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Optimization and Immobilization of α-amylase from *Bacillus licheniformis*

Tarek E. El-Banna[1], Ahmed A. Abd-Aziz[1], Mohamed I. Abou-Dobara[2] and Reham I. Ibrahim[1]
[1]Microbiology Department, Faculty of Pharmacy, Tanta University
[2]Botany Department (Microbiology), Faculty of Science, Mansoura University (Damiatta Branch), New Damietta, Egypt

ABSTRACT

The production of α-amylase by *Bacillus licheniformis* was optimized. Maximum α-amylase production could be achieved after an incubation period of 48 hrs, at 40 ^{o}C and pH 7.0. Starch (1 %) was found to be the best carbon source among the tested carbohydrates. The organism grew well and produced high levels of α-amylase using beef extract as a nitrogen source. The produced α-amylase was immobilized on various carriers by different methods and the properties of the enzyme were compared before and after immobilization. Compared to the free enzyme, the optimum pH after immobilization enzyme changed to acidic range and the optimum reaction temperature was shifted slightly to 70 - 80 ^{0}C. The thermal stability of the immobilized enzyme was found to be higher than that of the free one. Among the tested salts, $CaCl_2$ exerted a stimulating effect on the activity of α-amylase.

Keywords: Immobilization, α-amylase, *Bacillus licheniformis*.

INTRODUCTION

α-amylase (EC3.2.1.1, 1,4-α-D-glucan glucanohydrolase, endoamylase) hydrolyzes starch, glycogen and related polysaccharides by randomly cleaving internal α-1,4-glucosidic linkages. It is widely distributed in various bacteria, fungi, plants, and animals and has a major role in the utilization of polysaccharides (Kelly *et al.* 1997). α-amylases are used commercially for the production of sugar syrups from starch that consists of glucose, maltose and higher oligosaccharides (Guilbot and Mercier, 1985).

Immobilized enzymes are becoming increasingly popular as reusable, selective analytical chemical reagents in solid phase flow–through reactors, as membranes in sensors and as films in dry reagent kits. For industrial application, the immobilization of enzymes on solid support can offer several advantages, including repeated usage of enzyme, ease of product separation, improvement of enzyme stability, and continuous operation in packed-bead reactors (Abdel-Naby, 1993). A wide variety of carriers have been used for immobilization of amylase (Emne'us and Gordon, 1990; Kurakake *et al.* 1997; Tien and Chiang, 1999; Dey *et al.* 2003).

A bacterial species identified as *Bacillus licheniformis* able to produce α-amylase could be isolated. Optimization of different cultural conditions is expected to improve α-amylase production. The aim of this study was to investigate the effect of some factors on α-amylase production by *B. licheniformis* and immobilize the produced α-amylase onto various carriers to compare the effectiveness of the different methods for immobilization. The properties of the immobilized enzymes were also compared with those of free enzyme.

MATERIALS AND METHODS

This study was conducted throughout the years 2005 and 2006 and carried out in the laboratories of Botany Department, Faculty of Science, New Damietta, Mansoura University, Egypt.

B. licheniformis was isolated from a fertile soil sample collected from Egypt and identified according to Bergey's Manual of Systematic Bacteriology (Sneath, 1986).

The organism was grown in 250 ml Erlenmeyer flasks, each containing 50 ml of nutrient starch broth medium with the following composition (g/l): beef extract, 3.0; peptone, 10.0; NaCl, 5.0; starch, 10.0; distilled water, 1000 ml. The pH was adjusted to 7.0. The flasks were inoculated with 1ml of the spore suspension obtained from nutrient agar cultures and incubated at 40°C with shaking at 150 rev min^{-1} for 48 hr. The filtrate was then separated by centrifugation at 10,000 rpm for 10 min.

Estimation of α-amylase

α-amylase was assayed by measuring the amount of reducing sugar released from 1 % starch. The amount of reducing sugar was estimated according to Miller (1959). The reaction mixture contained 0.4 ml of 1 % (w/v) soluble starch plus 0.5 ml each of diluted culture supernatant or partial purified enzyme preparation and 1.1 phosphate buffer (0.2 M, pH 7). The mixture was incubated at 50°C for 20 min., centrifuged at 10,000 rpm for 10 min. and aliquots of the supernatants were assayed for reducing sugars. One unit of the enzyme activity was defined as the amount of enzyme that released 1umole of reducing sugar (expressed as glucose equivalents) per milliliter per minute under assay conditions. Enzyme and substrate controls were included routinely.

