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STUDIES ON PRODUCTION OF ALKALINE SERINE PROTEASE FROM *Bacillus sphaericus*

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ABSTRACT

Screening studies on seventy eight strains of *Bacillus sphaericus* (*Bs*) of different origins have revealed that the majority of cultures produced extracellular alkaline protease activities in variable levels when grown in nutrient broth- yeast extract- salt medium (NYSM) under shake culture conditions. Physiological studies on enzyme formation by two highly mosquitocidal strains namely the international strain 2362 and a local Egyptian isolate NRC 69 have shown that the enzyme was produced on a variety of different media including standard media, powdered legumes seeds broths as well as media based on agro-industrial by-products. The highest enzyme levels were obtained in both *Bs* cultures upon using fodder yeast, a by-product of ethanol fermentation industry, as a mono component medium for growth and ENZYME production. Maximum enzyme levels were obtained in shake cultures with medium containing 4% (w/v) fodder yeast under high aeration levels, inoculum size ranges 4-8 × 10^6 CFU/ml after three days of incubation at 30 °C. The enzyme exhibited maximum activity at 55°C incubation temperature and pH 8.5 suggesting an alkaline protease activity with retention of about 60 % of the activity at pH 10 for the enzymes of both bacterial strains. Enzyme stability at 60 °C was markedly improved in the presence of calcium chloride. Inhibition studies showed that the alkaline protease activity was inhibited (72% - 79%) by an active-site inhibitor of serine protease, phenyl methylsulfonyl fluoride (PMSF) but not by soybean trypsin inhibitor or iodoacetamide.. These results suggest that the alkaline protease activity produced belongs to serine protease group of enzymes prevalent in some species of *bacilli*.
Keywords: *Bacillus sphaericus*, alkaline protease, production

INTRODUCTION

Spore forming *Bacilli* produce a variety of protein hydrolyzing enzymes (Maurizi and Switzer, 1980). These enzymes are involved in substrate degradation, protein turnover, spore coat processing and various other functions related to metabolic regulation and differentiation (Koide *et al.*, 1986).

Some strains of *Bacillus sphaericus* (*Bs*) produce parasporal body, which is highly toxic if ingested by susceptible mosquito larvae such as those of *Culex* and *Anopheles spp* (Dumusois and Priest, 1993). The proteases of *Bs* are relevant to the use of this bacterium as a biological agent for mosquito larvae in two ways. First, the release of non-specific proteases by the bacterium would assist the dissolution of the larval cadaver and provide nutrients for the growth of the bacterium in the larval body during recycling. Second, since this bacterium does not use sugars as a source of carbon and energy (Russel *et al.*, 1989), the organism relies heavily on protein degradation to serve adequate supply of carbon and energy source from the resulting amino acids and peptides and also for providing carbon skeletons

required for various metabolic activities. Consequently, large-scale production of *Bs* for use as biological control of mosquitoes requires adequate supply of proteinaceous materials in the growth medium rather than cheaper starchy agricultural by-products (Bhumiratana, 1990). On the other hand, the formation of extracellular proteases has been also reported in other biological control bacteria belonging to *Bacillus thuringiensis* (Kunitate *et al.*, 1989; Kuppusamy and Balaraman, 1990; Donovan *et al.*, 1997; Reddy *et al.*, 1998 and Oppert, 1999) thus emphasizing an additional possible role in their insecticidal activities.

Some reports (Yoshida *et al.*, 1977; Yoshida, 1983 and Almog *et al.*, 1994) have shown the formation of sfericase (a partially characterized serine protease) during the stationary phase of growth by some cultures of *Bs*. More recently Mas *et al.*, 1997 in a detailed study could identify a protease enzyme produced by *Bs* strain SSII-1 and proved to be involved in the *in vivo* degradation of the 100 KDa mosquitocidal toxin (Mtx). The N-terminal sequence of this enzyme suggests that it was related to sfericase.

In spite of the apparent significance of the extracellular proteases in metabolism of *Bs*, only meager information are available regarding their production physiology and properties in this bacterial species. Thus, the present work was undertaken to elucidate the physiological aspects of the enzyme formation and production by *Bs* with special reference to those strains with potential biological control prospective against mosquitoes.

MATERIALS AND METHODS

Source of *Bs* strains

The international strains were obtained from Prof F.G.Priest, Heriot Watt University, UK. The Egyptian strains were isolated from Egyptian environments and identified according to international Keys at the National Research Centre of Egypt and Institute Pasteur, France (El-Bendary, 1999).

Media

Standard media

Nutrient broth (NB); NYSM contains (g/l): peptone (5), Beef extract (3), Yeast extract (0.5), $MnCl_2$ (0.01), $CaCl_2$ (0.1), $MgCl_2$ (0.2); and Minimal medium contains (g/l): $(NH_4)_2SO_4$ (8), K_2HPO_4 (56), KH_2PO_4 (24), $MgSO_4$ (0.8), $MnCl_2$ (0.3), $FeCl_2$ (0.14), $ZnSO_4$ (0.2), and supplemented with yeast extract (0.5), thiamin (0.2), and biotin (2µg).

