Purification and Some Properties of aThermostable Glucose-Producing α-Amylase from Bacillus sp. Cos.

Opurum C.C and Odibo F.J.C

***Abstract*— Thermophilic microorganisms are known for their high temperature requirement. One outstanding attribute of these organisms is the production of thermostable enzymes, and because of this feature, enzymes produced by thermophiles have found a number of commercial and research applications. Thus there is a continuous search for thermophilic micro-organisms capable of producing thermostable enzymes with novel characteristics. In the present study, a thermostable α-amylase-producing bacterial strain was isolated from soil. The isolate was phenotypically identified as Bacillus sp. Cos by adopting standard methods and was used in the production of amylase in sub-merged shake flask culture. The enzyme was purified 8-fold by cold acetone (-10OC) precipitation, ion-exchange chromatography on DEAE -Sephadex (A-50) and on Bio-gel P-4 gel filtration column. The homogeneity and molecular weight was estimated to be 38.02KDa by SDS-PAGE. The enzyme was optimally active at 70OC and pH7.0. It was not activated by any of the tested metal ions but was strongly inhibited by Hg2+. The enzyme hydrolyzed some tested polysaccharides producing only glucose. The Km values (mg/ml) for glycogen, starch, amylopectin, pullulan and amylose were 0.71, 0.83, 0.83, 1.67 and 10.0, respectively. The production of only glucose from various polysaccharides by Bacillus sp. Cos thermostable α-amylase, its optimum pH and temperature of activity at 7.0 and 70OC, coupled with its independence on metal ions for activation and stabilization suggests its applicability in starch saccharification for industrial production of glucose and fructose syrups.**

***Index Terms*— α-Amylase, glucose-producing, thermostable.**

I. INTRODUCTION

Micro-organisms are increasingly playing significant roles as the most important sources of enzymes used predominantly in the industrial sector, diagnostic and scientific researches [1], [2]. Enzymes produced by micro-organisms generally have gained recognition because of their relative ease of large scale production and purification, low-cost of production in a short time [3] and stability at various extreme conditions [4], [5].

Microbial enzymes that are secreted extracellularly are highly reliable for industrial processes and applications. More so, the production and expression of recombinant enzymes are much easier with micro-organisms as the host cell [4]. These underscore the import of screening and selection of the right micro-organism for high yield of the desired enzymes. For the production of enzymes of industrial importance, isolation and characterization of new and promising strains using cheap carbon and nitrogen sources is a continuous process [6].

The awareness of the potentials of using micro-organisms as biotechnological sources of enzymes of industrial relevance has stimulated interest in the exploration of extracellular enzymatic activity in a myriad of microorganisms [7]. They have opened new frontiers of many biotechnological processes and commercial applications including renewable energy, pharmaceuticals, in detergent industries, warp sizing of textiles, fibres, paper industries [8], baking, clarification of haze formed in beer or fruit juice and for pretreatment of animal feed to improve digestibility [9]. Conversion of starch into sugar, syrups and dextrins also form the major part of starch processing industry [10].

Amylases are starch degrading enzymes and are extensively distributed in micro-organisms, plants and animals [11]. These enzymes degrade starch and related polymers to yield products characteristics of individual amylolytic enzymes [12]. They can be categorized into two groups. 1) Endo-acting or endo-hydrolases, e.g. α-amylases (EC3.2.1.1; 1, 4- α-glucan-glucanohydrolases) are extracellular enzymes which hydrolyze α-1, 4-glycosidic bonds. These enzymes hydrolyze starch by randomly cleaving the interiors (internal α-1, 4-glucosidic linkages) of the molecule producing reducing sugars and malto-oligosaccharides. 2) Exo-amylase or exo-hydrolases e.g., β- amylase, glucoamylase and α-glucosidase. Glucoamylases (α-1, 4-glucan-glucohydrolases) act on starch by splitting glucose units from the non- reducing end. β-Amylase is usually of plant origin but has been produced by some micro-organisms [12],[13]. It produces predominantly maltose and β-limit dextrins from starch. However, enzymatic hydrolysis of starch typically requires the coordinated action of a cocktail of amylolytic enzymes, preferably thermostable ones.

