Purification and Characterization of α-Amylase from *Bacillus subtilis* Isolated from Cassava Processing Sites

1 1 1 2*

* Adeleke Bartholomew Saanu, Department of Microbiology, Federal University of Technology, Akure, Nigeria, Tel: +2347065058110; E-mail: microbade@yahoo.com

September 29, 2017; October 26, 2017; October 30, 2017

This study was designed to purify and characterize of α-amylase from pure strain of . The crude α-amylase was purified by ammonium sulphate precipitation, then loaded on DEAE Sephadex A-50 ion exchange chromatography and gel filtration. The effect of pH, temperature and metal ions were investigated on the purified enzyme. The single protein band on SDS-PAGE suggested that the enzyme was homogenous. Two different activity peaks were observed in ion exchange chromatography designated pool A and pool B with the 8% and 4% yield, 15.93 and 6.44 purification fold and specific activity 2.55 µmol/min/mg and 1.03 µmol/min/mg respectively. The two fractions revealed the same optimum pH 7.0 for the α–amylase activity while the enzyme was relatively stable at pH 4.0 and 7.0 between 20 to 40 minutes and 60 to 80 minutes for pool A and pH 8.0 between 40 and 100 minutes for pool B. At 40°C, optimum temperature was reached, and amylase activity was maintained at 75% and 70% temperature stability between 60 to 80 minutes for pool A and B, less than 20%, the residual activity at 60°C and 70°C was recorded. The incubation of α–amylase with Na^+ and Zn^{2+} ions enhanced/activate the enzyme activity correspondingly, Al^{3+} and K^+ ions exhibited varied degree of inhibition while Ca^{2+} and Hg^{2+} ions caused total inhibition on α–amylase activity. The ability of purified α-amylase from under wide range of temperatures and pH suggests its applications in industries and bioremediation of effluent discharge on food processing sites.

Bacillus subtilis; Cassava processing site; α-amylase; Purification; Characterization

Introduction

Globally, indiscriminate dumping or disposal of wastes to the environment affects both macro-and-microorganisms causing ecological imbalance and displacement from their niche. The survival of microorganisms depended on several factors such as biotic, and abiotics. Soil stands as major reservoir of different microorganisms. Biodegradation of wastes on/in soil by the action of microorganisms could prompt a safer environment [1]. Microorganisms isolated from soil stand as potential source of industrial enzymes due to their abundance in nature. However, utilization of these enzymes such as amylases, cellulases, linamarases, mannanase and pectinase etc in our industries are limited due to the poor exploration. Amylases (EC 3.2.1) is a hydrolytic enzyme that catalyze the hydrolysis of amylose and

DK-600), and the reaction was terminated by adding 1 ml of 3,5-dintrosalicylic acid (DNSA) reagent tube. T e tubes were incubated for another 5 minutes in a boiling water bath for colour development and cooled rapidly. T e activity of the reaction mixture was measured against blank at 540 nm. T e amount of liberated reducing sugars (glucose equivalents) were estimated by the dinitrosalicylic acid (DNS) method of Miller. One unit of α-amylase was defned as amount of enzyme producing 1 micromole of glucose equivalent per minute under the experimental conditions.

Protein determination: T e protein estimation of the crude extract was determined by Bradford method and Bovine Serum Albumin (BSA) was used as standard Bradford.