Growth estimation

Growth was determined spectrophotometrically by measuring optical density of the culture at 600 nm. The final pH was also recorded at the end of each experiment.

Factors affecting α-amylase production by *B. licheniformis*

(1) Incubation period

The basal medium containing 1% soluble starch was used; 25 ml medium in 250 ml conical flasks were

* *Corresponding author: aboudobara@mans.edu.eg*

inoculated with 1ml of an overnight culture in triplicate manner. The culture was incubated in an incubator shaker at a shake rate of 150r.p.m. Samples were taken at 12hr intervals (12, 24, 36, 48, 60, and 72).

(2) Effect of different nitrogen sources

Different nitrogen sources were added to the medium according to the nitrogen content of peptone (16.16% nitrogen) and beef extract (12% nitrogen). These nitrogen sources included peptone, tryptone, beef extract, yeast extract, NH_4Cl, $(NH_4)_2S0_4$, NH_4N0_3 and $KN0_3$). Media were inoculated and incubated at 40 °C for 48 hrs.

(3) Effect of different carbon sources

Different carbon sources including; D-glucose, D-fructose, lactose, D-xylose, D-galactose, mannitol, sucrose, potato starch, rice starch, flour) were separately incorporated into flasks containing the production medium to the final concentration of 1% (w/v). The flasks were inoculated and incubated at 40°C for 48 hrs.

(4) Effect of different temperatures

Temperature effect was carried out by inoculating the flasks containing α-amylase production medium at pH 7 with the tested strain and incubated at different temperatures, notably, 30, 35, 40, 45 and 50 °C for 48hrs.

(5) Effect of pH

Different flasks of basal amylase production media of different initial pH values (4, 5, 6, 7, 8 and 9) were prepared. The pH was adjusted by using 0.1N NaOH or 0.1 N HCl. The inoculated flasks were incubated at 40°C for 48hrs.

Physicochemical properties of the α-amylase

(1) pH

To study the effect of pH on the activity of α-amylase, citrate phosphate buffer with pH's (from 3 to 5), phosphate buffer with different pH's (from 6 to 8), and carbonate buffer with pH 9.5, were used in the standard assays. Aliquots of the enzyme preparations were mixed with the different buffers at each pH and the enzyme activity was assayed as previously described. Activities were expressed as a percentage of the maximal activity.

(2) Temperature

Reaction mixtures were incubated at different temperatures (40, 50, 60, 70, 80°C), while the assay was carried out under the standard assay methods. Activity was expressed as percentage of the maximal activity**.** The effect of temperature on the stability of α–amylase was determined by incubating the enzyme solution in the absence of substrate for different times at 60 °C at pH 7. The residual activity was assayed as described before

(3) Effect of metal salts

Half ml of the dialyzed precipitated α–amylase was mixed with 0.5 ml of calcium chloride, sodium chloride, cobalt chloride, nickel chloride, and EDTA solutions of different concentartions (1 mmol, 5 mmol, and 10 mmol) of each metal salt. The α–amylase activity was determined as previously mentioned.

(4) K_m and V_{max}

The K_m and V_{max} of α–amylase were determined at pH 7 and 40°C by varying the substrate concentration from 1.0 to 15mg of starch per ml. Data were plotted by the method of Lineweaver–Burk plot.

(5) Partial purification of the enzyme

The crude enzyme was precipitated with ammonium sulphate. The precipitation was carried out at 4°C under constant stirring and the precipitated proteins were centrifuged at 10,000rpm for 10min. The fraction was dialyzed against 0.1M phosphate buffer (pH 7) to remove the remaining salt. Specific activity of the enzyme was estimated in all precipitates. This partially purified enzyme was used for the preparation of the immobilized enzyme.

(6) Protein estimation

The quantitative estimation of protein was carried out according to Bradford, (1976). Protein concentrations were determined from a standard curve with bovine serum albumin (BSA).

