Trop J Nat Prod Res, February 2022; 6(2):219-226 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Purification and Characterization of *α*-Amylase from *Bacillus cereus* SM22 Isolated from Deteriorating Cocoyam

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ARTICLE INFO

Article history:
Received 17 June 2021
Revised 08 December 2021
Accepted 20 February 2022
Published online 06 March 2022

ABSTRACT

Amylases can be sourced through various organisms that thrive on common food items such as cocoyam. This study optimized, purified, and characterized α-amylase from *Bacillus cereus* SM22 isolated from decayed cocoyam tubers. Healthy cocoyam tubers were homogenized, filtered, allowed to stand, and then decanted. The residue was dried at 50^{o}C for 48 hours, powdered and used as cocoyam starch. Inoculum size, temperature, pH, starch concentration, nitrogen and carbon sources were used to optimize the α-amylase activity/production by *Bacillus cereus SM22*. The *α*-amylase activity and protein concentration of the cell-free culture supernatant were determined. The supernatant was purified on DEAE-Sephacel and BioGel P-100 and the native molecular weight of the α-amylase was estimated. Also, the α-amylase was optimized and kinetic parameters; Michaelis constant (K*m*) and maximum velocity of the reaction (V*max*) were determined. The result showed that pH 7.0, 37^{o}C, 1% cocoyam starch, 2% inoculum, peptone and ammonium nitrate were necessary for *α*-amylase production by *Bacillus cereus* SM22. Similarly, pH 6.0, 50^{o}C, NaCl (100 or 240 mM), Mg^{2+} (80-120 mM) and Ca^{2+} (20-80 mM) were required for maximum α-amylase activity. However, Al^{3+} and EDTA completely inhibited the α-amylase activity. The study successfully purified and optimized α-amylase from *Bacillus cereus* SM22 isolated from decayed cocoyam tubers. It demonstrated the necessary factors for optimal performances of *Bacillus cereus* SM22 and *α*-amylase in a given culture medium. Also, the low K_m and high V_{max} for starch substrate make the α-amylase from *Bacillus cereus* SM22 an excellent, cheap, and robust candidate for industrial applications.

***Keywords*:** Amylolytic bacterium, Characterization, Cocoyam, Isolation.

Introduction

An α-amylase is an enzyme that hydrolyzes the α-1,4-glycosidiclinkages in starch molecules into oligosaccharide units such as glucose and maltose.[1] Industrially, it has been shown to have diverse important applications.[2] The enzyme has a variety of sources such as plants, animals, and microorganisms (fungi, yeast, and bacteria). However, different sources produce amylases that vary in activity, specificity, and requirements.[3] Over the years, different α-amylase-producing microorganisms have been isolated and characterized. Studies have shown that some secrete their amylases extracellularly to help them digest the starch substrates into smaller units of glucose for energy source and growth.[4] Most amylases used in the industry are produced from the activities and properties of diverse microorganisms with peculiar genetic properties in their respective environments. These microorganisms have high enzymatic activities under certain standardized conditions (such as pH, temperature, molarity, and pressure). Also, the production process is often simple and cost-effective.[3] In as much as most industrial processes favour the use of microorganisms because of their innate potentials, there is also a need for the use of robust enzymes which can withstand the heat and harsh conditions of the industrial processes. Consequently, *Bacillus* spp (*B. cereus, B. circulans, B. subtilis*, and *B. licheniformis)*, and *Clostridium thermosulfurogenes* have been extensively used for the commercial production of thermostable α-amylase.[5] This study aimed at isolation, purification, and characterization of α-amylase from a robust amylolytic bacterium, *Bacillus cereus* SM22 using chromatographic techniques.

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Citation: Evurani SA, Philip B, Eze O, Emencheta SC, Anyim G, Okpalanwa CF. Purification and Characterization of α-Amylase from *Bacillus cereus* SM22 Isolated from Deteriorating Cocoyam. Trop J Nat Prod Res. 2022; 6(2):219-226. doi.org/10.26538/tjnpr/v6i2.9

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Materials and Methods

Sample Collection and Culturing

The pure culture of *Bacillus cereus* SM22 isolated from decayed cocoyam tubers was used in this study. It was obtained from the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria.

Cocoyam starch preparation

The modified method of Singh et al.[6] was adopted for the preparation of cocoyam starch. Healthy cocoyam tubers were collected at Obafemi Awolowo Research and Teaching Farm, Ile-Ife, Nigeria. Samples were rinsed in clean water and peeled. Then 500 g were homogenized using pestle and mortar with 1 L of distilled water. The homogenate was filtered with cheesecloth and allowed to stand for 3 h before decanting. The residues were dried at 50 ^{o}C for 48 h, ground into powder and then used as the starch source for the study.[7]

Biochemical and *α-amylase Assays*

The *α*-amylase activity was determined according to the combined methods of Nelson and Somogyi.[8,9]

Protein assay
Protein concentration was determined based on the method of Bradford.[10] The assay mixture consisted of the crude enzyme (0.05 mL), distilled water (0.75 mL) and Bradford reagent (0.2 mL) which were gently mixed and incubated at room temperature for 5 min. Absorbance was measured at 595 nm and the protein concentration in the sample was extrapolated from the standard curve using Bovine serum albumin (BSA) as standard.

Optimization of α-Amylase Production by Bacillus cereus
Determination of the inoculum size
Different inoculum sizes (0.5-3.0%) from 24 hours broth culture of the test isolates were used for the determination of α-amylase production level. Sterile basal medium (50 mL) constituting soluble starch (5 g/L), yeast extract (5 g/L), $(NH_4)_2SO_4$, (2.5 g/L), $MgSO_4.7H_2O$ (0.2 g/L), K_2PO_4 (3 g/L), and $CaCl_2.2H_20$ (0.25 g/L)[11] was inoculated with *B. cereus* SM22 (0.5-3.0%) and fermented at 35°C for 48 h with steady agitation with a rotary shaker at 150 rpm. After fermentation, the medium was thereafter centrifuged at 6000 rpm for 30 min and the supernatant was tested for α-amtlase activity.[8,9]

Determination of optimum pH for α-amylase production
To test the effect of different pH values on the production of α-amylase, the pH of the basal medium (50 mL) was varied between 3.0 and 9.0 before sterilization. The sterile basal medium was inoculated with 0.5 mL of 24 hours broth culture of *B. cereus SM22* and incubated at 35°C for 48 h with steady agitation at 150 rpm. The medium was thereafter centrifuged at 6000 rpm for 30 min and the supernatant was tested for α-amylase activity.[8,9]

