Journal of Bacteriology Research Vol. 3(8), pp.129-137, 25 October, 2011
Available online at http://www.academicjournals.org/JBR
ISSN 2006- 9871 ©2011 Academic Journals

Full Length Research Paper

A potential new isolate for the production of a thermostable extracellular α- amylase

Ahmed, A. Alkando, Hanan Moawia Ibrahim*

Central Laboratory, Ministry of Science and Technology, Sudan.

Accepted 15 July, 2011

Two hundred and seventy *Bacillus* species were isolated from soil samplse in Khartoum State and tested for α- amylase production. 20 potential isolates of α- amylase producer were obtained during primary screening. Secondary screening of these isolates yielded a high thermostable alkaline α-amylase producing isolate. Of all the species tested, *Bacillus licheniformis* gave maximum α-amylase activity of 0.7947 U/mg/ml at pH of 8 in iodine method, and (0.024 U/mg/ml) in 3.5.dinitrosalicylic acid (DNS) method. Characterization of the extra-cellular crude α- amylase was further evaluated for its biochemical properties as an enzyme for industrial use. The production of α- amylase following growth of the microorganism was found to be at optimum temperature and pH of 70°C and 9.0, respectively.

Key words: Thermostable α- amylase, alkaline α- amylase, *Bacillus licheniformis*.

INTRODUCTION

Amylases (1-4,α D-glucan glucano hydrolase EC 3.2.1.1) hydrolyses internal α–1-4- glycosidic linkage in starch and related substrate in an endo-fashion producing oligosaccharides, glucose and α–limit dextrin (Fogarty et al., 1999). α-amylases constitute a class of industrial enzyme having approximately 30% of the world enzyme production (Van der Maarel et al., 2002) and represent one of the three largest groups of industrial enzymes and account for approximately 60% of total enzymes sales in the world (Rao et al., 1998) and is an important enzyme, particularly in the process of starch hydrolysis. Though they originate from different sources (plants, animals and microorganism) and show varying pattern of action depending on the source and origin (Hagenimana et al., 1992), in industry, they are mainly produced from microbes. Enzymes from microbial sources generally meet industrial demands due to their higher yield and thermostability (Burhan et al., 2003). Microbial enzymes present a wide spectrum of characteristics that make them useful for specific application. Microbial α- amylase are among the most important hydrolytic enzymes and have been studied extensively. Each application of α-amylase from microbial source requires unique properties with respect to specificity, stability, temperature and pH dependence (McTigue et al., 1995). It can be produced in amounts meeting all the demands of the market because the diversity of enzymes available from microorganisms is very great. They find potential applications in industry such as food, fermentation, textile (in desizing fabric), paper and detergent (Pandy et al., 2000; Hag et al., 2003). The spectrum of amylase applications has expanded in several fields such as clinical, medicinal and analytical chemistry (Reddy et al., 2003). Despite the fact that many different α- amylases have been purified and charac-terized so far and some of them have been used in biotechnological and industrial applications, the presently known α- amylases are not sufficient to meet most industrial demands (Karbalaei-Heidari et al., 2007). High value of microbial extracellular enzyme is placed on extreme thermostability and thermostability of amylase for use in the bioprocess of starch (Adeyanju et al., 2007). Thermo stability is a feature of most of the enzymes sold for bulk industrial usage and thermophilic organisms are therefore of special interest as a source of novel thermo-stable enzymes. Recent research with thermostable α-amylase has concentrated on the enzymes of thermo-piles and extreme thermopiles (Sadhukhan et al., 1992; Arnesen et al., 1998; Lin et al., 1998), and little is known about the properties of the enzymes produced by these organisms (Eduardo et al., 2000). Screening of micro-organisms with higher α- amylase could therefore,

*Corresponding author. E-mail: hara111@yahoo.com. Tel: +249-9-11171941. Fax: + 249-155183855.

facilitate the discovery of novel α- amylase suitable for new industrial applications such as bread and baking industries (Gupta et al., 2003).

The objective of the present study is: to isolate and carry out a taxonomic characterization of a bacterial strain isolated from Sudanese soil which was found to be a potential source of thermostable α- amylase.

MATERIALS AND METHODS

All the chemicals used in this study are of analytical grade unless otherwise stated. This research was conducted between 2006 and 2009 at the Central Laboratory, Ministry of Science and Technology, Sudan.

$$\frac{\text{Average diameter index of microorganism - average diameter of colony}}{\text{Average diameter of colony}} \quad (1)$$

Isolation of organism

Soil samples were taken from 3 to 5 cm depth after removing 5 cm from the earth surface. These samples were collected into sterilized plastic bags and then transferred to labeled screw-caped bottles. Isolation of the microorganisms was performed by the soil dilution plate technique (Clark et al., 1958). The nutrient agar base containing soluble starch (1%) was used for bacteria isolation. The stock of the bacterial isolate was mixed with sterilized glycerol, and kept in 1 ml aliquots and preserved at -20°C for further use.