Immobilization Methods

(1) Immobilization on sodium – alginate (Entrapment)

An equal volume of tested enzyme solution and sodium alginate solution was mixed to give a 4% (w/v) final concentration of sodium alginate solution. The mixture obtained was extruded drop wise through a Pasteur pipette (1 mm diameter) into a gently stirred 2% (w/v) $CaCl_2.2H_2O$ solution for 2 hrs to give bead size of 3 mm. The calcium alginate beads containing the enzyme were thoroughly washed with distilled water and used for further studies (Roy *et al.* 2000)**.**

(2) Immobilization on Chitin (Physical Adsorption)

Chitin beads were prepared as follows; 0.5gram of chitin was shaked in 10ml phosphate buffer containing 2.5% glutaraldehyde for two hrs at room temperature. The solid material was filtered and washed to remove excess glutaraldehyde. The treated chitin was mixed with 30ml of the enzyme solution for 24hrs at 4°C. The mixture was centrifuged at 10,000 rpm for 5 min and the supernatant was used for further study. The precipitate was washed by phosphate buffer containing small amount of 0.1M sodium chloride then subjected to the immobilized enzyme activity (Ohtakara *et al.* 1988).

(3) Immobilization on Sephadex

One gram of Sephadex was added to 30ml of the partial purified enzyme solution in phosphate buffer (pH 7) and kept at 4°C for 24 hrs. The unbound enzymes were removed by washing three times with the same buffer (Kusunoki *et al.* 1982)**.**

(4) Immobilization on Dowex (Ionic binding)

One gram of Dowex was incubated with 30ml of the partial purified enzyme solution in phosphate buffer (pH 7) and kept at 4 °C for 24 hrs. The unbound enzymes were removed by washing three times with the same buffer to remove unbounded protein (Ohtsuka *et al*., 1984).

Physical properties of the immobilized α–amylase

Effect of pH, temperature, thermal stability and kinetic constant Km and Vmax and some metal salts on physical properties of the immobilized α–amylase was studied as mentioned before.

Statistical analysis and data presentation

The experiments were carried out in three replicates with standard deviation and standard error presented the statistical procedure used where appropriate was the T-test (Little and Hills, 1978).

RESULTS

The time course for the production of α–amylase by *B. licheniformis* in the basal liquid medium containing 1 % soluble starch as a substrate is shown in the Figure (1). The α–amylase activity of the *B. licheniformis* increased during the growth phase of the culture and the optimum production (2.60u/ml) was reached after 48 hrs. Thereafter, enzyme production declined significantly ($p<0.05$, t-test), reaching the minimum level (0.63u/ml) after 72hrs. Maximum growth (OD_{600} 0.276) was measured after 48 hrs and the growth declined significantly to reach the minimum level (OD_{600} 0.060) after 72hrs.

The best nitrogen source for production of α–amylase from *B. licheniformis* was beef extract followed by peptone plus beef extract, and peptone with activities (2.90 u/ml, 2.84 u/ml and 2.50u/ml, respectively) and the maximum growth measured (OD_{600} 0.390) with peptone plus beef extract as shown in Table (1).

B. licheniformis was able to grow in a basal liquid medium supplied with the different carbon sources, which were glucose, xylose, galactose, mannitol, rice, flour, potato, sucrose, fructose, lactose and starch (Table 2). The best carbon source for production of α–amylase and growth for *B. licheniformis* was starch with activity (5.15 u/ml). Flour, glucose, lactose, galactose and xylose

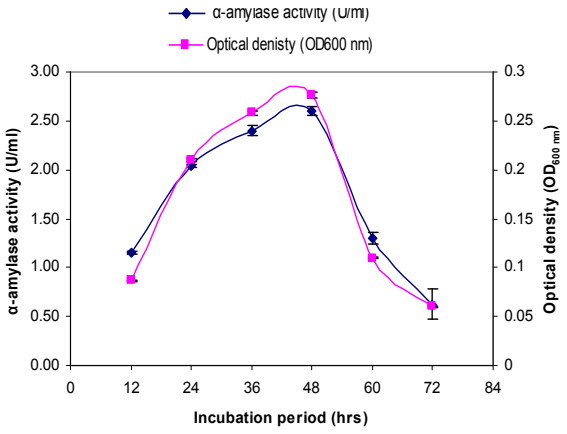

Figure (1): Effect of different incubation periods on α–amylase production and growth of *B. licheniformis*. Values are shown as means of triplicates ± standard error.