Determination of optimum temperature for α-amylase
To test the effect of different temperature values on the production of α-amylase, the sterile basal medium (50 mL, pH 7.0) was inoculated with 0.5 mL of 24 h broth culture of *B. Cereus* SM22 and incubated at 25, 37, and 40–70 °C for 48 h with steady agitation at 150 rpm. The medium was thereafter centrifuged at 6000 rpm for 30 min and the supernatant was tested for α-amylase activity.[8,9]

Effect of cocoyam starch concentration on α-amylase production
The effect of different starch concentrations on α-amylase production was tested using 0.5-2.5% cocoyam starch constituted into the basal medium. The basal medium was inoculated with 0.5 mL of 24 hours broth culture of *B. cereus* SM22 and incubated at 37 °C for 48 h with steady agitation at 150 rpm. The medium was thereafter centrifuged at 6000 rpm for 30 min and the supernatant was tested for α-amylase activity.[8,9]

Effect of carbon sources on α-amylase production
The effects of different carbon sources were evaluated using 1 %w/v of maltose, glucose, starch, galactose and sucrose, respectively. The basal medium (50 mL) containing the various carbon sources was inoculated with 0.5 mL of 24 hours broth culture of *B. cereus* SM22 and incubated at 37°C for 48 h with steady agitation at 150 rpm. The medium was thereafter centrifuged at 6000 rpm for 30 min and the supernatant was tested for α-amylase activity.[8,9]

Effect of nitrogen sources on α-amylase production
The effects of different nitrogen sources, each with a concentration of 10 g/L (w/v) [KNO_3, $NaNO_2$, NH_4Cl, $Ca(NO_3)_2$, $(NH_4)_2SO_4$, yeast extract, and peptone] were tested. The basal medium (50 mL) containing the various nitrogen sources was inoculated with 0.5 mL of 24 hours broth culture of *B. cereus* SM22 and incubated at 37°C for 48 h with steady agitation at 150 rpm. The medium was thereafter centrifuged at 6000 rpm for 30 min and the supernatant was tested for α-amylase activity.[8,9]

Purification of α-amylase
Ion-exchange chromatography on diethyl-aminoethyl (DEAE)sephacel
The cell-free culture supernatant obtained from the basal medium was dialyzed and concentrated. The concentrated sample was layered on DEAE-Sephacel ion exchange column (1.0 x 10.0 cm) equilibrated with phosphate buffer (10 mM, pH 7.0). Fractions of 1 mL each were collected at a flow rate of 20 mL/h.The bound enzyme was eluted by using a linear gradient from 0 -1.0 M of NaCl in the elution buffer (10 mM, pH 7.0). Fractions that had *α*-amylase activity[8,9]were pooled together and concentrated. Also, the protein profiles of the fractions were measured at 280 nm using a UV-visible spectrophotometer.[10]

Gel filtration on biogel P-100
The post-DEAE Sephacel concentrated sample was re-dissolved in 1 mL phosphate buffer (10 mM, pH 7.0) and layered on BioGel P-100 column (1.0 × 50 cm) pre-equilibrated with phosphate buffer. Fractions of 5 mL each were collected at a flow rate of 10 mL/h. Elution was performed by using the same buffer and fractions with α-amylase activity[8,9] were pooled together and lyophilized to afford partially purified α-amylase.

Determination of native molecular weight
The native molecular weight of the partially purified α-amylase was determined by gel filtration on a Bio-Gel P-100 column (1.0×50 cm). The void volume (V_o) was determined with blue dextran (2 mg/mL) from a plot of absorbance of fractions at 620 nm against fraction volume. The column was calibrated with low molecular range protein standards:[10] gamma globulin (135 kDa), α-chymotrypsinogen A (25 kDa), ovalbumin (45 kDa) and bovine serum albumin (66 kDa). Each protein standard was prepared in tris buffer (10 mM, pH 7.0); and 1 mL of each standard was loaded on the column.The bound proteins were eluted with the same buffer at a flow rate of 10 mL/h. Fractions (1 mL each) were collected and the protein profile was monitored at 280 nm. The partition coefficient (K_{av}) of each standard and the partially purified α-amylase was calculated. The K_{av} values of each standard protein were plotted against the logarithm of the molecular weights from where the molecular weight of the amylase was extrapolated.

Characterization of the purified α-Amylase from bacillus cereus SM22
Effects of pH on activity and stability of purified α-amylase
Different buffer systems; citrate buffer (10 Mm, pH 3.0-5.0); phosphate buffer (10 mM, pH 6.0-7.0) and tris buffer (10 mM, pH 8.0-11.0)] were prepared and used for the determination of α-amylase activity. For measurement of pH stability, the enzyme was incubated at room temperature for 1 h in buffers having different pH values. The residual activity was thereafter determined as described.[8,9]

Effects of temperature on the activity and stability of α-amylase
To determine the effect of different temperatures on starch hydrolysis by α-amylase, an aliquot of the enzyme (in phosphate buffer, pH 7.0) and starch substrate were incubated at 0 -100 °C for 20 min. Thereafter residual activity of the enzyme was determined[8,9] and plotted against the different temperatures and optimum temperature was extrapolated. Negative control was prepared without incubation. The peak of the *α*-amylase activity was detected and considered as the optimal temperature.

Thermal stability of the α-amylase
The thermal stability of the α-amylase was determined by incubating the enzyme preparation at different temperatures (50, 60, 70, and 80 °C) for 60 min. Aliquots were withdrawn at 10 min intervals and the residual activity was measured as described.[8,9]

Effects of different metal ions on the α-amylase
The effects of different metal ions (NaCl, $MgCl_2$, $CaCl_2$, and $Al_2(SO_4)_3$) on α-amylase activity were determined under optimal conditions of temperature and pH using different concentrations (0-300 mM) of metal ions prepared in phosphate buffer (10 mM, pH 6.0). The reaction mixture was incubated at 50 °C for 10 min. Negative control was prepared without the metal ions.[11]

Effects of EDTA on the α-amylase activity
The effect of EDTA on α-amylase activity was determined using different concentrations (0-300 mM) of EDTA prepared in phosphate buffer (10 mM, pH 6.0) and incubated at 50 °C for 20 min.[11]

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ISSN 2616-0692 (Electronic)

Effect of raw starch on the crude α-amylase production
The effect of α-amylase on raw starches was determined by incubating an aliquot of the α-amylase with 1% gelatinized and ungelatinized raw starch (0.1 mL). Different sources of starch used include cassava, cocoyam, corn, soluble starch, plantain, and yam.[8,9]

Determination of kinetic parameters
The apparent kinetic parameters (K_m and V_{max}) were determined according to the method of Abdel-Fattah et al.[11], using an aliquot of the enzyme with different concentrations of cocoyam starch (0-200 mg/mL) as substrate at 50°C and pH of 7.0. The products formed were estimated and used for the apparent kinetic parameters such as maximum rate V*max* (mU/mg/min) and Michaelis-Menten constant; K*m* (mg/mL).