Screening and identification of bacteria strain

The isolated *Bacillus* strains were primarily screened for α- amylase synthesis after incubation at 60°C for 48 hr, the plates were flooded with a solution of 0.5% (w/v) I_2 and 5.0% (w/v) KI (Thippeswamy et al., 2006). Colonies exhibiting halo starch hydrolysis were picked up. The clear zone surrounding the colony was measured in (cm) from the edge of the colony to the limit of clearing and also the diameter of colony was recorded. The relative amylolytic activity is expressed as an index of activity calculated according to the follow equation: Identification of the selected *Bacillus* strain was identified on the basis of standard morphological and biochemical tests according to the method described in Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974). Subculture was prepared by a full loop of the suspension of vegetative cells streaked aseptically onto a nutrient agar (NA) several times until pure colonies were obtained. The pure isolates were maintained on NA slants and routinely sub-cultured every four weeks.

Inoculum preparation

50 ml of vegetative inoculum was transferred to 250 ml sterilized cotton-plugged Erlenmeyer flasks and then rotated at 200 rpm at 35°C in a rotary shaking incubator for 24 h.

Fermentation technique

α-amylase fermentation was carried out by submerge fermentation. 10% from the bacterial inoculums was inoculated to 500 ml shake-flasks containing 100 ml of a defined medium (Horikoshi medium II) (Horikoshi, 1999). Soluble starch 1%(w/v), peptone 0.5%(w/v), yeast extract 0.5%(w/v), $MgSO_4.7H_2O$ 0.02%(w/v), K_2HPO_4 0.1%(w/v), and $Na_2CO_3$1%. The flasks were incubated at 37°C in an orbital shaker at 150 rpm for 24 to 48 hr with an initial pH of 7.0. The culture broth was then centrifuged (Hettich Mikro 20) at 30 rpm for 30 min at 4°C, the free-cell supernatant was used as an extracellular crude enzyme (Hanan et al., 2004).

Determination of amylolytic potentially isolate activity (Iodine method)

α- amylase activity was determined by Iodine method to assay the amylolytic activity (Fuwa, 1954). One unit of the activity is defined as the amount of enzyme that could produce a change of 0.01 absorbency at 700 nm in the standard assay.

Saccharification activity: Dinitrosalsylic acid (DNS method)

The DNS method used involved estimating the amount of reducing sugar produced (DeMoraes et al., 1999), using 1% soluble starch as substrate. Glucose was used as standard. One unit of enzyme activity was defined as the amount of enzyme that formed 1 mg of reducing sugar in 1 min.

Optimisation of α-amylase activity assay conditions (DNS)

The influence of pH, time of incubation, temperature and soluble starch concentration were measured to optimize the enzyme assay conditions. Optimum pH was determined at different pH range (5 to 10). Reaction time was determined at different time intervals ranging from 5 to 60 min with 2% soluble starch in phosphate buffer at pH 6.0 as earlier obtained at 60°C. The optimum temperature was determined by incubation of 2% (w/v) soluble starch in phosphate buffer pH 6.0 with the crude enzyme at various temperatures ranging from 40 to 100°C for 10 min. Substrate concentration was determined at various ranges from 0.5 to 3% (w/v).

Biomass estimation

The pellets were washed with acetate buffer three times in order to remove the starchy material attached to the pellet. The suspension were then filtered using a pre-weighted filter paper and dried in an oven at 60°C for 1 to 2 days to a constant weight for the measurement of cell concentration (mg/ml) (Herbert, 1961).

High Performance Liquid Chromatography (HPLC)

For all analysis that involved HPLC, the conditions were set as follows: the column employed was of analytical column Supelcosyl LC-NH2 (5 µm) with length of 250 mm and 4.6 mm internal diameter. The mobile phase consisted of acetonitrite and water in ratio 75:25 (v/v). The flow rate was set at 1 ml/min. (Kouame et al., 2004). The internal column temperature was maintained at 30°C and the evaporation temperature was 60°C. The eluent from the column was monitored by refractive index detector, and the data werte recorded by the integrated computer system attached to the HPLC (Waters corp.).

Reaction conditions

Extracted crude α- amylase was incubated with 1% soluble starch, while glucose and maltose were incubated at 70°C without the addition of crude enzyme. Then 3.5 ml of 0.3 mM NaOH was added to stop the reaction. Samples were filtered through millipore filter