Table (1): Effect of different nitrogen sources on α-amylase production and growth of *B. licheniformis.*

Nitrogen source	α-amylase (U/ml)	Optical density (OD_{600nm})	Final pH
Ammonium sulphate	0.069±0.001	0.020±0.002	7.4
Ammonium chloride	0.062±0.006	0.046±0.003	6.9
Ammonium nitrate	0.261±0.025	0.094±0.007	6.9
Potassium nitrate	0.370±0.029	0.110±0.008	7.3
Tryptone	0.029±0.004	0.040±0.003	8.4
Peptone	2.500±0.115	0.225±0.017	7.1
Beef extract	2.900±0.115	0.388±0.029	7.5
Yeast extract	0.737±0.009	0.124±0.009	7.9
Peptone + beef extract	2.840±0.046	0.390±0.029	8.8

Each results is the means of three replicates ± standard error (SE)

Table (2): Effect of different carbon sources on α-amylase production and growth of *B. licheniformis*.

Carbon source	α-amylase (U/ml)	Optical density ($OD_{600\ nm}$)
D-glucose	2.24 ± 0.154	0.016 ± 0.001
D-xylose	1.58 ± 0.021	0.140 ± 0.001
D-galactose	2.00 ± 0.053	0.280 ± 0.003
Mannitol	0.37 ± 0.022	0.020 ± 0.000
Sucrose	0.04 ± 0.003	0.020 ± 0.000
D-fructose	0.43 ± 0.018	0.060 ± 0.001
Lactose	2.20 ± 0.056	0.210 ± 0.002
Starch	5.15 ± 0.061	0.630 ± 0.006
Rice	0.11 ± 0.001	0.010 ± 0.000
Flour	2.49 ± 0.002	0.280 ± 0.003
Potato	0.39 ± 0.050	0.080 ± 0.001

Each results is the means of three replicates ± standard error (SE).

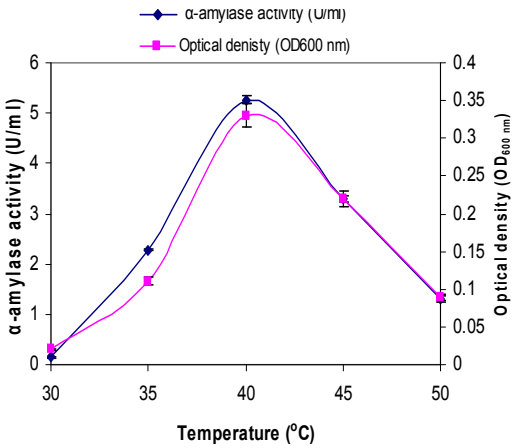

Figure (2): Effect of different temperatures on α–amylase production and growth of B. licheniformis. Values are shown as means of triplicates ± standard error

were also found to be good sources for the production of α-amylase. Little growth was observed with glucose, mannitol, sucrose and rice powder.

An optimum temperature for α–amylase production from *B. licheniformis* was 40 °C (Fig. 2). At 50 °C the activity was declined significantly ($p < 0.05$, t-test). Maximum growth was attained at 40°C.

The optimum pH for α–amylase production was 7 with activity 6.8 u/ml (Fig. 3). The enzyme activity decreased significantly at pH 9.0 ($p < 0.05$, t-test). The range for maximum growth was found to be pH 7 to pH 8.

Physicochemical properties of crude and partially purified immobilized α–amylase

Free α–amylase activity increased with increasing pH within the pH range of 3 to 7 till reached the optimum value of pH 7 (Fig. 4). Generally, the optimum pH of the immobilized α–amylase was shifted to acidic range in comparison to the free enzyme (Fig. 4). The relative activity decreased and reached minimum levels at pH 8 and pH 9.5 for free and immobilized enzyme, separately (Fig. 4).