Statistical analysis
The research data was duly analyzed and presented using Statistical Package for the Social Sciences (SPSS) version 23.0 and Microsoft Excel for Windows.

Results and Discussion

Optimization of inoculum size, pH and temperature for α-amylase production by Bacillus cereus SM22
The optimum inoculum size required for the growth and release of α-amylase by *Bacillus cereus* SM22 was 2% with the maximum α-amylase activity of 276.01 ± 2.21 Units/mL (Figure 1). Inoculum size is an important factor affecting the biosynthesis of enzymes. The higher the inoculum size, the higher the number of cells in the production media and vice versa. Lower inoculum size would require a longer time for the cells to grow into optimum number to be able to transform the substrate into the desired product(s).[12] The best inoculum size for this study was achieved at 2%. Also, Kashyap *et al*[13] had reported a similar inoculum size of 2% for maximum growth in the culture medium.
The optimum pH of the medium for the growth and synthesis of α-amylase by the bacterium was pH 7.0 with the α-amylase activity of 503.97±47.91 Units/mL; while the optimum temperature was 37°C with the activity of 336.83±17.2 Units/mL (Figure 2).
Furthermore, temperature is another important factor in the development of biotechnological processes. Temperature influences protein denaturation, enzyme inhibition, and cell growth.[14]

Effects of starch on α-amylase production by Bacillus cereus SM22
The cocoyam starch concentration required for optimum synthesis and release of α-amylase by *B. cereus* SM22 in the media was 1 %. Therefore, with 1 % cocoyam starch composition in the production medium, an optimum activity of 259.8±2.56 Units/mL was recorded (Figure 3).Similarly, maximum α-amylase activity had also been reported in the range of 1-1.5 % starch composition.[15] Steady increase in a starch concentration above 25 % caused inhibition of α-amylase activity.[16]

Effects of different carbon sources on α-amylase production
The maximum α-amylase activity was recorded with cocoyam starch (Figure 4) with enzyme activity of 835.9 units/mL, while galactose produced the least activity. This showed that the starch molecule is an ideal substrate with a maximum activity of 835.9±0.03 units/mL. The high affinity for starch by this organism could indicate that α-amylase is inducible in the presence of starch substrate.

Effects of organic and inorganic nitrogen sources on α-amylase production
For optimal microbial growth in a culture medium, a suitable nitrogen source is needed for the synthesis of biological macromolecules such as protein. In this study, for organic nitrogen sources, peptone was found to induce maximum α-amylase synthesis (293.65 Units/mL) than other nitrogen sources (Figure 5, A). On the other hand, ammonium nitrate induced maximum α-amylase activity (60.795 Units/mL) than other inorganic nitrogen sources (Figure 5, B).

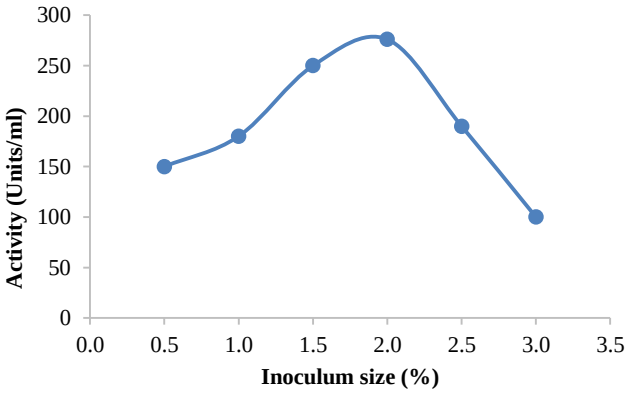

Figure 1: Effect of inoculum size on α-amylase activity at 35°C and pH 7.0.

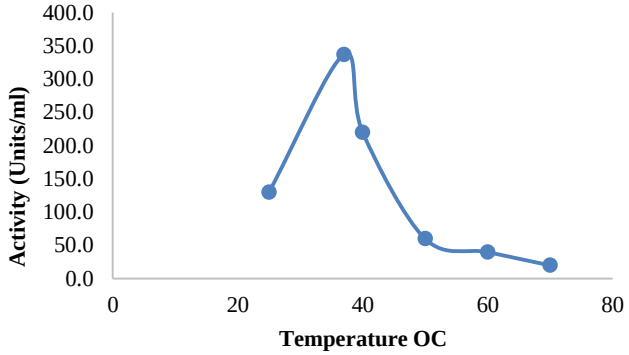

Figure 2: Effect of temperature on α-amylase activity at pH 7.0.

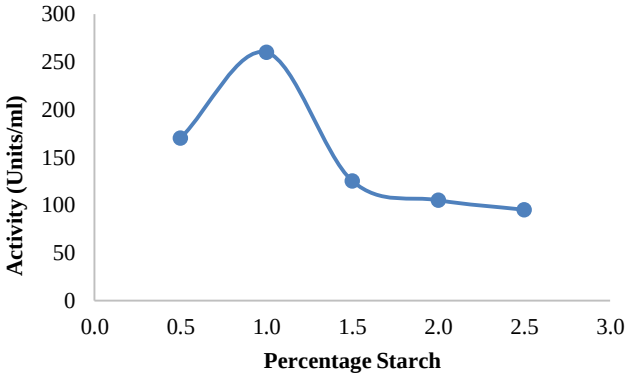

Figure 3: Effects of cocoyam starch and carbon sources.