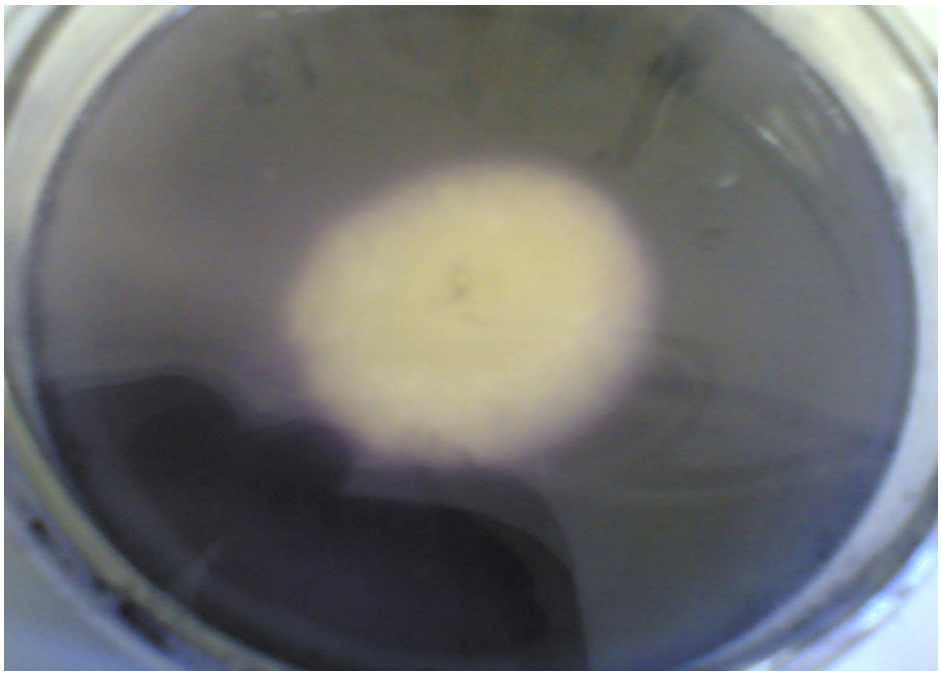

Figure 1. Zone of clearance of Strain (2) which further identified as *B. licheniformis.*

units of 0.2 μm pore size prior to injection to the HPLC.

RESULTS

The first step was to isolate the desired microorganism that produces α- amylase. Among the four selected *Bacillus* strains (1, 2, 3, and 4) that showed the highest amylase synthesis, Strain 2 was selected because it gave a larger diameter zone of clearance (6.5 cm) (Figure 1) and the highest relative amylolytic activity (calculated with Equation 1) of 1.5 compared to the other species. Therefore Strain 2 was chosen to fulfill the aim of this research which is amylase production. These culture were further screened for amylase production by submerge fermentation using the hydrolysis method.

Characterization and identification of the isolated amylolytic bacterial strain

The morphological and biochemical characteristics of the isolated amylase producing bacteria strain (2) are shown in Table 1. On the basis of standard method, the strain was assigned a code and identified as *Bacillus licheniformis* according to the method described in Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974). This strain (*B. licheniformis*) was selected for further studies (Figure 2).

Amylolytic activity (Iodine method)

Figure 3 shows the growth profile of *B. licheniformis* in Horikoshi medium II during the 120 h cultivation. Maximum growth of the bacterium was obtained within 24 to 84 h of cultivation based on biomass dry weight (0.13 to 0.148 mg/mL). The activity of the enzyme reached a maximum within 36 h after inoculation (0.6867 U/mg/ml) and plateau off for the next two days. Beyond 96 h of growth, no increase in enzyme activity was recorded. The two profiles were paralleled and showed that the fermentation kinetics of α- amylase production by *B. licheniformis* might be classified as the growth associated type (Figure 3). Enzyme production was found to be concomitant with growth.

The result of the crude enzyme of *B. licheniformis might* also contain a mixture of amylolytic enzymes as well asα-amylase as the iodine assay may not be specific for α- amylase activity. DNS method which determined the reducing sugar was used as a specific method. The results of enzyme activity using DNS method (Figure 4) showed thesame results as Iodine method indicating that the enzyme might be α- amylase.

Optimisation of DNS assay conditions

The influences of pH, time of incubation, temperature and substrate concentration were measured to optimize DNS assay conditions. The results showed a broad peak optimum pH with ranges between 6.0 and 9.0 and some activity was gained (0.01 U/mg/ml) at pH 10.0. The maximum *B. licheniformis* crude enzyme activity (0.0237 U/mg/ml) was initially observed between pH 6.0 and 9.0 (Figure 5).

Table 1 Morphological and biochemical characteristics of the isolated amylolytic bacterial strain.

Characterization tests	Bacterial reaction
Gram reaction	+
Cell shape	Rod
Motility test	+
Spore	+
Growth in air	+
Catalase	+
Oxidase	-
Starch hydrolysis	+
Nitrate reduction	+
VP	+
Citrate utilization	+
Urease	+
Oxidative- fermentation test	F
Casein hydrolysis	+
Indole production	-
Anaerobic growth	+
Acid from sugar fermentation	
Glucose	+
Sucrose	+
Melibiose	+
Salicin	+
Raffinose	-
Cellobiose	+
Galactose	+
Xylose	+
Mannose	+
Growth at 50 C	+
Growth at 10% NaCl	-
Probable identity	*Bacillus licenformis*