Media based on agro-industrial by-products and legumes seeds

These media were tested as possible production media that are low priced and locally available around the year. The legumes seeds and agro-industrial by-products were used as mono-component media at 2% (w/v) in distilled water.

Growth conditions.

Unless otherwise is indicated, exponentially growing cultures of the strains understudy were used to inoculate 250 ml conical flasks each containing 25 ml of the experimental media. The inoculated flasks were incubated on a rotary shaker for 3 days at 30 °C. At the end of incubation period, the full grown cultures were centrifuged at 5000 rpm for 25 minutes, the pellets were discarded and the clear supernatants was used as an enzyme source.

Study of physiological factors affecting protease formation by *Bs* strains. The following factors were studied.

1. Screening of various *Bs* cultures with respect to their abilities to4 produce alkaline protease (AP). The study was carried out using NYSM medium for growth and comparative enzyme production.
2. Potentials of some agro-industrial by-products and legumes seeds as growth media for enzyme production. A group of locally available agro-industrial by-products and legumes seeds that are rich in their protein contents were tested as possible media for AP production. With the exception of sweet cheese whey, which used as such, all the ingredients were tested at 2% (w/v) final concentration.
3. Effect of initial pH of the medium. The medium was buffered with 0.05 M of phosphate buffer at pH values ranging between 5.5 and 8.0.
4. Effect of aeration level. The air: medium ratios (v:v) in the experimental flasks (250 ml) were varied between 40:1 and 1.5:1. The experimental flasks in duplicates were then incubated either under shaking conditions on a rotary shaker or under static conditions at 30 °C.
5. Effect of inoculum size. The inoculum size was varied by varying the initial colony-forming unit (CFU) between $4\times10^6 - 32\times10^7$/ ml of medium.

Elucidation of some properties of the enzyme.

1. The optimum pH of the crude enzyme was determined by the azocasein assay in 0.1 M of phosphate, Tris-HCl and carbonate-bicarbonate buffers to cover the pH range 5.7-10.7.
2. Effect of reaction temperature on alkaline protease activity (APA) was determined by incubating the reaction mixture at various temperatures (30 °C –90 °C).
3. Thermal stability was determined by incubating enzyme samples for ten times at various temp before estimating residual activity compared with a control.
4. pH stability. Enzyme solution in 0.1 M of Tris-HCl buffer or carbonate bicarbonate buffer at pH values ranging between 7 and 10.7 were incubated at room temp for 24 hours after which the remaining activity was assayed.

5. Effect of inhibitors on APA was determined by incubating enzyme solution with inhibitor (see table 6 for concentration) for 1 hour at 30 °C and residual activity was determined by the azocasein assay.

Protease assay

The alkaline protease activity (APA) was assayed according to Dumusois and Preist (1993).

Samples from grown cultures were clarified by centrifugation at room temperature and the supernatant fraction was used as the source of enzyme either directly or after storage in freezer. APA was determined initially at pH 9 and thereafter at the pH optimum for the protease(s). Azocasein (0.2% w/v in 0.1 M Tris-HCl buffer) 1 ml, and enzyme source (0.5 ml) were incubated initially at 40 °C for 30 minutes and thereafter at the optimum temperature and time. The reaction was stopped by the addition of 1 ml of 20% (w/v) TCA (at 0 °C) and left on ice for 10 min. The mixture was centrifuged and the optical density of the supernatant fluid was measured at 420 nm. APA was expressed as the change in optical density at 420 nm per 10 min per milliliter of culture supernatant as an enzyme source

RESULTS AND DISCUSSION

Screening of *Bs* strains for production of alkaline protease activities.

Seventy-eight *Bs* cultures including sixteen international strains of *Bs* and sixty-two egyptian isolates were screened for their APA using NYSM medium.

As shown in Table 1, three international strains of *Bs* (2362, Gha1, and KellenQ) and eleven egyptian isolates (NRC 4, NRC 5, NRC 11, NRC 28, NRC 43, NRC 53, NRC 56, NRC 69, NRC 70, NRC 72 and NRC 76 exhibited relatively the highest APA strains.

One international strain (2362) and one egyptian isolate (NRC 69) were selected to be investigated throughout the present work.

These strains are highly pathogenic against *Culex pipiens* and *Anopheles stephensi* and they have been studied before for production of mosquitocidal toxins (El-Bendary, 1999). Therefore the authors selected these two strains to study the factors affecting the production of alkaline protease as representatives of the bacterial species *Bacillus sphaericus*.