The activity of free α–amylase increased with increasing temperature starting from 40°C till reached the optimum at 60°C (Fig. 5). The optimal reaction temperature of the free α–amylase shifted from 60 °C to 70 °C for the enzyme immobilized on sephadex and to 80 °C for immobilization on dowex, chitin and Ca alginate (Fig. 5). The minimum level of the free and immobilized α–amylase activity occurred at 90°C (Fig. 5).

The effect of some metal salts on the activities of free and immobilized α–amylase was estimated in Table (3). Generally, some metal salts increased the activity of free and immobilized α–amylase such as $CaCl_2$ and NaCl

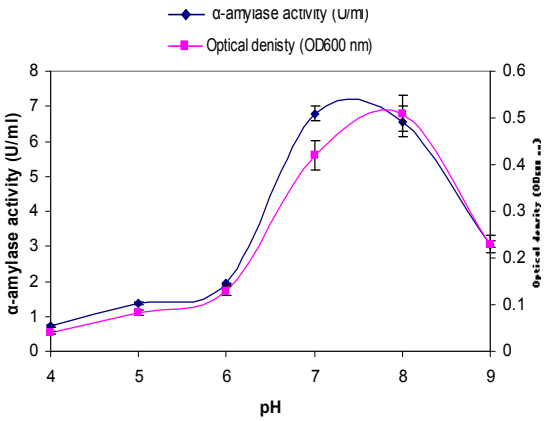

Figure (3): Effect of different pH values on α–amylase production and growth of B. licheniformis. Values are shown as means of triplicates ± standard error.

with the three used concentrations (1, 5 and 10 mM). The other metal salts, notably $CoCl_2$, $NiCl_2$ and EDTA inhibited the activity of free and immobilized α–amylase. The minimum levels of the activity were reported when using EDTA in all concentrations.

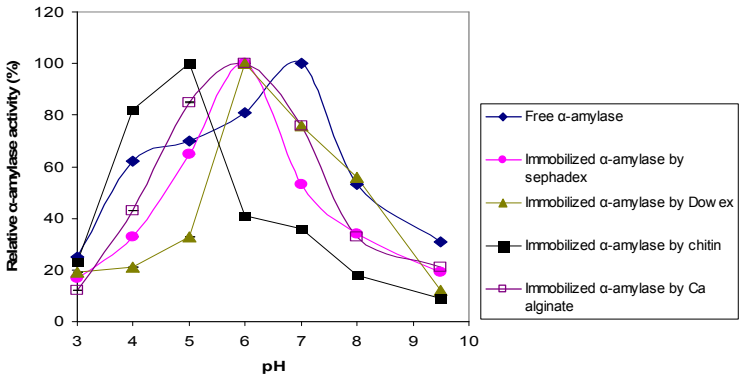

Figure (4): Effect of pH on activity of α-amylase of B. licheniformis before and after immobilization. Values are shown as means of triplicates ± standard error (SE).

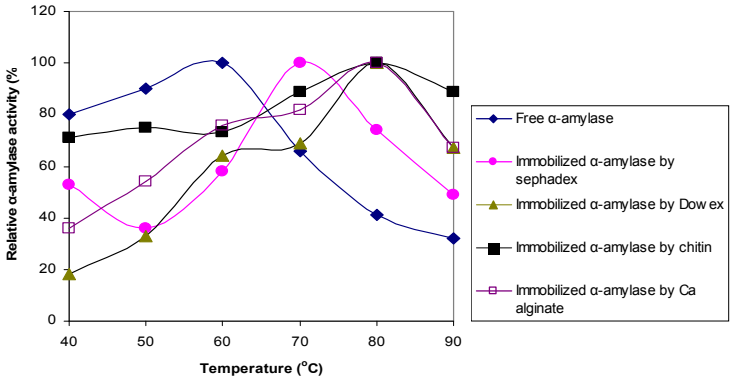

Figure (5): Effect of temperature on activity of α-amylase of B. licheniformis before and after immobilization. Values are shown as means of triplicates ± standard error (SE).