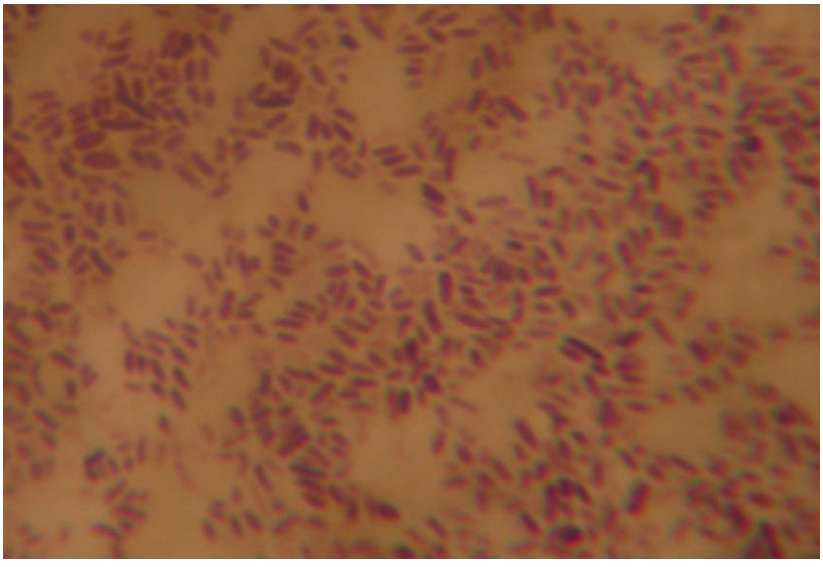

Figure 2. *B. licheniformis* (microscopic characteristics).

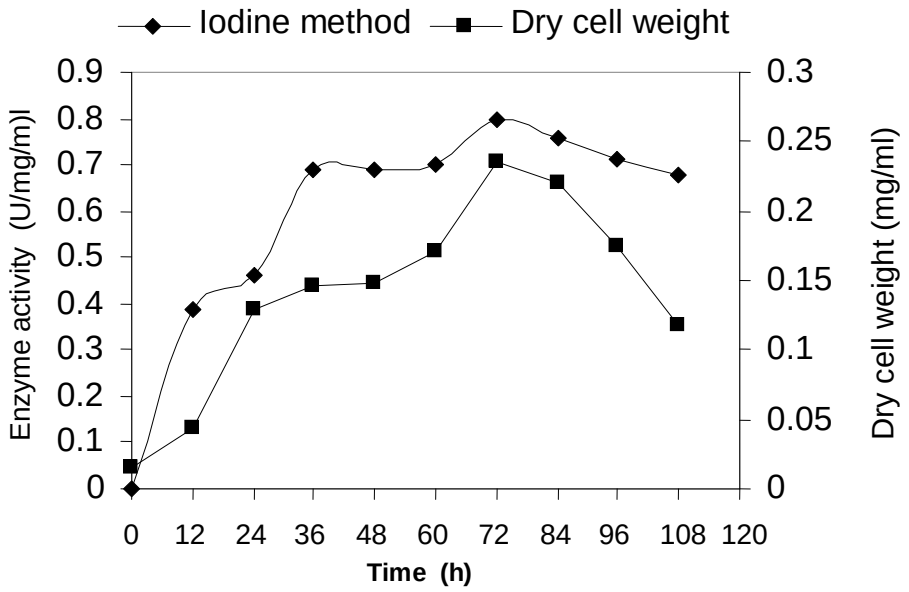

Figure 3. Biomass and crude enzyme activity profiles during the growth of *B. licheniformis* using hydrolysis method (Iodine method).

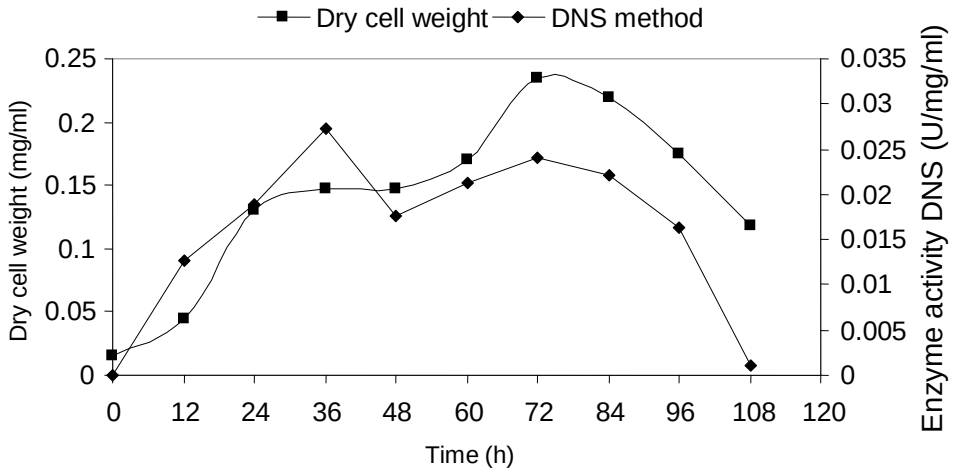

Figure 4. Time course of *B. licheniformis* crude enzyme activity and growth curve using saccharification method (DNS method).

Based on the substrate concentration, the extracted crude enzyme activity is shown to be linear between 0.5 to 1.0%. Hence, a practical assay substrate concentration is taken to be 1.0% of 0.7941 U/mg/ml of α- amylase activity (Figure 6).α- amylase from *B. licheniformis* was found to be active (0.018 to 0.017 U/mg/ml) in a broad temperature range of 50 to 90°C and showed a maximum activity (0.0252 U/mg/ml) at around 70°C (Figure 7). The enzyme is active even at high temperatures of up to 90°C.