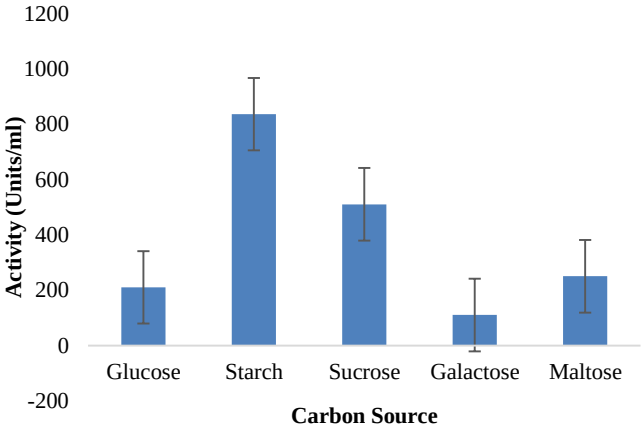

Figure 4: Effects of carbon sources on crude α-amylase production (37°C, pH 7) by *Bacillus cereus SM22.*

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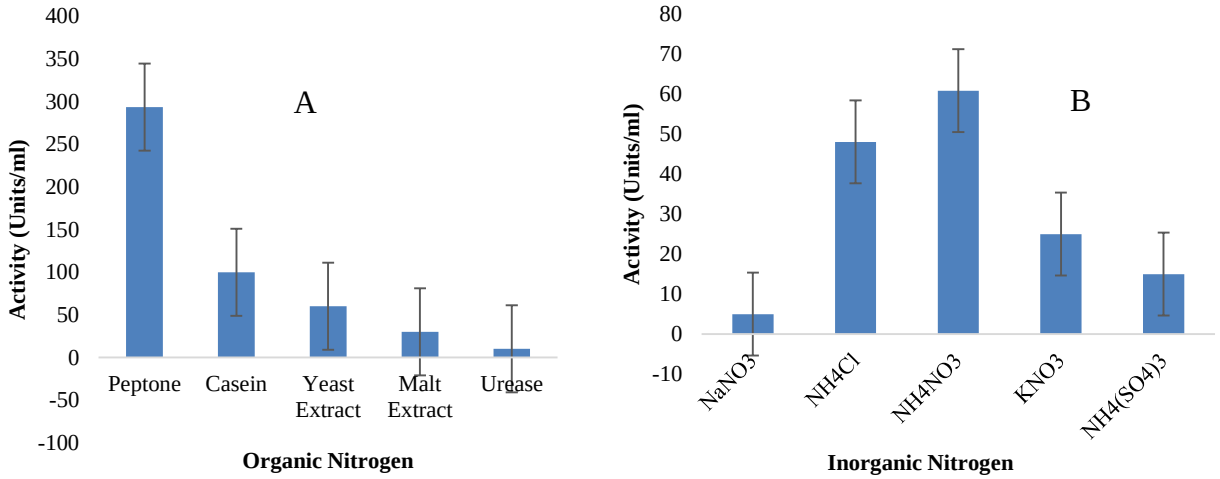

Figure 5: Effects of organic (A) and inorganic nitrogen sources (B) on crude α-amylase Production (37°C, pH 7) by *Bacillus cereus.*

Effects of raw starches on the crude α-amylase production
The effect of cocoyam starch was compared with corn, cassava, plantain, and yam starches. The result showed that cocoyam starch was the best nutrient for *Bacillus cereus* to secrete maximum α-amylase (882.891 U/mL) in the medium (Figure 6).

Elution Profile of cell-free supernatant of B. cereus SM22 culture on DEAE sephacel
The crude enzyme concentration from the cell-free supernatant was 4.808 units/mg protein. The purification run on DEAE Sephacel produced only one peak (Fig. 7); which was pooled and further purified via BioGel P-100 (Fig. 8). Also, a single peak was obtained on gel filtration. The final purification step yielded 42% of overall recovery with the specific activity of 46.6 Units/mg of protein (Table 1).

The native molecular weight of α-amylase from B. cereus SM22
The native molecular weight of the α-amylase from *B. cereus SM22* was estimated to be 56 kDa. Further purification on SDS PAGE revealed that the molecular weight was 54 kDa (Figure 9).Native molecular weights of 55.2 kDa[17], 58 kDa[18] and 63 kDa[19] for α-amylases isolated from *Bacillus lichenniformis*, *Bacillus amyloliquefaciens*, and *Bacillus subtilis* BS5 respectively have been reported.

Characterization of the purified α-Amylase from Bacillus cereus SM22
Effects of pH on activity and stability of the purified α-amylase
The purified α-amylase showed activity over a wide range of pH (3.0–11.0) with optimum activity at pH 6.0. The relative activities at acidic (3.0) and basic (11.0) pH were 322.56±0.01 Units/mL and 241.45±1.75 Units/mL, respectively (Figure 10).

Effect of temperature on the α-amylase activity
The optimum temperature obtained from the activity of the purified α-amylase was 50 °C (Figure 11). The enzyme also retained 100% activity at this temperature for 1 h. The stability of the α-amylase at the incubation of 50 °C was an indication that the enzyme was moderately heat stable. This was further supported by the ability of the enzyme to retain 100 % activity even after sixty minutes (60) 1 h of incubation at 50 °C. At temperature above 50 °C, there was a steady reduction in activity with a complete loss at 100 °C (Figure 12). The result shows that the α-amylase produced by the *B. cereus* SM22 was found to be more robust and heat stable than 56.6% heat stability reported from *B. subtilis* BS5.[20]

Effect of metal ions on the purified α-amylase activity
The effects of metal ions on the activity of α-amylase showed that though sodium ion inhibited the α-amylase activity at a very low concentration, NaCl had a stimulatory/optimum effect on the activity of the enzyme at 100 and 240 mM.(Figure 13, A).

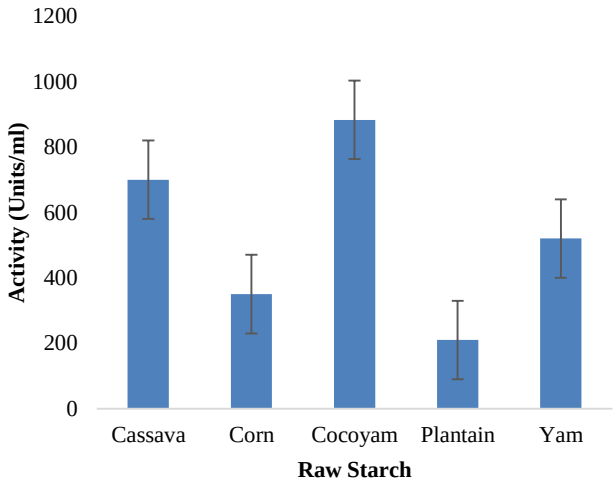

Figure 6: Effects of raw starches on crude α-amylase production (37°C, pH 7) by *Bacillus cereus* SM22.