Table (3): Effect of metal ions on relative activity (%) of free and immobilized α-amylase of *B. licheniformis*. Values are shown as means of triplicates

Chemical	Concentration (mM)	None	Free α-amylase	Immobilized α-amylase by			
				Sephadex	Dowex	Chitin	Ca alginate
$CaCl_2$	1	100	104	109	106	109	112
	5	100	107	111	107	111	117
	10	100	109	118	109	119	119
NaCl	1	100	92	102	104	106	103
	5	100	95	104	106	110	107
	10	100	97	106	107	114	109
$CoCl_2$	1	100	94	96	89	95	91
	5	100	87	91	71	84	78
	10	100	82	87	54	79	65
$NiCl_2$	1	100	72	83	81	71	69
	5	100	54	65	34	53	51
	10	100	43	54	18	42	27
EDTA	1	100	24	36	39	34	28
	5	100	12	27	21	14	8
	10	100	4	7	10	7	4

Table (4): Km and Vmax values of free and immobilized of α-amylase of *B. licheniformis*. Values are shown as means of triplicates.

Value	Free α-amylase	Immobilized α-amylase by			
		Sephadex	Dowex	Chitin	Ca alginate
Km (mg/ml)	25	14.28	12.5	25	25
Vmax (μmole/ml/min.)	1.66	12.5	10	20	30.30

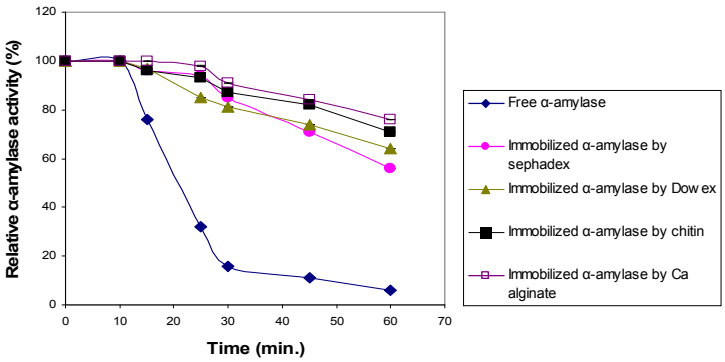

Figure (6): Thermal stability of α-amylase of B. licheniformis before and after immobilization. Values are shown as means of triplicates ± standard error (SE).

The Km and Vmax values for free and immobilized α–amylase are listed in Table (4). In general, the calculated Vmax values of the immobilized enzymes were higher than that of the free enzyme while the Km values were lower in the cases of dowex and sephadex carriers.

Thermal stability of free and immobilized α–amylase was completely stable at 60°C for 10 min (Fig. 6). The free enzyme lost 24% of the relative activity after 15 min. while the immobilized ones retained 96% and 97% in case of utilization of sephadex and dowex; to 87% and 91% of the activity after 30 minutes when chitin and Ca-alginate were used for immobilization respectively (Fig. 6). After 60 min., the free enzyme lost more than 90% of its activity.

DISCUSSION

Growth and α-amylase production by *Bacillus licheniformis* increased reaching maximum values after 48 hrs, after which, the production of the enzyme decreased. The observed peaking and throughing of the production of extracellular enzymes can be attributed to (1) The products of action of one component inducing the synthesis of another, (2) Differential inhibition by products of substrate hydrolysis, (3) decrease in growth observed after 48 hrs of growth of *B. licheniformis*. This probably resulted from cellular lysis, an observation previously reported. These results indicate that the production of extracellular α-amylase by *B. licheniformis* was growth associated and this is in agreement with

other investigators (Bajpai and Bajpai, 1989; Stephenson *et al.* 1998; Riaz *et al.* 2003).

The optimal production of α-amylase and growth of *B. licheniformis* could be obtained when either beef extract or peptone plus beef extract or peptones was used as nitrogen sources. Many investigators had recorded that when peptone was used as nitrogen source, bacilli species produce high amount of α-amylase (Chandra *et al.* 1980; Babu and Satyanarayana, 1995; Aiyer, 2004). Beef extract and peptone containing media are complex and contain high nutritional amino acids and this may lead to high α-amylase production and good growth. Proteose peptone was recorded as good nitrogen source for amylase production and growth of thermophilic *Bacillus* sp. WN11 (Bajpai and Bajpai, 1989).