In selected reaction conditions, namely at a temperature of 70°C, substrate concentration of 1% and pH at 9.0 were used as optimal conditions to assay for B. *licheniformis* α- amylase activity using DNS method.

HPLC analysis

The result of the reaction mixture of glucose, maltose and *B. licheniformis* crude enzyme with soluble starch revealed that the retention time of glucose (Figure 8) and maltose were 8.841 and 2.588 respectively, where the area were 36909 and 11.5902 respectively, (Figure 9). *B. licheniformis* crude enzyme resulted in 8.561 retention time and an area of 279965 (Figure 10) which were nearly similar to the reading for standard glucose and

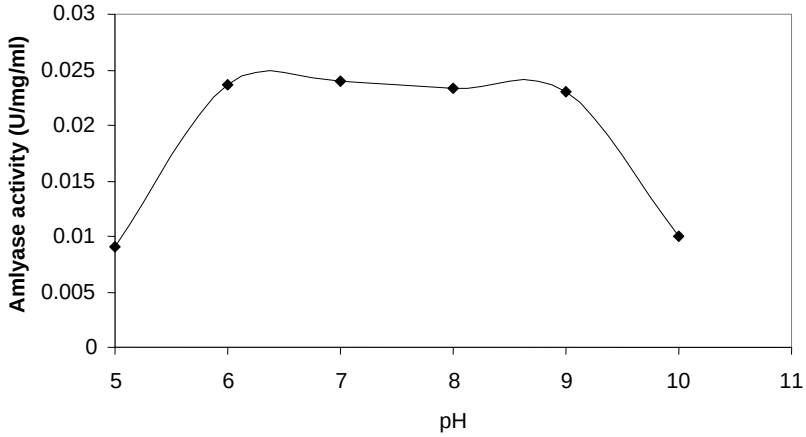

Figure 5. Kinetic evaluation of different pH on the production of α-amylase by *B. licheniformis*.

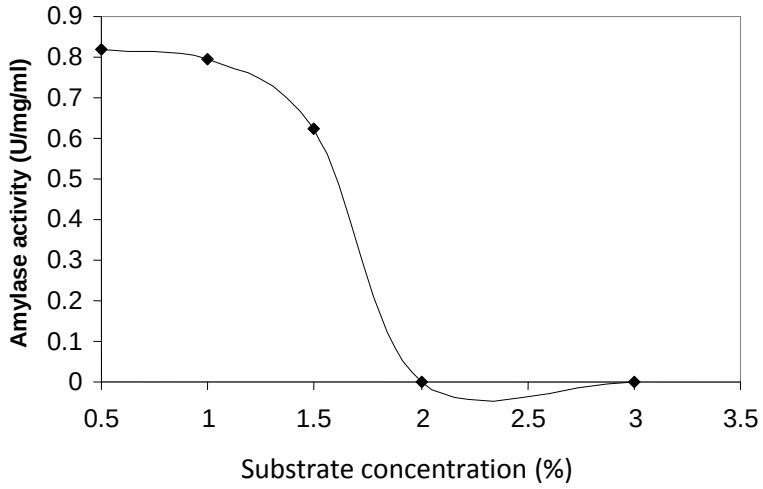

Figure 6. Kinetic evaluation of different substrate concentration on the production of α-amylase by *B. licheniformis*.

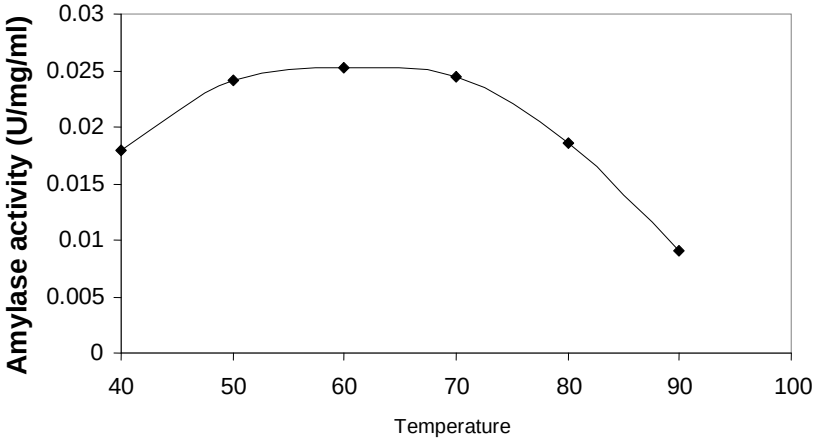

Figure 7. Kinetic evaluation of different temperature on the production of α-amylase by *B. licheniformis*.