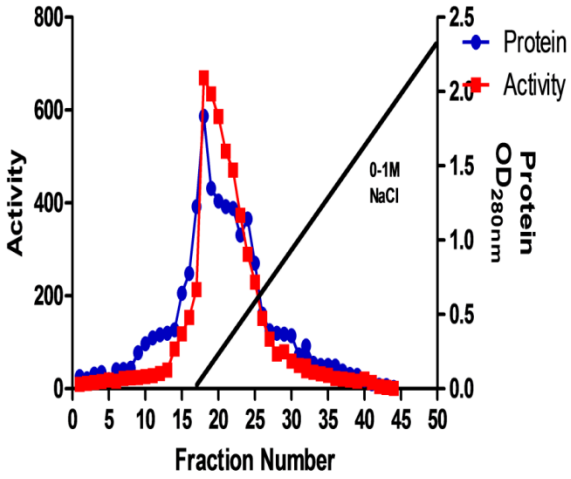

Figure 7: Elution Profile of cell-free supernatant of *Bacillus cereus* SM22 culture on DEAE Sephacel.
Fractions of 22 mL each were collected. Fractions were assayed for amylase activity (left vertical axis) and total protein content (right vertical axis) and plotted against the tube fractions (horizontal axis).

ISSN 2616-0684 (Print)
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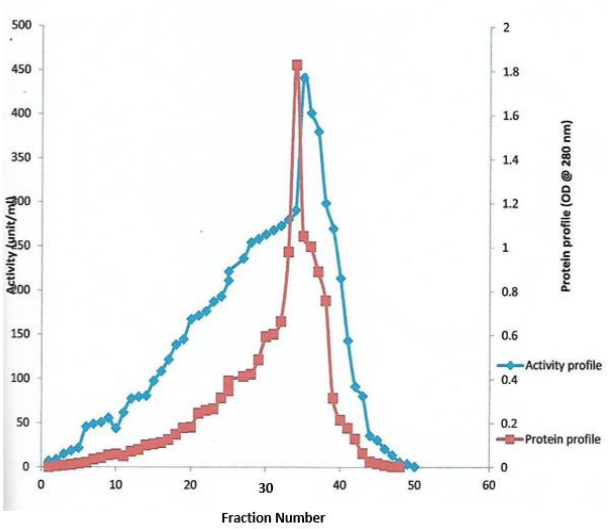

Figure 8: Elution profile of cell-free supernatant from *B. cereus* SM22culture on BioGel P-100.

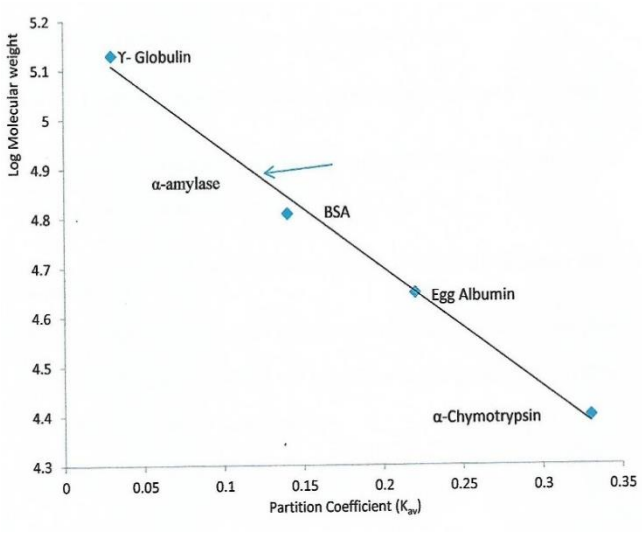

Figure 9: A plot of log (molecular weight) against molecular weight.

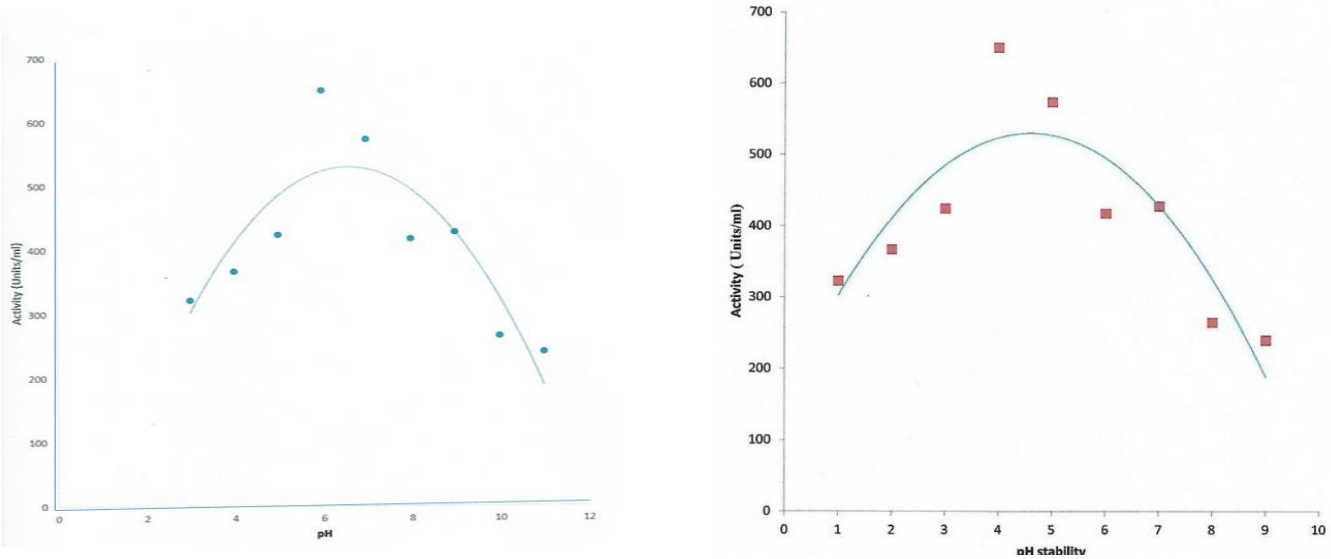

Figure 10: Effect of pH (left) and pH stability (right) on α-amylase activity produced by *B. cereus* SM22 at 50°C.