Purifcation and Characterization of α - amylase from *Bacillus subtilis*

Purifcation of α-amylase from *Bacillus subtilis*: T e purifcation of crude extract (enzyme supernatant) obtained afer cold centrifugation was done in three stages. In stage one, 400 ml of the supernatant of crude enzyme was precipitated by adding 240 g ammonium sulphate to obtained 60% ammonium sulphate concentration. T e concentrates were then cold centrifuged at 6000 rpm for 30 minutes, the precipitate obtained was washed with 100 ml phosphate bufer (pH 6.0) and then dialyzed in the same bufers. In stage two, the partially purifed α–amylase obtained was purifed on ion exchange column chromatography containing DEAE (diethylamineethyl) Sephadex A-50 (2.0 × 2.5 cm, Pharmacia). T e eluates obtained from stage two were washed with 300 ml ion-free water, followed by 200 ml 0.01 M Tris-HCl bufer (pH 8.0). T e gel was eluted with NaCl. T e absorbance of each of the fraction at time interval was measured. In stage three, 2.5 ml of partially purifed α–amylase was loaded onto a column chromatography (2.5 cm in diameter and 30.0 cm high) using Sephadex G-150 (Pharmacia). Phosphate elution bufer at 50 mM pH 6.0 was applied with the fow rate 20 ml/hour. A fraction of 5.0 ml was collected at interval of 30 minutes and the highest

	10.72	1.95	2.01	20.9	21.55	1.03	3.59	6.44

B are shown (Figures 5 and 6). The α–amylase from pool A and pool B increased progressively up pH 4 with specific activity 39.17 µmol/min/mg. The optimum activity was attained at pH 7.0. The relative activities of pool A and pool B at pH 6.0 was reduced by 34% and 47% and pH 9.0 was reduced by 10% and 48% respectively when compared with the value obtained at pH 7.0. The stability of the enzyme was checked at different pH ranged from 5.0 to 8.0 at room temperature for two hours. The α – amylase activity obtained from pool A was relatively stable between 20 to 40 and 60 to 80 minutes of incubation at pH 4.0 and 7.0 while α–amylase activity obtained from pool B was stable between 40 to 100 minutes of incubation at pH 8.0 respectively. pH of the growth medium plays an important role by inducing morphological change in the nature of microorganism and their enzyme secretion. The decrease in pH beyond optimum might be due to the concomitant alteration in the conformation of the enzyme protein caused by changes in pH of its environment resulting in changes in the three dimensional structure of the enzyme active sites and substrates binding speed to produce maximum product. pH 6.8-7.2 optimum for purified amylase from *Bacillus subtilis* has been reported [20]. Roy and Rowshanul [16] reported pH 6.0 optimum for purified xylanase from *Bacillus cereus*. It was reported that the stability of most plant enzymes decreased significantly at pH values below 4.0 and above 7.5, whereas, the majority of the corresponding microbial enzymes are rather stable at pH 7.0 [21]. Mukesh et al. [8] reported α–amylases stability over a wide range of pH 4 to 11. The optimum pH 7.0 and above for α–amylase activity from *B. subtilis, B. licheniformis, B. amyloliquefaciens* and thermophilic *Anoxybacillus favithermus* has been reported [16,22-26] pH 8.0 optimum has been reported for amylase activity from *Termus* sp., *Bacillus* KSM-K38 and *Bacillus* spp [27,28] Wide range (pH 3.5 to 12) optimum pH for α–amylases has been reported [7,29-32]. The result obtained from this study agrees with the findings of Agülo˘glu Fincan and Bukhari and Rehman [32,33] who reported pH 7.0 optimum for purified α–amylase from *Bacillus subtilis* from local environment.

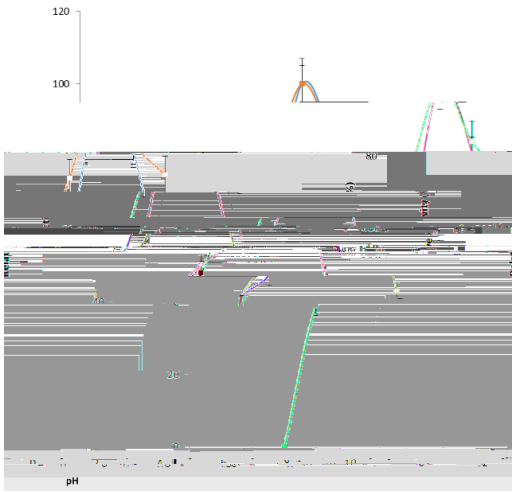

Figure 4: Effect of pH on activity of purified α–amylase.