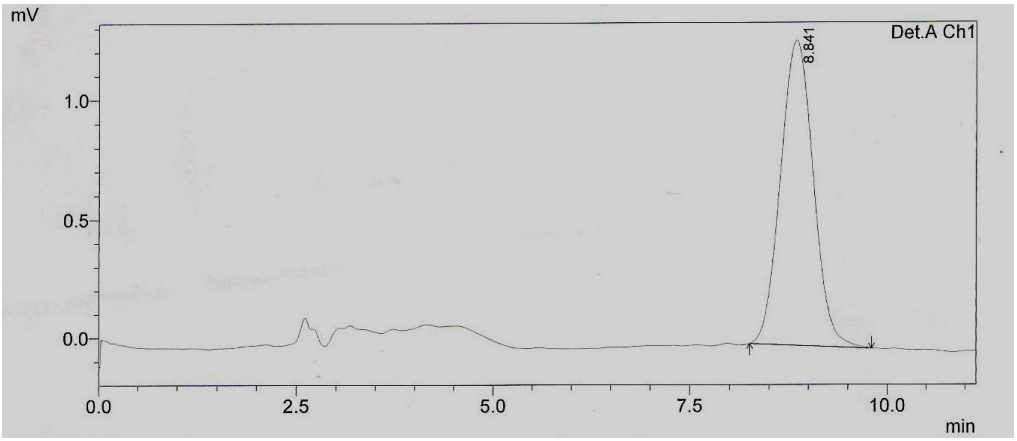

Figure 8. HPLC chromatogram of standard glucose.

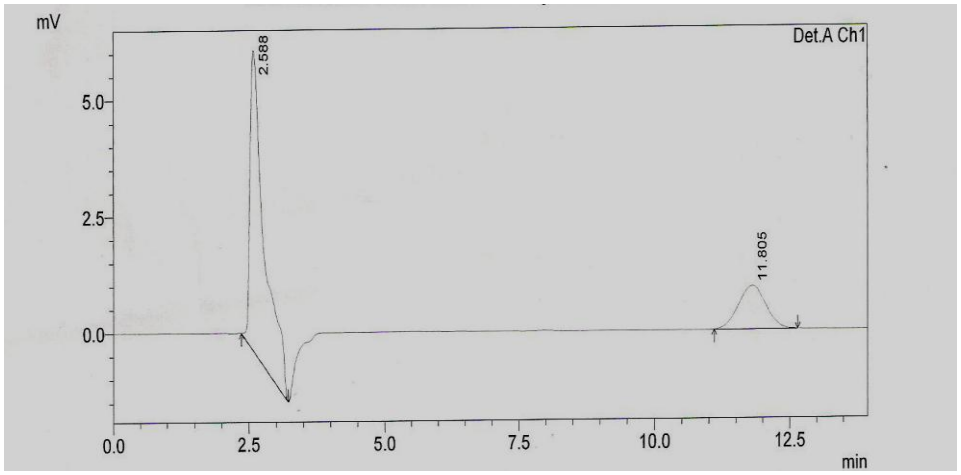

Figure 9. HPLC chromatogram of standard maltose.

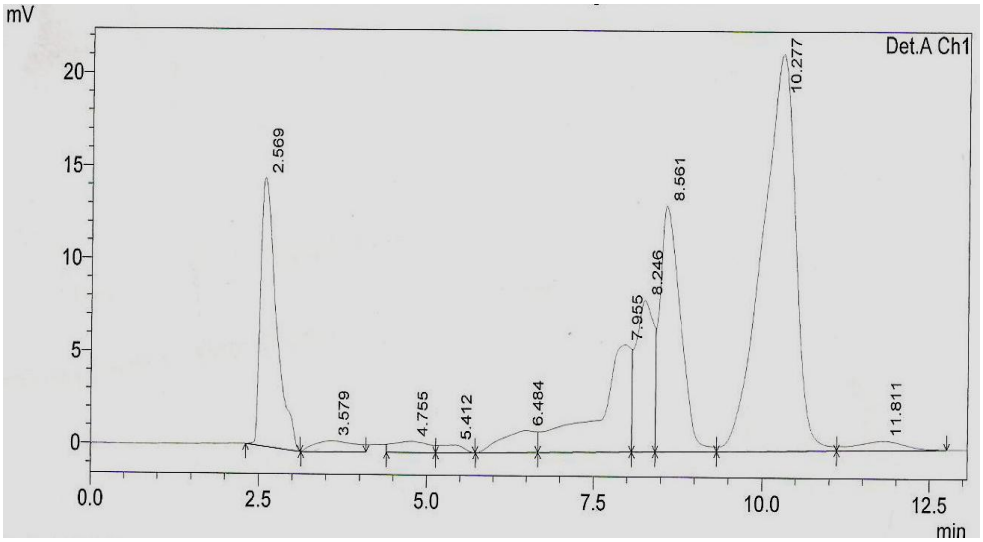

Figure 10. HPLC chromatogram of glucose produced from *B. licheniformis* crude α- amylase with soluble starch after 20 min incubation time at 70°C and pH 6.0.

maltose. It is therefore concluded that the end product of *B. licheniformis* crude enzyme with soluble starch could produce glucose and maltose as an end product. It can be clearly seen that *B. licheniformis* is mainly an α-amylase producer.