Thermostable enzymes have been reported in several microbial genera including the archaea [14]- [21], though a lot of studies have been carried out on members of the Bacillaceae [22] - [25]. Majority of the characterized α- amylases hydrolyze starch to a mixture of glucose, maltose, malto-oligosaccharides and α- limit dextrins thereby necessitating the use of a glucoamylase in achieving complete saccharification. In this paper we report the characterization of a thermostable, liquefying and saccharifying glucose-producing α-amylase from a *Bacillus sp*. isolated from soil inundated with cassava starch processing wastewater.

II. MATERIALS AND METHODS.

Isolation of Thermophilic Amylolytic *Bacillus* species.

Cassava wastewater-impacted soil sample was collected from a cassava processing plant at a depth of 5cm in sterile sampling container. Ten (10g) grams of the soil sample were

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suspended in 90ml of sterile saline (0.85% NaCl). Ten-fold serial dilution was prepared to dilutions 10^{-4}, and 0.1ml of 10^{-3} and 10^{-4} dilutions were pour-plated in duplicates on sterile starch agar medium of the following composition (%): peptone, 1; soluble potato starch, 1.5; NaCl, 0.1; KH_2PO_4, 0.3; $MgSO_4.7H_2O$, 0.3; agar 2.0. The inoculated plates were incubated at 55^OC in a humidified incubator for 72 h. Colonies that developed at the end of incubated were purified by repeated sub-culturing on starch agar medium and pure isolates were stored in nutrient agar slant at 4^OC. **Screening of Isolates for Amylase Production and Choice of Working Strain.**

Preliminary amylase production by the isolates was carried out in six (6) 100ml Erlenmeyer flasks containing starch medium of composition (%): peptone, 1; soluble potato starch, 1.5; NaCl, 0.1; KH_2PO_4, 0.3; $MgSO_4.7H_2O$, 0.3. The sterilized medium was each inoculated with 24 h culture of the isolates and incubated at 55^OC for 72 h in a rotary shaker at 160 rpm.

The crude enzyme preparation (cell-free supernatant) was obtained after centrifugation (4000xg, 15min, 4^OC) of the broth using a refrigerated centrifuge. The crude enzyme from each of the isolates was assayed for amylolytic activity by the DNS method [26]. The isolate with the highest amylase activity was chosen as the working strain for further studies.

Characterization and Identification of the Working Strain.

The characterization and subsequent identification of the working strain were based on cultural, morphological and biochemical characteristics as described by Bergey's Manual of Determinative Bacteriology [27].

Production of Amylase from the Isolate.

The amylase was produced in submerged fermentation in three (3) 250ml Erlenmeyer flasks containing starch medium of composition earlier stated. The sterile medium was inoculated with three (3) loopfuls of 24 h vegetative cells of the isolate. The inoculated flasks were incubated for 72 h in a rotary shaker at 160 rpm. The 72 h brew was centrifuged at 4000 rpm for 15min at 4^OC using MSE 25 high-speed refrigerated centrifuge and the supernatant served as the crude enzyme.

Amylase Assay.

Amylase activity was estimated by the 3', 5'- dinitrosalicylic acid method of Bernfeld [28]. The reaction mixture comprised 0.5ml of 1% (w/v) gelatinized soluble potato starch in 0.2 M phosphate buffer (pH 7.0) and 0.5ml enzyme solution. The reaction was stopped with 1ml DNS after 10 min of incubation at 55^OC, then heated for 10 min in boiling water and cooled in running tap water. Five (5ml) milliliter of distilled water was added to the reaction mixture and subsequently, the optical density (OD) was measured with a spectrophotometer at 540 nm. The OD reading was converted to milligram glucose with a standard glucose curve (0-1mg/ml). A unit of amylase activity is defined as the amount of enzyme that produced one microgram of glucose per min under the assay condition [29].

Estimation of protein.

Protein was estimated by the method of Bradford [30] as described by Ali *et al.* [31]. To 0.1ml of the enzyme, 5ml of Bradford reagent was added and mixed by vortexing. The absorbance (OD) was measured with a spectrophotometer at 280nm. Bovine albumin was the standard protein. The concentration of the Amylase enzyme was calculated from a standard curve (bovine albumin concentrations versus absorbance).