Table 1: Purification scheme of *α*-amylase extracted from *Bacillus cereus* SM22

S/N	Purification Step	Volume (mL)	α-Amylase (U/mL)	Total α-Amylase Activity (U/mL)	Protein (mg/mL)	Total Protein (mg/mL)	Specific Activity (U/mL)	Yield (%)	Purification (fold)
1	Crude	50	14.53	726.50	3.022	151.6	4.79	100	1
2	Lyophilized crude	1	653.0	653.0	15.61	41.83	89.88	8.70	8.70
3	Pooled fraction (Ion - exchange)	11	49.66	546.29	1.81	19.91	27.44	75.19	5.71
4	Lyophilized fraction (ion-exchange)	2	301	602	6.51	13.02	46.22	82.86	9.60
5	Pooled fraction (Gel-filtration)	8	37.98	303.84	0.82	6.52	46.60	41.822	9.70

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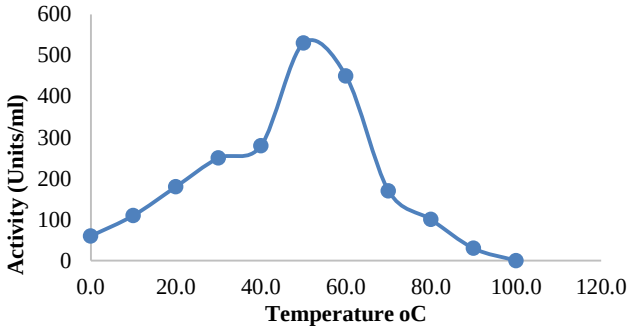


Figure 11: Effect of temperature on the α-amylase activity produced by *B. cereus* SM22 at pH 6.0.

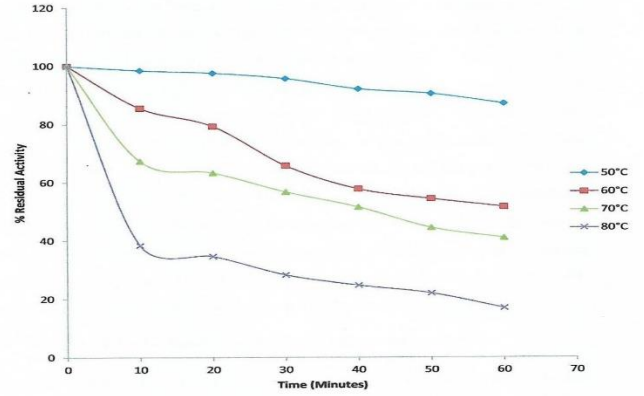


Figure 12: Effect of heat stability on the α-amylase activity produced by *B. cereus SM22* at pH 6.0.

Also, Mg^{2+} also enhanced the activity of the enzyme at 80-120 mM but inhibited the enzyme at 140 mM and above. (Figure 13, B). However, Ca^{2+} inhibited the activity of the enzyme at 100-300 mM though, it tends to slightly enhance the activity at lower concentrations (20-80 mM). The Al^{3+} slightly inhibited the activity of α-amylase at 20-200 mM but a total loss of activity was recorded at concentrations above 200 mM. The stimulatory effects of Na^{+} and Ca^{2+} have been reported.[21,22]

Effect of EDTA on purified α-amylase activity

EDTA inhibited the activity of the enzyme at various concentrations tested with maximum inhibition resulting in complete loss of activity at 260-300 Mm (Figure 15). EDTA is a chelating agent and tends to form inactive complexes with metal ions such as cofactors in enzyme structures, thus lowering the enzyme-substrate affinity.[23] The high sensitivity of *α*-amylase to EDTA could be that the enzyme contains inorganic group(s) which might have formed inactive complexes with the EDTA molecules.

Effects of raw starches on the α-amylase produced by B. cereus

The study revealed the effectiveness of raw starches on α-amylase activity with significant activity (850.71 Units/mL) obtained with un-gelatinized raw cocoyam starch while gelatinized soluble induced the least activity (156.6 Units/mL) (Figure 16).

Kinetic Parameter (K_m and V_{max})

Further studies with gelatinized and un-gelatinized starches including soluble starch revealed the effectiveness of raw starches on α-amylase activity which can be harnessed in hydrolytic processes using raw starch as substrate.

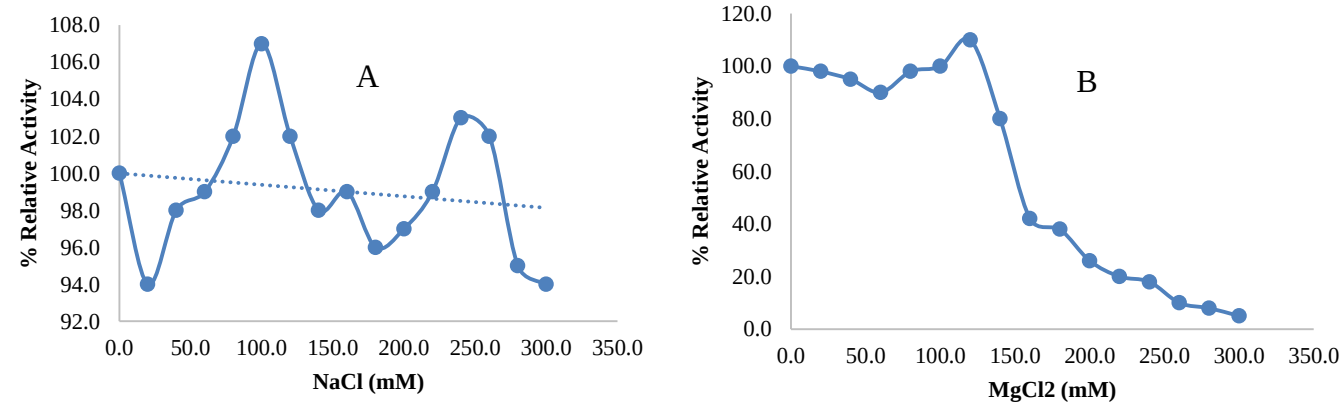


Figure 13: Effects of NaCl (A) and $MgCl_2$ (B) on the α-amylase produced by *Bacillus cereus* SM22 at pH 6.0 and 50 ^{o}C.