DISCUSSION

Many bacteria produce extra-cellular amylases during fermentation of starch. From the result of the morphological and biochemical tests, the strain was identified as *B. licheniformis*. Robyt (1984) stated that many bacteria including *B. licheniformis* produce extracellular amylases during the fermentation of starch. *B. licheniformis* is a ubiquitous bacterium of importance in the environment as a contributor to nutrient cycling due to its production of amylase and other enzymes. The biosynthesis of α-amylase by B*. licheniformis* appears to be related since the enzyme in this isolate is primarily produced during the exponential phase. This observation is similar to the pattern of amylase synthesis by *Clostridum acetobutylicum* (Annous and Blaschek, 1991) while Adeniran and Abiose (2009) produced a potential α-amylase from a fungi isolate. Some *Bacillus* produce the enzyme in the exponential phase, where as some others in the mid-stationary phase. Though the pattern of growth and enzyme profile of *Bacillus spp*. have similarities, the optimized conditions for the enzymes differ widely, depending upon the strain.

The production and stability of the enzyme is very sensitive to pH and temperature (Declerck et al., 2003). Slight changes in temperature and pH have adverse effect on the growth of microorganisms as well as on the produc-tivity of α-amylase (Anyangwa et al., 1993). The activity of *B. licheniformis* crude α- amylase is in a broad temperature range showing that the enzyme is active at high temperatures of up to 90°C which was quite high comparable with other thermostable amylase (Jin et al., 1990; Saxena et al., 2007). Ivanova et al. (1993) reported an optimum temperature of 90°C for *B. licheniformis* CUMC 305. This broad-temperature activity characteristic of the α-amylase produced lends the isolate *B. licheniformis* as a potential microbe to produce α-amylase required for high temperature applications. At elevated temperatures, they improve the solubility of starch, decrease the viscosity, limit microbial contaminants and reduce reaction time (Thippeswamy et al., 2006). This isolate showed that it is an alkaline *Bacillus* that produces alkaline enzyme at pH 9.0 and it also showed activity at pH 10. This is advantageous since the higher pH will lessen the tendency of gelatinized starch to retrograde (Yu et al., 1988). The concentration of the substrate resulted in 1% as optimum and it has been reported that the synthesis of carbohydrate-degrading enzymes in most species of *Bacillus* is subject to catabolic repression by readily metabolizable substrates such as glucose (Lin et al., 1998).

HPLC chromatogram confirmed the availability of glucose and maltose, indicating that the crude enzyme in B. *licheniformis* is mainly α- amylase.

Conclusion

The newly isolated *B. licheniformis* strain which produces α-amylase is novel and offers interesting hydrolytic properties since the enzyme was active between pH 7 to 10. Besides pH tolerance, the most striking feature of the enzyme is its thermostability. Also significant thermostability of the enzyme make it potential for industrial applications such as starch liquefaction for sweeteners and syrups, textile desizing and paper industries, which requires the process to be carried out in multiple steps at high temperature.

ACKNOWLEDGEMENT

The authors greatly acknowledge the Ministry of Science and Technology, Sudan for their financial support.

REFERENCES

Adeniran AH, Abiose SH (2009). Amylolytic potentially of fungi isolated from some Nigerian Agriculture waste. Afr. J. Biotechnol., 8: 667-672.

Adeyanju MM, Agboola FK, Omafuvbe BO, Oyefuga OH, Adebawo OO (2007). A thermostable Extracellular α- amylase from *Bacillus Licheniformis* isolated from cassava steep water. Biotechnology, 6(4): 473-480.

Annous BA, Blaschek HP (1991). isolation and characterization of *Clostridum acetobutylicum* mutants with enhanced amylolytic activity. Appl. Environ. Microbiol., 57: 2544-2548.

Anyangwa EM, Mapsev C, Musanage P, Elemva M (1993) The effect and removal of starch in the sugar refining industry. J. Int. Sugar, 95: 210-213.

Arnesen S, Eriksen SH, Olsen J, Jensen B (1998). Increased production of α- amylase from *Thermomyces Lanuginosus* by the addition of tween 80 Enz. Microbial. Technol., 23: 249-252.

Buchanan RE, Gibbons NE (1974). Bergey's manual of determinative bacteriology. Baltimore: The Williams and Wilkins Co.

Burhan A, Nisa U, Gokhan C, Omer C, Ashabil A, Osman G (2003). Enzymatic properties of a novel thermostable, thermophilic, alkaline and chelator resistant amylase from an alkaliphilic Bacillus sp. isolate ANT-6. Process Biochem., 38: 1397-1403.

Clark HE, Bordner GEF, Kabler PW, Huff CB (1958). Applied Microbiology. International Book Company, NY, pp: 27-53

Declerck N, Machius M, Joyet P, Wiegand G, Huber R, Gaillardin C (2003). Hyperthermostabilization of *Bacillus Licheniformis* α- amylase and modulation of its stability over a 50°C temperature range. Protein Eng., 16(4): 287-293.

DeMoraes LMP, Fillo SA, Ulhoa CJ (1999). Purification and some properties of an α- amylase glucoamylase fusion protein from *Saccharomyces cerevisiae.* World J. Microbiol. Biotechnol., 15: 561-564.