Amylase Purification.

The crude enzyme preparation was precipitated from the broth with two (2) volumes of cold acetone (-10^OC). The resultant precipitate was separated by centrifugation (3000 x g, 20 min.) and re-suspended in 18ml of 0.2 M phosphate buffer (pH 7.0). The buffered suspension was applied onto a DEAE -Sephadex (A-50) column (2 x 18 cm) previously equilibrated with 0.2 M phosphate buffer (pH7.0) and eluted with the same buffer under linear NaCl gradient (0.8M) at a flow rate of 1.5ml/min. Fractions (10ml) were collected and assayed for amylase activity using 3', 5'- dinitrosalicyclic acid (DNS) reagent, and protein absorbance at 280 nm. Fractions showing amylase activity were pooled and concentrated by dialysis against 4.0 M sucrose solution.

The concentrated enzyme was further purified by gel filtration chromatography in a column of Bio-gel P-4 (1.2 x54 cm) which was equilibrated with 0.2 M phosphate buffer (pH 7.0). Elution was with the same buffer (300ml) at a flow rate of 1ml/min.

Fractions with high amylase activity were detected by the DNS method and active fractions were pooled and concentrated in dialysis bag using 4.0M sucrose. The concentrated enzyme was re-suspend in 0.2M phosphate buffer (pH 7.0), and this served as the purified enzyme.

Enzyme Characterization.

Homogeneity and Molecular Weight of Enzyme.

The homogeneity of the amylase was determined by SDS -polyacrylamide gel electrophoresis (SDS-PAGE) following the method of Laemmli, [32] as described by Tole *et al*. [8]. The electrophoresis was run with 10% polyacrylamide gel in Tris-HCl buffer (pH 8.3) containing 0.25mM Tris-, 0.192M glycine and 0.1% SDS. The prepared sample of the purified enzyme (60 µl) was applied in the well and electrophoresed at a constant current of 80mA for 12 h. The standard proteins, Bovine albumin, α- lactoalbumin and pepsin with the molecular weight 66000, 14000 and 34000, respectively were applied in the wells on both sides of the sample. The gel was fixed for 60 min in a *fixing solution* consisting of glacial acetic acid: methanol: H_2O (10:20:70 v/v/v), and thereafter rinsed with distilled water. The protein was stained overnight with staining solution containing 0.25% Coomassie brilliant blue R- 250, 50% methanol and 7.5% acetic acid. It was de-stained for 12 h in a de-staining solution containing 20% methanol and 7.5% acetic acid. The molecular weight of the enzyme was estimated by plotting the log molecular weight of the standard proteins against relative mobility.

Effect of Temperature on Enzyme Activity and Stability.

The effect of temperature on the enzyme activity was determined by incubating the reaction mixture (0.5ml enzyme and 0.5ml of 1.0% w/v gelatinized soluble potato starch in phosphate buffer (pH 7.0) at a temperature ranging from $40\text{-}100^OC$ for 10 min. The DNS method earlier described was used to measure the residual enzyme activity.

The temperature stability profile was evaluated with a 0.5ml portion of the enzyme contained in a thin-walled Pyrex glass tube. The tubes were held at various temperatures ($40\text{-}100^OC$) for 30 min. and the residual amylase activity was determined as earlier described.

Effect of pH on Enzyme Activity and Stability.

The pH activity profile was determined by separately incubating 0.5ml of the enzyme with 0.5ml of 1.0 %(w/v) gelatinized soluble potato starch in the following buffers of varying pH values: 0.2 M phosphate buffer, pH 3.0-8.0 and 0.2 M carbonate-bicarbonate buffer, pH 9 -10.0. After 10 min of incubation at 55^{O}C, the amount of reducing equivalent released was estimated by the DNS method.

Effect of Metal Ions on Enzyme Activity.

The effects of Cu^{2+}, Mn^{2+}, Ca^{2+}, Hg^{2+}, Fe^{2+}, Pb^{2+}, Co^{2+}, $Ba^{2+}$and Sr^{2+} on the activity of the enzyme were evaluated. The reaction mixture contained 0.5ml enzyme, 0.5ml of 1.0% (w/v) gelatinized potato starch in 0.2M phosphate buffer (pH) and 0.5ml of 2mM solution of the metal salts. The enzyme activity was assayed after incubation of the mixture at 55^{O}C for 30min and the reducing equivalent released was estimated by the DNS method as earlier described. The activity of the enzyme assayed without any metal ion was used as a control and the activity was taken as 100 % [33].