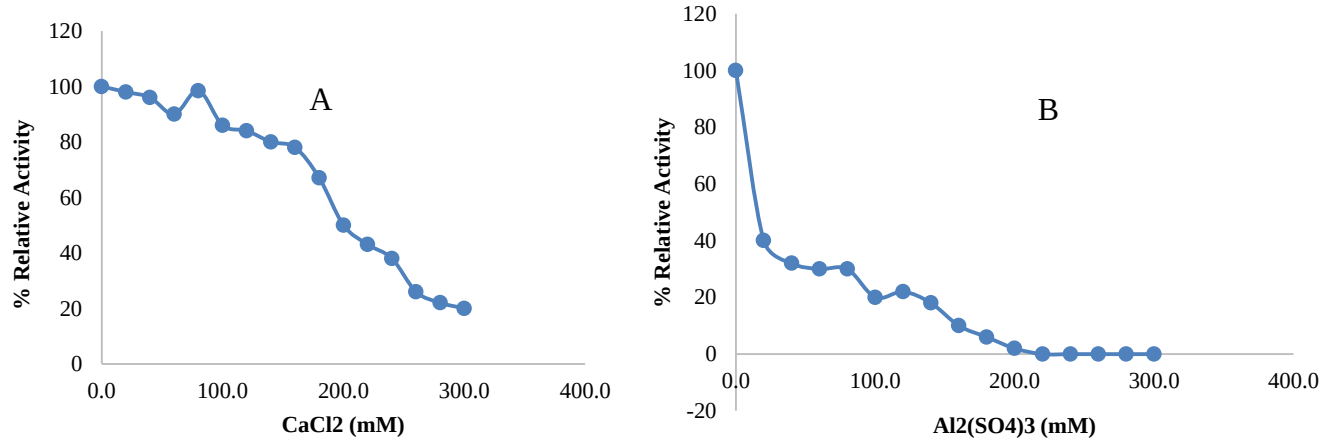


Figure 14: Effects of $CaCl_2$ (A) and $Al_2(SO_4)_3$ (B) on the α-amylase produced by*Bacillus cereus SM22* at pH 6.0 and 50 ^{o}C.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

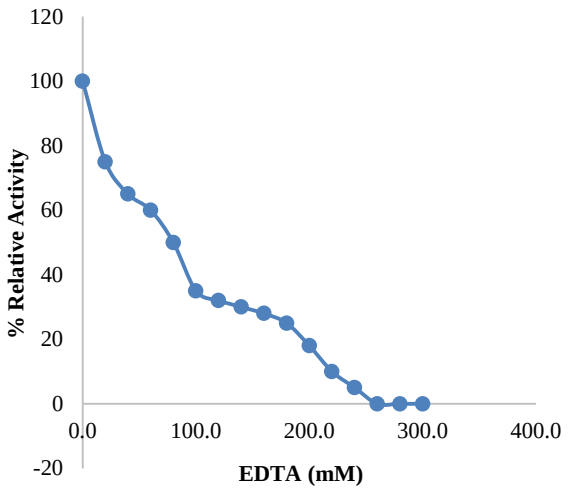


Figure 15: Effects of EDTA on the α-amylase produced by*Bacillus cereus SM22* at pH 6.0 and 50°C.

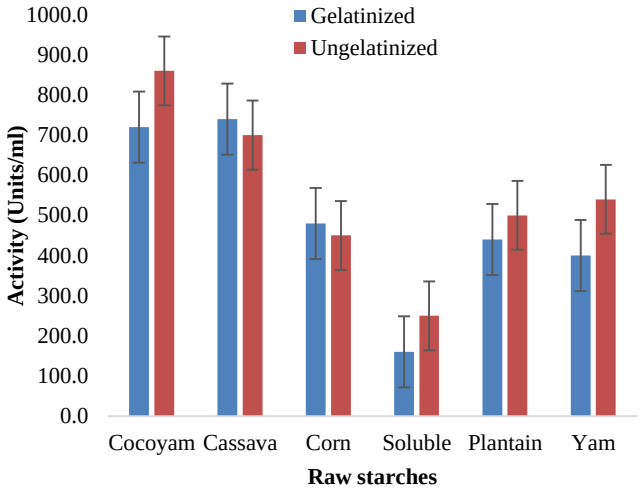
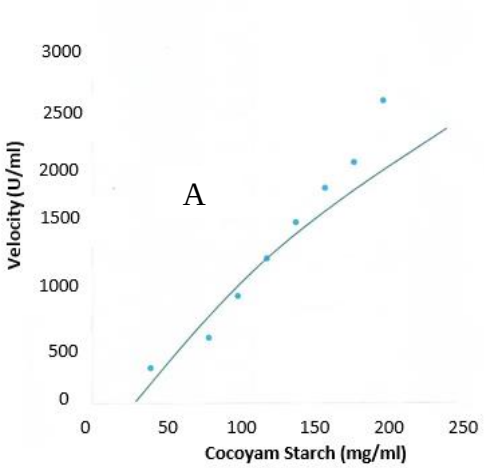


Figure 16: Effects of starches on the α-amylase produced by*Bacillus cereus* SM22 at pH 6.0 and 50°C.



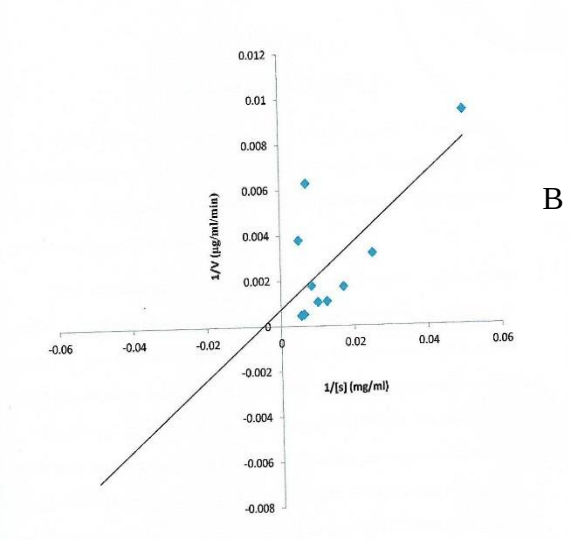


Figure 17: Effects of different concentrations of cocoyam starch (A) on *α*-amylase activity; and the Lineweaver-Burk plot (B) of α-amylase produced by*Bacillus cereus SM22* at pH 6.0 and 50°C.

The activity of the partially purified α-amylase increased with an increase in substrate concentration; and the trend continued until a maximum concentration was reached where there was no further increase in enzyme activity (Figure 17A). At this point, the activity of the enzyme was inhibited by saturation concentration. The Michaelis-Menten constant ($k_m$andV_{max}) of the purified α-amylase for cocoyam starch were estimated from the Lineweaver-Burk plot of the activity of purified α-amylase at a varying concentration of substrate as shown in Figure 17B. The V_{max} and K_m obtained were 4834.64 ± 95.71 and 91.09 ± 3.72 Unit/mg protein, respectively. Low K_m values for α-amylases produced by some *Bacillus* spp had been reported [24] and indicates that the high affinity of the enzyme with its substrate in the formation of the enzyme-substrate complex. The low K_m value obtained from this study has shown robust potentials for harnessing the α-amylase produced by *B. cereus* SM22 in active biotechnological processes for efficient bioconversions of starch molecules into useful products.