The best carbon source for α-amylase production from *B. licheniformis* was found to be starch**.** Starch is known to induce amylase production in different bacterial strains (Saito and Yamamoto, 1975; Fogarty and Bourke, 1983; Wind *et al.* 1994; Aiyer, 2004; Ryan *et al.* 2006). Low levels of α-amylase were detected when the organism was grown with mannitol. Similar observations were recorded by Meers (1972), Chandra *et al.* (1980), and Wind *et al.* (1994). Glucose and fructose showed repression effects on enzyme production. It has been reported that the synthesis of carbohydrate degrading enzymes in most species is subjected to catabolite repression by readily metabolize substance such as glucose and fructose. Among the undefined carbon sources that led to high levels of amylase were flour, potato and rice. Undefined carbon sources are known to induce a high level of amylase production in many bacterial strains (Fogarty *et al.* 1974; Mamo and Gessesse, 1999; Tonkova *et al.* 1993; Ryan *et al.* 2006).

The optimal temperature for production of α-amylase and growth of *B. licheniformis* was 40 ^{o}C. Similar observations were recorded by Bajpai and Bajpai (1989); Anyangwa *et al.* (1993), and Lin *et al.* (1998). Bacterial cells have various mechanisms that allow them strictly to control excretion (Mamo amd Gessesse, 1999)**.** Change in the nature of cell envelope can affect the release of extracellular enzymes to the culture medium (Antranikian, 1990)**.** Temperature is one of the factors that induces such changes on cell membranes and cell walls (de Vrij *et al.* 1990; Nordstrom, 1993). It is also reported that in Bacillaceae, the surface protein layer (S-layer) is involved in the control of exoenzyme release (Egelseer *et al.*, 1996a & b).

Extracellular α-amylase production and growth of *B. licheniformis* was maximal at pH 7 and this result is in accordance with the work of Bajpai and Bajpai (1989) and Lin *et al.* (1998). The composition of cell wall and plasma membrane of microorganisms is known to be affected by the culture pH (Ellwood and Tempest, 1972a & b)**.** The change of the initial pH of the medium may lead to change of the nature of the cell membrane and/ or cell wall and hence affecting the α-amylase production and the growth of *B. licheniformis*. On the other hand, Mamo and Gessesse (1999) reported high level of α-amylase at pH 5.0 and 6.0 and when the pH was increased low level of α-amylase was obtained.

The pH optimal for the free α-amylase of *B. licheniformis* was found to occur at pH 7. These results are in accordance with those reported by Vihinen and Mäntsälä (1989), Hamilton *et al.* (1999), Saito (1973), and Khoo *et al.* (1994). On the other hand, the immobilized α-amylase showed optimum pH in the acidic range in comparison to the free enzyme. These effects may depend on the ionic environment around the active site of the enzyme bound to the carrier (Abdel-Naby, 1993). Similar shifts of pH optima were reported by Yoshida *et al.* (1989), Sadhukhan *et al.* (1993), and El-Banna *et al*. (2007).

The free α-amylase of *B. lichenifromis* showed temperature optima at 60 ^{o}C and the immobilized α-amylase showed higher relative activity at the temperatures 70 ^{o}C and 80 ^{o}C, respectively. Increase in temperature optima had been found in immobilized enzymes (Yoshida *et al.* (1989), Sadhukhan *et al.* (1993), and El-Banna *et al*. (2007). The increase in temperature optima for the activity of immobilized enzyme could be due to the fact that actual temperature in the micro-environment of the gel matrix in cases of Ca-alginate as a carrier was lower than in the bulk solution (Kennedy, 1987).

The relative activity of free α-amylase of *B. licheniformis* was declined to 76% after 15 min., to 32% after 25 min. at 60 ^{o}C and these results are similar to that had been obtained by Shaw and Chuang (1982), Shaw and Ou–Lee (1989), Gerhartz (1990), and El-Banna *et al*. (2007). The thermoinactivation at 60 ^{o}C of free and immobilized enzyme showed remarkable achievement of thermostability by the immobilization with *B. licheniformis* with different carriers. The high thermostability of immobilized α-amylase is consistent with the results obtained for other enzymes (Sheffield *et al.* 1995). The thermal stability of the enzyme increased the tolerance to thermal denaturation, therefore, had been imparted by the gel entrapment covalent cross-linking of the enzyme protein.