Relative Rate of Hydrolysis of Various Polysaccharides by the Enzyme.

The relative rate of hydrolysis of various substrates by the enzyme was studied. The reaction mixture comprises 0.5ml of the amylase and 0.5ml of each of the following substrates (1% w/v): starch, glycogen, amylose, amylopectin and pullulan in 0.2 M phosphate buffer (pH 6.0). After incubation at 70^{O}C for 30min, the reducing equivalent was determined.

Effect of Substrate Concentration on Amylase Activity.

The effect of various concentrations (0.2 - 1.0 mg/ml) of amylose, glycogen, pullulan, amylopectin and starch on amylase activity was investigated. The reaction mixture contained 0.5ml enzyme and 0.5ml of each of the concentrations of the substrates in 0.2M phosphate buffer (pH 7.0). The reaction mixture was incubated at 70^{O}C for 30 min and the reducing equivalent estimated by the DNS method. The *Km* values of the enzyme for the substrates were determined by the Lineweaver-Burk linear transformation of the Michaelis-Menten equation.

Determination of the Hydrolytic Products of the Amylase.

The purified enzyme solution (0.5ml) was incubated separately with equal volume of 1% (w/v) amylose, pullulan, amylopectin, glycogen and soluble potato starch in 0.2M phosphate buffer (pH 7) for 18 h at 70^{O}C. Hydrolysis was stopped by chilling the reaction mixture in ice and 0.02ml of the digest was spotted alongside the sugar standards: glucose, maltose, maltotriose and maltotetraose on Whatman No.1 chromatography paper.

The chromatogram was run for a total of three (3) ascents with a solvent system comprising pyridine-butanol-water (4:6:3 v/v) and then allowed to air-dry. Sugars were detected by spraying with aniline reagent (aniline-phthalic acid-butanol-water). The chromatogram was allowed to air-dry and subsequently heated in an oven at 105^{O}C for 5min.

Determination of the Type of Amylase.

The type of amylase produced by the candidate isolate was confirmed by Phadebas Amylase test. The enzyme (0.2ml) was mixed with 4ml of 20 mM phosphate buffer (pH 7). One tablet of Phadebas reagent was added to the mixture and vigorously agitated for 10 sec. This was incubated at 55^{O}C for 30 min and the reaction was stopped by adding 1ml of 0.5M NaOH. The reaction tube was shaken and allowed to stand for 30min. Retention of deep blue coloration was positive for α-amylase.

III. RESULTS.

Choice of Working Strain.

A total of six thermophilic amylase-producing bacteria were isolated from cassava wastewater- impacted soil by spread plate technique on starch agar medium. The isolates were screened for amylase activity by DNS method. The isolate exhibiting the highest activity and chosen for further studies was identified using standard cultural, morphological and biochemical characterization methods and was identified as *Bacillus* sp. Cos (Table 1). The isolate was a Gram-positive, rod-shaped, spore former (sub-terminally arranged) and starch hydrolyzer. It grew in 10% glucose and NaCl at 28 and 37^{O}C.

Amylase Purification and Molecular Mass

The thermostable α-amylase was purified by cold acetone (-10^{O}C) precipitation, ion-exchange (on DEAE- Sephadex A-50), and gel filtration chromatography. The elution profile of the enzyme on Bio-gel P-4 gel filtration chromatography (Fig.1) showed one major peak for α-amylase activity. The purification results summarized in Table 2 indicated that the enzyme retained 2.47% of its original activity after gel filtration chromatography. The enzyme was purified 8-fold with a specific activity of 181.4 U/mg protein. The enzyme purity and homogeneity was confirmed by the observed single band on SDS-PAGE (Picture not shown). The molecular weight was estimated to be 38 KDa.

Effect of Temperature and pH on Enzyme Activity and Stability.