Conclusion

The study purified, and characterized α-amylase from *Bacillus cereus* SM22 isolated from decayed cocoyam tubers. For efficient production of α-amylase in the growth medium, the following conditions were required: pH 7.0, 37°C, 1% cocoyam starch, 2% inoculum, peptone and ammonium nitrate. Conversely, the optimum conditions required for the maximum performance of α-amylase from *Bacillus cereus* SM22 were pH 6.0, 50°C, NaCl (100 or 240 mM), low concentrations of Mg^{2+} (80-120 mM) and Ca^{2+} (20-80 mM), no Al^{3+} and EDTA. With these important factors, the *B. cereus* SM22 andα-amylase were found to perform maximally in the culture media. Above all, the low $K_m$and high V_{max} values make the enzyme from this bacterial strain an excellent candidate for robust industrial applications. Further studies is required to enable potential commercialization of the enzyme.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

We wish to acknowledge the staff of the relevant departments of University of Nigeria, Nsukka and Obafemi Awolowo University, Ile-Ife for their professional assistance in this study.

References

1. Lim SJ, Hazwani-Oslan SN, Oslan SN. Purification and characterisation of thermostable α-amylases from microbial sources. Bio Res. 2019; 15(1):2005–2029.
2. Kalsoom F, Anjum FR, Anam S, Khan S, Hasan F. Effect of temperature, pH and metal ions on amylase produced from selected indigenous extremophile bacteria in Pakistan. Int J Biosci. 2018; 13(03):262–275.
3. Gopinath SCB, Anbu P, Arshad MKM, Lakshmipriya T, Voon CH, Hashim U, Chinni SV. Biotechnological Processes in Microbial Amylase Production. BioMed Res Int. 2017; 2017:1-9.
4. Elyasi FB, Ahmadi Y, Yari KA, Dilmaghani A. Microbial Alpha-Amylase Production: Progress, Challenges and Perspectives. Adv Pharm Bull. 2020; 10(3):350-358.
5. Özcan D and SİpahİoĞlu HM. Simultaneous production of alpha and beta amylase enzymes using separate gene bearing recombinant vectors in the same *Escherichia coli* cells. Turk J Biol. 2020; 44(4):201-207.
6. Singh H, Singh SN, Signh N. Structure and structural properties of thinned sorghum starch. Int J Food Prop. 2009; 12(4):713-725.
7. Udechan IS, Sahoo AK, Hend GM. Extraction and characterization of sorghum (*Sorghum bicolor* L. Moench) Starch. Int Food Res J. 2012; 19(1):315-319.
8. Nelson N. A Photometric adaption of the *somogyi* method for the determination of glucose. J Bio Chem. 1944; 153(2):373-380.
9. Somogyi M. A new reagent for the determination of sugars. J Bio Chem. 1945; 160(1):61-68.
10. Shen CH. Chapter 7 - Detection and analysis of nucleic acids. In: Diagnostic Molecular Biology. London, United: Academic Press, First Edition. 2019. 167-185 p.
11. Abdel-Fattah YR, Nadia AS, Nabil ME, El-Gendi H, Rania SA. Production, purification, and characterization of thermostable *α*-amylase produced by *Bacillus licheniformis* Isolate AI20. J Chem. 2013; 2013:1-11.
12. Nguyen HT and Tran G. Optimization of fermentation conditions and media for production of glucose isomerase from Bacillus megaterium using response surface methodology. Sci. 2018; 2018:1-11.
13. Kashyap P, Sabu A, Pandey A, Szakas G, Soccol CR. Extracellular L-glutaminase production by *Zygosaccharomyces rouxii* under SSF. Pro Biochem. 2002; 38(3):307-312.
14. Moore CE, Meacham-Hensold K, Lemonnier P, Slattery RA, Benjamin C, Bernacchi CJ, Lawson T, Cavanagh AP. The effect of increasing temperature on crop photosynthesis: from enzymes to ecosystems. *J Exp Bot. 2021;* 72(8):2822-2844.
15. Balkan B and Ertan F. Production and properties of α-amylase from *Pencillium chrysogenum* and its applications in starch hydrolysis. Prep Biochem Biotechnol. 2005; 35(2):169-178.
16. Mohammed MAAZ, Shivayogeeshwar N, Gurumurthy DM, Rajeshwara AN. Identification, characterization of novel halophilic *Bacillus cereus* Ms6: a source of extracellular α-amylase. Adv Env Bio. 2011; 5(5):992-999.
17. Yasser RAF, Soliman NA, El-Toukhy NM, El-Gendi H, Ahmed RS. Production, purification, and characterization of thermostable α-amylase produced by *Bacillus licheniformis* Isolate AI20. J Chem. 2013; 2013:1-11.
18. Liu YH, Lu FP, Wang JL, Gao C. Acid stabilization of *Bacillus licheniformis* alpha amylase through introduction of mutation. Appl Microb Biotechnol. 2008; 80(5):795-803.
19. Gangadharan D, Sivaramakrishnan S, Nampoothiri KM, Sukumaram RK, Pandey A. Response surface methodology for the optimization of α -amylase production by *Bacillus amyloliquefaciens.* Biores Tech. 2008; 99(11):4597-4602.
20. Femi-Ola TO and Olowe BM. Characterization of alpha amylase from *Bacillus subtilis* BS5 isolated from *Amitermesevuncifer Silvestri.* Res J Microbiol. 2011; 6(2):140-146.
21. Dakhmouche DS, Bennamoun L, Labbani FZK, Ait KA, Nouadri T, Pauss A, Meraihi Z, Gillmann L. An alkalothermophilicamylopullulanase from the yeast *Clavisporalusitaniae* ABS7: purification, characterization and potential application in laundry detergent. Catalysts. 2021; 11(12):1438.
22. Liao SM, Liang G, Zhu J, Lu B, Peng L-X, Wang Q-Y, Wei Y-T, Zhou G-P, Huang R-B. Influence of calcium ions on the thermal characteristics of α-amylase from thermophilic Anoxybacillus sp. GXS-BL. Prot Pep Lett. 2019; 26(2):148–157.
23. Reilley DJ, Hennefarth MR, Alexandrova AN. The case for enzymatic competitive metal affinity methods. *ACS Catalysis.* 2020; *10*(3):2298-2307.
24. Aladejana OM, Oyedeji O, Omoboye O, Bakare M. Production, purification and characterization of thermostable alpha amylase from *Bacillus subtilis* Y25 isolated from decaying yam (*Dioscorearotundata*) tuber. NotulSci Bio. 2020; 12(1):154-171.