The free α-amylase from *B. licheniformis* showed good affinity towards starch with a Km and Vmax values of 25 mg/ml and 1.66 umole/min./ml, respectively. The Vmax of the immobilized α-amylase increased with the four carriers while the Km decreased with dowex and sephadex carriers. This increase is most likely a consequence of either structural changes in the enzyme introduced by the applied immobilization procedure and /or lower accessibility of the substrate to the active site of the immobilized enzyme (Abdel-Naby, 1993). The latter, as explained above, may result either from diffusional resistance of the matrix or steric hinderance in the immediate vicinity of the enzyme molecules (Abdel-Naby, 1993).

The results obtained were similar to those reported for immobilized glucoamylase by Koji *et al.* (1999). For practical application, an immobilized system with lower Km value and faster rate of reaction is preferred.

Among the tested metal salts, $CaCl_2$ was the most activator metals. The relative activity of free and immobilized α-amylase from *B. licheniformis* increased in the presence of all concentration of calcium salt. These results are in accordance with the work of other investigators (Lin *et al.*, 1998; El-Banna *et al.* 2007)**.** Ca^{2+} ions has a positive effect on the activity of the α-amylase enzyme. The binding of Ca^{2+} ions has been shown to increase the α-helical structure of the α-amylase enzyme leading to increased stability. Na^{+} ions similar to Ca^{2+} increased the activity of the α-amylase. The ions like Co^{2+}, and Ni^{2+} slightly inhibited amylase activity. Some amylases are metalloenzymes, containing a metal ion with a role in catalytic activity by Ni^{2+} and Co^{2+} ions could be due to competition between the exogenous cations and the protein-associated cation resulting in decreased metalloonzyme activity. The inhibitory effect of the chelating agent of EDTA demonstrated the ion requirement of this amylase similarly. EDTA has been found to be a potent inhibitor of amylases from other bacteria (Yoshida *et al.*, 1989; Sadhukhan *et al.*, 1993; Shaw *et al.*, 1995; and El-Banna *et al.*, 2007).

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إنتاج وتسكين الفا-أميليز من باسيلس لايكينيفورمانس

طارق السعيد البنا[1]، أحمد أحمد عبد العزيز[1]، محمد إسماعيل أبودبارة[2]، ريهام إبراهيم علي[1]
[1]كلية الصيدلة، جامعة طنطا. [2]كلية علوم دمياط، فرع دمياط، جامعة المنصورة

الملخص العربـــى

إستهدف هذا البحث دراسة تأثير بعض العوامل علي إنتاج ألفا-أميليز من باسيلس لايكينيفورمانس. ولقد وجد من الدراسة أن الظروف المثلي لأعلي إنتاجية للألفا-أميليز بعد فترة تحضين 48 ساعة ودرجة حرارة 40 درجة مئوية ودرجة أس هيدروجيني 7 وكذلك إتضح أيضا أن النشا بتركيز 1 % أحسن مصدر كربوني ومستخلص اللحم أفضل مصدر نيتروجيني لإنتاج ألفا-أميليز من باسيلس لايكينيفورمانس.

إمتدت الدراسة لتسكين ألفا-أميليز المنتج من باسيلس لايكينيفورمانس بإستخدام الجينات صوديوم وكيتين وسفاديكس ودويكس ومقارنة صفات الإنزيم الخام قبل وبعد التسكين. ولقد وجد أن درجة الأس الهيدروجيني المثلي للألفا-أميليز الذي تم تسكينه قد تحولت إلي الوسط الحامضي وكذلك درجة الحرارة المثلي تحولت بين 70- 80 درجة مئوية. ولقد وجد أن الثبات الحراري للألفا-أميليز الذي تم تسكينه أعلي من القيم الخاصة بالألفا-أميليز الخام قبل التسكين. ومن الدراسة أيضا تبين أن كلوريد كالسيوم بتركيز 1 و5 و10 مليمول له تأثير منشط للألفا-أميليز الخام قبل وبعد التسكين.