The effect of temperature on amylase activity and stability profile was investigated at varying temperatures ranging from 40-100^{O}C at pH 7.0. The results showed an increase in the enzyme activity with increasing temperature. The highest activity was recorded at 70^{O}C beyond which the activity started to decrease with an increase in temperature to 58% at 100^{O}C. Under the assay condition, the temperature stability profile showed a decrease in enzyme activity with an increase in temperature. The enzyme retained 40% of its activity at 100^{O}C (Fig.2).

The result of the effect of pH on the enzyme activity and stability is presented in Fig.3. In like manner, the enzyme activity profile increased with increase in pH and maintained 100% activity between pH 6.0-7.0. Above pH 7.0, the activity decreased, retaining 98% and 70% activity at pH8.0 and 10, respectively. The peak of stability profile was observed at pH 7.0; the enzyme retained 70% activity at pH10.0 for 30min of reaction time.

Effect of metal ions on Enzyme Activity.

The results of the effect of metal ions on the enzyme activity are shown in Fig.4. None of the tested metal ions enhanced the enzyme activity. Instead, Hg^{2+} was highly inhibitory to the enzyme activity.

Relative Rate of Hydrolysis of Various Polysaccharides by the enzyme.

The relative rate of hydrolysis of a number of polysaccharides was studied and the result is shown in Figure 5. Glycogen was the best hydrolyzed followed by starch, amylopectin, pullulan with amylose being the least hydrolyzed.

Paper Chromatography of the Hydrolysis Products of the Amylase.

Presented in Fig.6 is the chromatogram of the hydrolysis products of several polysaccharides: pullulan, amylose, soluble starch, glycogen and amylopectin by the α-amylase of

Bacillus sp. Cos. G_1, G_2, G_3 and G_4 are sugar standards (Std) and represent glucose, maltose, maltotriose and maltotetraose, respectively. The result indicates that glucose was the hydrolysate produced by the enzyme.

Effect of Substrate Concentration on Amylase Activity.

The enzyme activity was influenced by the concentration of the substrates evaluated. The velocity of the enzyme reaction with the tested substrates was linear up to 0.2 mg/ml. A reciprocal plot of the enzyme velocity against substrate showed that the enzyme had *Km* values of 0.71 mg/ml, 0.83 mg/ml, 0.83 mg/ml, 1.67 mg/ml and 10.0 mg/ml for glycogen, soluble starch, amylopectin, pullulan and amylose, respectively.

Determination of the Type of Amylase.

The enzyme was confirmed to be α-amylase based on the positive result of Phadebas test, as shown by the retention of deep blue coloration.

IV. Discussion.

Microorganisms have proved to be the major source for the production of enzymes of industrial and biotechnological importance. Thus, the selection of the right organism is very vital for a high yield of desirable enzymes. For the production of enzymes for industrial use, isolation and characterization of new promising strains with unique characteristics is a continuous process [34]. In the present study, the bacterial species isolated at a temperature of 55^{O}C was identified as *Bacillus sp. Cos* following standard methods. It was observed to be Gram-positive, rod-shaped, spore former (sub-terminally arranged) and starch hydrolyzer. The isolate showed poor growth in nutrient broth with 10% NaCl, an indication that the organism is not halophilic.

The thermostable α-amylase produced by *Bacillus* sp. Cos was purified 8- fold from the culture supernatant of by cold acetone precipitation and chromatographic techniques. The specific activity of the enzyme was 181.40U/mg and the molecular weight determined by SDS-PAGE was 38 KDa. A major peak for α-amylase activity revealed by the elution profile of the enzyme on DEAE- Sephadex A-50 ion-exchange column chromatography and Bio-gel P-4 gel filtration chromatography was indicative of the level of the enzyme purity.

There are a number of similar reports on the production and purification of thermostable amylases from Bacillus species isolated from the soil and other environmental samples [33], 35]-[37]. Abdel-Fattah *et al.* [38] purified and characterized a thermostable α-amylase from *Bacillus licheniformis* A120. The α- amylase was purified through various chromatographic techniques, and the molecular mass was estimated to be 55kDa. The enzyme had optimal temperature and pH of 60-80 ^{O}C and 6-7.5, respectively. The Vmax and the Km values of the purified amylase were 454 mU/mg and 0.709 mg/ml, with greater stability against different solvents. Tole *et al*, [8] similarly reported the isolation of an amylase producing bacteria from the soil around Tulja-Bhavani Temple of Tuljapur. The enzyme purified from this organism had a lower molecular weight (1.5 KDa) compared to the amylase reported in this study (38 KDa).