Eduardo Carlos de Souza T, Meire LLM (2000). Cultural conditions for the production of thermostable amylase by *Bacillus* sp. Braz. J. Microbiol., 31(4): doi: 10.1590/51517- 8382000000400011.

Fogarty WM, Dooylc EM, Kelly CT (1999). Comparison of the action pattern of two high maltose forming alpha-amylase on linear malto-oligosaccharides. Enzyme Microbial. Technol., 25(3-5): 330-335

Fuwa H (1954). A new method for micro determination of amylase activity by the use of amylose as substrate. J. Biochem., 41(5): 583-593.

Gupta R, Gigras P, Mohapatra HVK, Goswami and Chauhan. (2003).

Microbial amylases: a biotechnological perspective. Process Biochem., 38:1599- 1616.
Hagenimana V, Vezina LP, Simaed RE (1992). Distribution of amylasewithin sweet Potato (Ipomea batatas L) root tissue. J. Agric. Food Chem., 40: 1777-1783.
Hanan MI, Wan MWY, Aidil AH, Rosli Md Illias, Othman H, Othman O (2004). Optimization of Medium for the Production of β-Cyclodextrin glucanotransferase using Central Composite Design (CCD). Process Biochem., 40(2): 753-758.
Hag I, Ashraf H, Qadeer MA, Iqbal I (2003). Production of alpha amylase by *Bacillus licheniformis* using an economical medium. Bioresour. Technol., 87(1):57-61.
Herbert D (1961). The chemical composition of microorganisms as a function of their environment. Symp. Soc. Gen. Microbiol., 11: 391-416.
Horikoshi K (1999). Alkalophilic: Some applications of their product for biotehnology. Microbiol. Mol. Biol. Rev., 63(4): 735-750.
Ivanova VN, Dobreva EP, Emanuilova EL (1993). Purification and characterization of a thermostable alpha-amylase from *B. Licheniformis*. J. Biotechnol., 28: 277-289.
Jin F, Cheng X, Shi Y, Zhang C (1990). Isolation of new thermophilic aerobic bacteria which produce thermostable amylase. J. Gen. Microbial, 36: 415-424.
Karbalai-Heidari HR, Ziaee AA, Schaller J, Amoozegar MA (2007). Purification and characterization of an extracellular haloalkaline protease produced by moderately halophilic bacterium *salinivibrio sp* strain AF-2004 (Enzyme Microbial, Technol. 40: 266-272, doi: 10.1016/j.enzmictec. 2006.04.0006.
Kouame LP, Ahipo ED, Niamke SL, Kouame FA, Kamenan A (2004). Synergism of cockroach (*Periplaneta americana*) α-amylase and α-glucosidase hydrolysis of starch. Afr. J. Biotechnol., 3(10): 529-533
Lin LL, Chyaum CC, Hsu WH (1998). Production and properties of a raw- starch- degrading amylase from Thermofilic and alkalophilic *Bacillus sp.* TS-23. Biotechnol. Appl. Biochem., 28: 61-68.
McTigue MA, Kelly CT, Doyle EM, Fogarty WM (1995). The alkaline amylase of the alkalophilic *Bacillus* sp. IMD 370 Enzyme Microbial Technol., 17: 570-573.
Pandy A, Nigam P, Soccol CR, Soccol VT, Singh D, Mohan R (2000). Advance in microbial amylases; Review article. Biotechnol. Appl. Biochem., 31: 135-152.
Rao MB, Tanksale AM, Ghatge MS, Deshpande VV (1998). Molecular and Biotechnological aspect of Microbiol Protease. Microbiol.. Mol. Biol. Rev., 62: 597-635.
Reddy NS, Nimmagadda A, Sambasiva RKRS (2003). An overview of the microbial α-amylase family. Afr. J. Biotechnol., 2 645- 648.
Robyt JF (1984). Enzyme in the hydrolysis and synthesis of starch. In: Starch chemistry and technology. 2nd edi., Whistler, R. L., J. N. Br Miller and E. F. Paschall (Eds), 4: 88-90.
Sadhukhan R, Roy SK, Raha SS, Manna S, Chakrabarty SL (1992). Induction and regulation of amylase synthesis in a cellulolytic thermophilic fungus *Myceliophthora thermophillia* D14 (ATCC 48104). India J. Exp. Biol., 30: 482-486.
Saxena RK, Dutt L, Agarwal PN (2007). A highly thermostable and alkaline amylase from *Bacillus* sp. PN5. Bioresour. Technol., 98: 260-265.
Thippeswamy S, Girigowda K, Mulimami HV (2006). Isolation and identification of α- amylase producing *Bacillus* sp. From dhal industry waste. Indian J. Biochem. Biophys., 43(5): 295-298.
Van der Maarel MJEC, Van der Veen B, Uitdehaag JCM, Leemhuis H, Dijkhuizen L (2002). Properties and aplications of starch converting enzymes of α-amylase family. J. Biotecnol., 94:137-155.
Yu EKC, Hiroyuki A, Masanaru M (1988). Specific apha-cyclodextrin production by a novel thermostable cyclodextrin glycosyltransferase. Appl. Microbiol. Biotechnol., 28: 377-379.