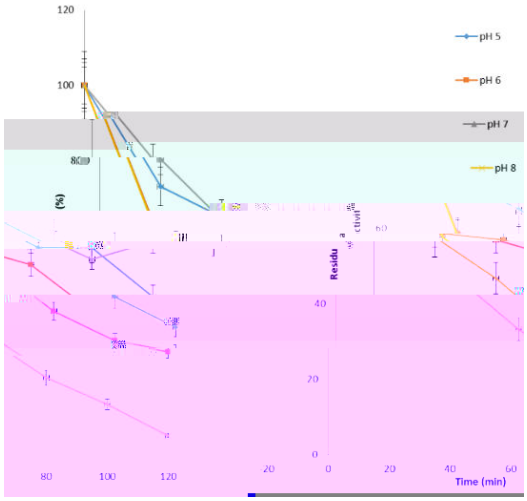

Figure 5: Effect of pH on the stability of purified α–amylase for pool A.

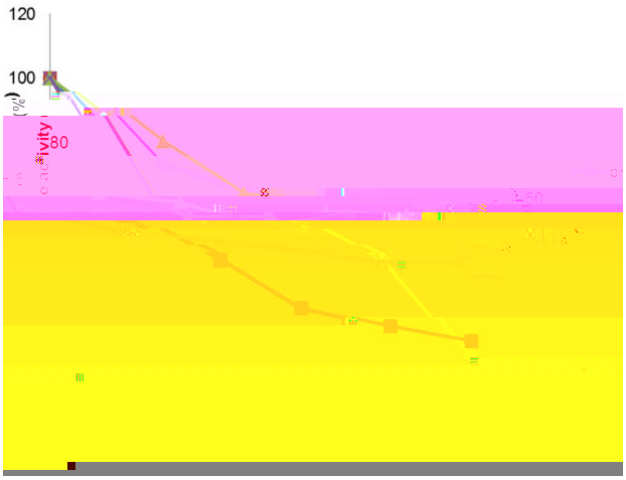

Figure 6: Effect of pH on the stability of purified α–amylase for pool B.

Effect of temperature on α–amylase activity and stability: Figure 7 shows the effect of temperature on purified α–amylase activity while the temperature stability of the purified α–amylase from pool A and pool B are shown in Figures 8 and 9. The α–amylase activity increased with increase progressively with incubation temperature until an optimum percentage relative activity was reached at 40°C and 60°C for pool A and pool B respectively (Figure 7).

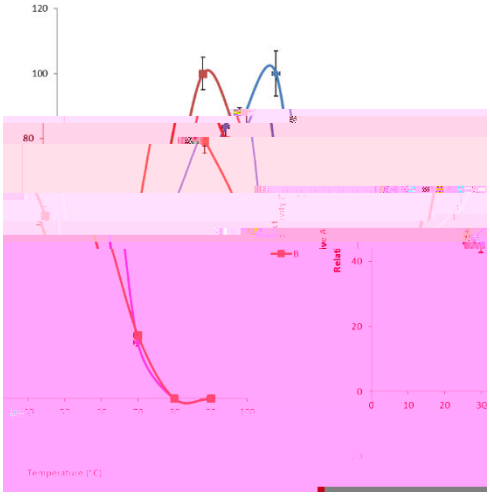

Figure 7: Effect of temperature on the activity of purified α–amylase.

Al^{3+}	37.61	63.54
Na^{+}	147.71	213.54
Ca^{2+}	0	0
K^{+}	21.10	97.92
Hg^{2+}	0	0
Zn^{2+}	156.88	277.08
Control	100	100

31. Ponnuswamy V, Prakash Vincent SG (2012) Purifcation and Characterization of Carboxymethyl Cellulase from *Bacillus* sp. Isolated from a Paddy Field. Polish Journal of Microbiology 61: 51-55.

32. Bukhari DA, Rehman A (2015) Purifcation and Characterization of α-Amylase from *Bacillus subtilis* Isolated from Local Environment. Pakistan