A thermostable raw starch digesting amylase from a *Streptomyces sp.* isolated in a milling factory was purified and characterized by chromatographic procedures. The specific activity of the enzyme was determined to be 11.7 U/mg with a molecular mass of 47 kDa. The optimum pH 6.0 and optimum temperature were found to be 6.0 and 50 to 60 ^{O}C, respectively. The enzyme showed sufficient activity even at 70^{O}C [39].

Bacillus sp. Cos α- amylase was optimally active at 70^{O}C and retained 40% of its activity at 100^{O}C for 30min. The enzyme also was most stable at pH 7-8 but retained 68% activity at pH4.0. This observation is similar to the report of Kiran *et al.*[33] (2018) on the amylase of *Bacillus subtilis* RK6 with optimum pH of 8.0, and was thermo-active, retaining its activity up to 90°C and stability at 60-90°C after preheating for 30 min.

Contrary to several reports [9], [11] on the activation and stabilization of α- amylase by Ca^{2+} and other divalent cations, the α- amylase from *Bacillus* sp. Cos was not activated by any of the tested cations. It could probably be that the cations do not contribute to the stability of the native three-dimensional configuration of the enzyme and enhancement of the enzyme active site in recognition of its substrates. The enzyme was, however, strongly inhibited by Hg^{2+}, retaining only 16% activity. A similar observation was made by Sandip *et al.*[40] in which the thermostable amylase produced by phosphate solubilizing *Bacillus thuringiensis* was inhibited in presence of Hg^{2+} and Cu^{2+} ions, but was enhanced by 1mM Ca^{2+}, Mg^{2+} and Zn^{2+} ions.

The stability and relatively high activity of the α- amylase from *Bacillus sp. Cos* in the absence of metal ions could be viewed as an advantage over other amylases because in the industrial starch saccharification, the requirement for metal ion, especially Ca^{2+}, contributes to the overhead cost of the process, making it capital intensive.

The enzyme was observed to hydrolyze different polysaccharides at different rates. Glycogen was the best hydrolyzed by the enzyme followed by starch, amylopectin, pullulan and amylose being the least.

A Lineweaver-Burk linear transformation of Michaelis-Menten equation indicates that the enzyme has *Km* values of 0.71mg/ml, 0.83mg/ml, 0.83mg/ml, 1.67mg/ml and 10.0mg/ml for glycogen, soluble starch, amylopectin, pullulan and amylose, respectively. Amylase activity was observed to increase with an increase in substrate concentration up to 0.2% and then gradually decreased. *Km* and *Vmax* are important coefficients that guide scientific research and engineering design. Besides, *Km* is independent of enzyme concentration and is a true characteristic of the enzyme under defined conditions of temperature and pH. The lower the *Km* value of an enzyme, the more firmly it binds to its substrate [33]. The smaller *Km* values recorded in the present study indicate the binding capacity and high affinity of the enzyme to the substrates with the exception of amylose. The result is similar to the findings of Sandip *et al.*, [40]. Barley amylase isozymes AMY 1 and AMY 2 were, however, found to have higher activity to DP 4900-amylose than reduced DP 18-maltodextrin and maltoheptaose of shorter chain length [41]. Thus, the longer the substrate the higher the activity. Our amylase, on the contrary, seems to prefer branched-chain substrates (glycogen, soluble starch, amylopectin and pullulan) to straight chain substrates, though only amylose was tested in this study.

Paper chromatography of the hydrolytic products of various polysaccharides by the enzyme revealed that glucose was the only product. Reports are very rare on α-amylase from

microbes producing only glucose on hydrolysis of polysaccharides. The production of only glucose suggests that the α-amylase produced by *Bacillus* sp. *Cos* is of the saccharifying type. Alpha-amylase is known to hydrolyze polysaccharides, producing glucose and other hydrolysates. With the industrial application of α-amylase produced by *Bacillus sp. Cos*, product recovery will be easier and inexpensive since only one product is produced. The hydrolytic pattern of the enzyme is similar to that of glucoamylase in the production of only glucose which is an indication of an exo-hydrolytic mechanism [42].

