incubation at 37 ℃.

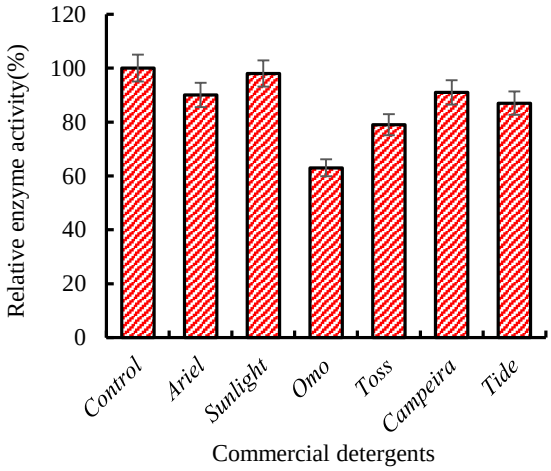

Figure 8. Effects of various detergents on the activity of α-amylase from *Bacillus subtilis* W3SFR5. Activity is reported as a fraction of the level; at which, the amylase activity is ordinarily present in absence of detergents (100%)

The enzyme stability decreased marginally in presence of Toss (72%) and was at its lowest level in presence of Omo (63%). An appropriate detergent enzyme must be compatible and stable with regularly used detergent chemicals to be effective during the washing process. Surfactants, oxidizing agents and other detergent additives are examples of these compounds. This study showed that W3SFR5 amylase was stable against Tween 20, Triton X100 and oxidizing agents such as H_2O_2. The current results supported detergent-compatible enzymes produced from *B. amyloliquefaciens* [28], *B. licheniformis* [21], *Aspergillus niger* [8] and *B. tequilensis* RG-01 [11]. Experiments on compatibility of the isolated W3SFR5 amylase with detergents demonstrated that the enzyme activity decreased with increasing incubation time, verifying its potential for commercial detergent formulations. Amylase produced by *Actinomadura keratinilytica* strain Cpt29 showed similar stability to almost all the investigated detergents[13]. Based on the findings of this study, nature of the enzyme which possessed critical attributes to the industries made the enzyme an excellent candidate for the detergent additives.

4. Conclusion

In this study, a potential amylase-producing bacterium was successfully isolated, which was identified as *B. subtilis* using 16S rRNA. After optimization of the culture conditions, a detergent-compatible amylase was isolated

from *B. subtilis* W3SFR5, purified by size exclusion chromatography and then characterized. The enzyme MW was reported as 65 kDa using SDS-PAGE. Amylase derived from *B. subtilis* strain W3SFR5 showed critical characteristics such as high activity and stability even at high pH values and temperatures and good resistance to metal ions, surfactants, organic solvents and commercial detergents. These characteristics are promising for potential industrial uses, especially detergent industries.

5. Acknowledgements

The authors gratefully acknowledge the African Union within Pan African University for providing the reagents, materials and laboratory to accomplish this study.

6. Conflict of Interest

The authors report no conflicts of interest.

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Research Article
pISSN: 2345-5357
eISSN: 2423-4214
OPEN ACCESS
APPLIED FOOD BIOTECHNOLOGY, 2022, 9 (3):9-19
Journal homepage: www.journals.sbmu.ac.ir/afb

جداسازی، تخلیص و تعیین ویژگی‌های آلکالین آلفا-آمیلاز از باسیلوس سوبتیلیس سوش W3SFR5 جداشده از ضایعات مرغ

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چکیده

سابقه و هدف: آمیلاز آنزیمی آب‌کافتی[1] است که نشاسته را به قندهای ساده تبدیل می‌کند. کاربرد این آنزیم در تولید نشاسته، آبجوسازی، فرمولاسیون مواد شوینده، تولید کاغذ و داروسازی، به عنوان کمک کننده هضم، است. هدف از مطالعه حاضر جداسازی، شناسایی و تعیین ویژگی آمیلاز قلیایی باکتری‌های پسماند مواد غذایی بود.

مواد و روش ها: جدا سازی باکتری‌ها با استفاده از رقت سازی سریالی ، غربالگری روی صفحات آگار غربال و تعیین ویژگی‌ها با ارزیابی‌های بیوشیمیایی و تعیین توالی 16 S rRNA انجام شد.. پس از بهینه‌سازی شرایط رشد باکتری‌ها با استفاده از روش یک عامل در زمان، آمیلاز قلیایی از محیط کشت استخراج و با استفاده از کروماتوگرافی Sephadex G-75 تا حدی خالص‌سازی شد. فعالیت آنزیمی تولید شده توسط تخمیر غوطه ور با استفاده از 3-5 dinitrosalicylic ارزیابی و به‌عنوان میانگین سه تکرار ثبت شد.

یافته‌ها و نتیجه‌گیری: جدایه باکتری W3SFR5 فعالیت آمیلولیتیک بالایی در کشت آگار نشان داد. تجزیه و تحلیل بیوشیمیایی و تعیین توالی 16 S rRNA ایزوله باکتریایی را به عنوان *باسیلوس سوبتیلیس* تایید کرد (شماره دسترسی در بانک ژن: OM258620). *باسیلوس سوبتیلیس* W3SFR5 در دمای ۳۰-۵۰ درجه سلسیوس و pH 6-9 تکثیر شد. آمیلاز *باسیلوس سوبتیلیس* W3SFR5 تا حدی خالص شده با وزن مولکولی ۶۵ کیلو دالتون و حداکثر فعالیت ویژه ۲۱۶/۰۲ $U\ mg^{-1}$ بود. دمای بهینه برای آنزیم ۶۰ درجه سلسیوس و pH 9 بود. آمیلاز W3SFR5 به‌طور فعال در دمای ۵۰-۷۰ درجه‌سلسیوس و pH 7-9 پایدار بود. علاوه بر این، ۵ میلی مولار Fe2+ فعالیت آمیلاز W3SFR5 را افزایش داد. این آنزیم در برابر حلال‌های آلی، سورفکتانت‌ها، بازدارنده‌ها و عوامل اکسیدکننده نسبت به اکثر آمیلازها مقاوم‌تر بود. علاوه بر این، نتایج نشان داد که آمیلاز W3SFR5 با اکثر شوینده‌های تجاری سازگار است، که نشان می‌دهد می‌توان از آن به عنوان یک افزودنی شوینده استفاده کرد.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

تاریخچه مقاله
دریافت ۱۹ سپتامبر ۲۰۲۲
داوری ۱۳ اکتبر ۲۰۲۲
پذیرش ۲۴ نوامبر ۲۰۲۲

واژگان کلیدی
- *باسیلوس سوبتیلیس*
- آمیلاز W3SFR5
- ویژگی‌های بیوشیمیایی
- سازگاری با مواد شوینده

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