V. Conclusion.

It is possible that the α-amylase from *Bacillus* sp. Cos is a novel enzyme, though it compared
favourably in most aspects with a number of the characterized α-amylases of microbial origin.
The production of only glucose from various polysaccharides by *Bacillus* sp. Cos α-amylase, its optimum pH and temperature of activity at 7.0 and 70^{O}C, coupled with its independence on metal ions for activation and stabilization suggests its applicability in starch saccharification for industrial production of glucose and fructose syrups.

Table 1. Morphological and Biochemical Characteristics of the Isolate

Experiments	Growth at 55^{O}C and pH 7.0
Starch agar medium	+
Peptone broth with 1% starch	+
Nutrient broth with 10% NaCl	+
Nutrient broth with 10% NaCl at 28 and 37OC	+
Nutrient broth with 10% glucose	+
Gram stain	+
Shape	Rod
Motility	Motile
Spores	Sub-terminal
Catalase test	+
Methyl red	+
Voges-Proskauer	+
Indole test	-
Gelatin liquefaction	+
Starch hydrolysis	+
Citrate utilization	+
Nitrate utilization	+
Mannitol fermentation	Acid
Arabinose ;;	-
Sucrose ;;	Acid
Maltose ;;	Acid
D-saccharose	Acid
D-glucose	Acid/gas
Lactose	-

Table 2. Summary of Purification of α-Amylase from Bacillus sp. Cos.

Purification Step	Volume (ml)	Total Activity (U/ml)	Total Protein (mg)	Specific Activity (U/mg) protein	Yield (%)	Purification
Crude Enzyme	250	9305	415	22.42	100	1
Acetone precipitation	18	680	8.84	76.96	7.3	3.43
Ion-exchange chromatography on DEAE Sephadex A-50	15	330.5	3.4	97.2	3.55	4.34
Gel filtration on Bio-gel P-4 Galactose	13	230.4	1.27	181.4	2.47	8.1

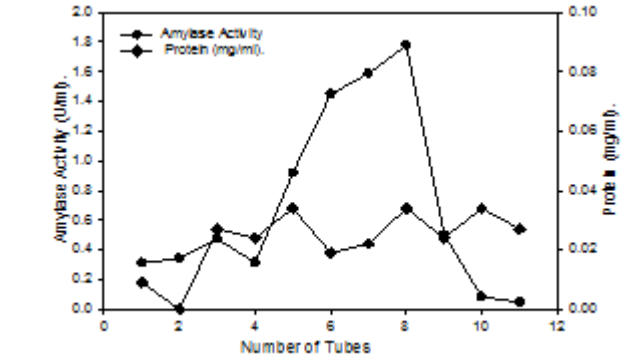

Fig.1. Elution Pattern of α-Amylase of *Bacillus sp.Cos* on Bio- Gel Filtration Chromatography.

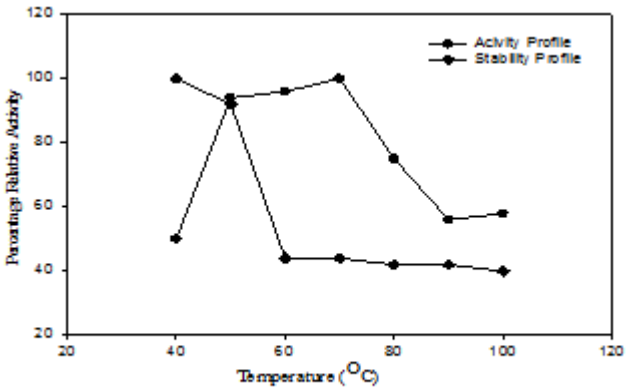

Fig.2. Effect of Temperature on the Activity and Stability of the Amylase.

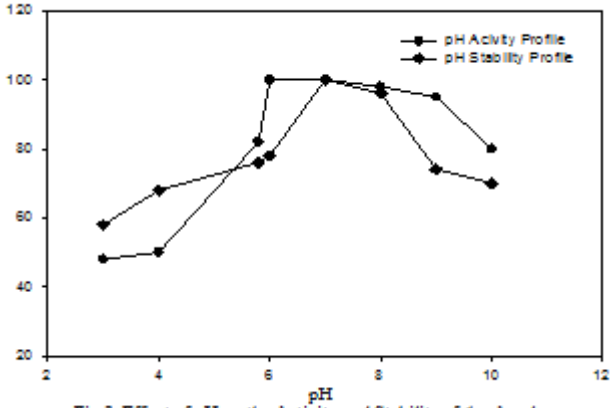

Fig.3. Effect of pH on the Activity and Stability of the Amylase.

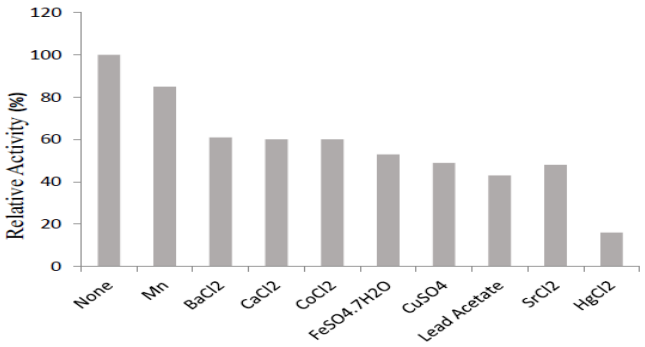

Fig.4.Effect of Metal Ions on the Enzyme Activity.

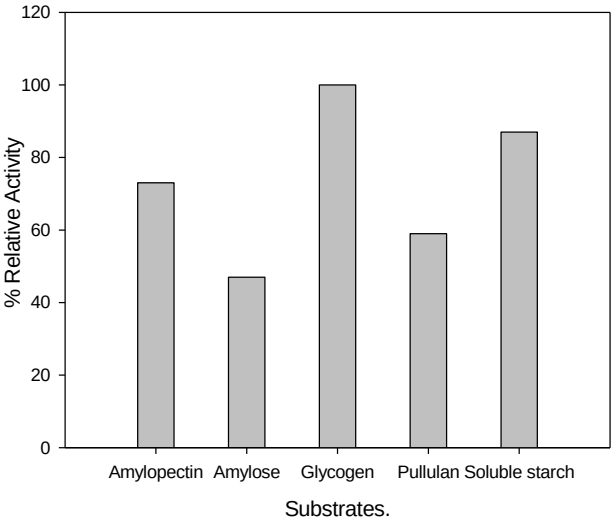

Fig.5. Relative Rates of Hydrolysis of Various Polysaccarides

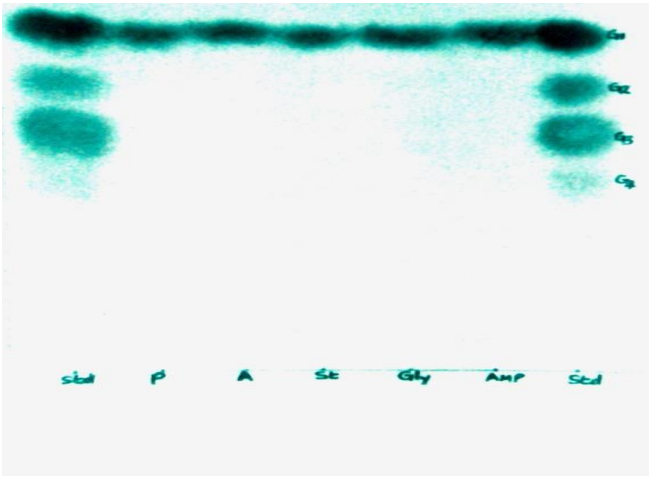

Fig.6. Paper Chromatograph of Hydrolysis Products of Various Amylaceous Polysaccharides: P (Pullulan), A(Amylose), St (Soluble Starch), Gly (Glycogen) and Amp (Amylopectin) by the α-Amylase of Bacillus sp Cos. G_1, G_2, G_3 and G_4 are sugar standards (Std) and represent glucose, maltose, maltotriose and maltotetriose, respectively.

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