Production of alkaline protease (AP) by *Bs* 2362 and NRC 69 on agro-industrial by-products and leguminous seeds.

Nine leguminous seeds (finely powdered), five agro-industrial by-products and baking yeast were evaluated as media for production of AP by *Bs* 2362 and NRC 69. Three standard media namely minimal, Nutrient broth and NYSM media were also included in this study for comparative purposes.

Table 2 shows that the fodder yeast was the most efficient medium ingredient for production of alkaline protease by the two strains under study.

The highest APA was found when bacteria were grown in 4% fodder yeast for three days (Figures 1 (A) & 1 (B)).

Table 1: Screening of alkaline protease activity (APA) of international strains and Egyptian isolates of *Bacillus sphaericus*. The cultures were grown on the standard NYSM medium for two days under shaking condition at 30 °C.

Strain no	Pathogenicity to mosquitoes*	Final pH of the culture	APA (OD units)#
International strains**			
ATCC14577	Non	8.76	0.04
ATCC10208	Non	9.03	0.28
2362	High	8.84	1.22
2297	High	8.84	0.77
1593	High	8.85	0.16
Gha1	High	8.92	1.28
LPIG	High	8.81	0.13
COK 31	High	9.00	0.27
IAP88I	High	9.10	0.43
LP12-AS	High	8.92	0.25
SSII-1	Low	9.09	0.33
KellenQ	Low	9.21	1.18
ATCC12300	ND	8.90	0.04
PI	ND	8.94	0.02
NRS 1693	ND	8.94	0.04
NRS 1199	ND	8.89	0.12
Egyptian isolates			
NRC 1	None	8.63	0.05
NRC 2	None	8.83	0.11
NRC 3	None	8.81	0.07
NRC 4	None	9.15	1.33
NRC 5	None	9.13	1.31
NRC 6	None	8.87	0.12
NRC 7	None	8.04	0.00
NRC 8	None	8.81	0.00
NRC 11	None	8.91	1.24
NRC 12	None	8.79	0.18
NRC 13	None	8.83	0.32
NRC 14	None	8.67	0.00
NRC 16	None	8.76	0.13
NRC 17	None	8.71	0.00
NRC 18	None	8.10	0.02
NRC 19	None	8.94	0.31
NRC 20	None	8.99	0.11
NRC 21	None	8.71	0.00
NRC 22	None	8.72	0.00
NRC 23	None	8.73	0.06
NRC 24	None	8.80	0.40
NRC 25	None	8.65	0.09
NRC 26	None	8.68	0.04
NRC 28	None	8.65	1.06
NRC 29	None	8.93	0.02
NRC 30	None	8.82	0.11
NRC 32	None	8.84	0.11

Table 1. continued.

NRC 33	None	8.14	0.02
NRC 35	Low	8.83	0.27
NRC 36	Low	8.94	0.28
NRC 38	Low	8.87	0.23
NRC 39	None	9.02	0.10
NRC 40	Low	8.86	0.19
NRC 41	Low	8.75	0.16
NRC 42	None	8.95	0.33
NRC 43	Low	9.14	1.40
NRC 44	Low	8.87	0.16
NRC 45	None	8.95	0.03
NRC 46	Low	8.89	0.19
NRC 48	Low	8.82	0.01
NRC 49	None	8.52	0.02
NRC 52	None	8.97	0.12
NRC 53	Low	9.02	1.33
NRC 54	None	8.71	0.02
NRC 55	None	9.04	0.31
NRC 56	None	9.06	1.30
NRC 57	Low	8.87	0.11
NRC 58	None	8.72	0.02
NRC 59	None	8.97	0.05
NRC 60	Low	8.88	0.02
NRC 62	None	8.45	0.06
NRC 63	Low	8.94	0.14
NRC 66	None	8.74	0.00
NRC 67	None	8.95	0.20
NRC 68	None	9.01	0.49
NRC 69	High	8.61	1.20
NRC 70	High	8.52	1.19
NRC 72	High	8.43	1.22
NRC 73	High	8.46	0.05
NRC 74	Low	8.20	0.04
NRC 75	High	8.81	0.18
NRC 76	High	8.59	1.06

* Data of pathogenicity were obtained from El-Bendary (1999). Pathogenicity to mosquitoes was determined against *Culex Pipiens* and *Anopheles stephensi* larvae.

Alkaline protease activity (APA) was expressed as the change in optical density at 420 nm per 30 min per milliliter of culture supernatant as an enzyme source.

** International strains were obtained from Prof. F. G. Preist, Biological sciences Department, Heriot Watt University, UK.

NRC-Cultures Originally isolated from Egyptian environments and identified according to international keys at the National Research centre of Egypt in collaboration with Institute Pasteur of France.

ND, Not determined.

Table 2: Comparative (APA) of two highly mosquitocidal strains BS 2362 and NRC 69 grown on standard media as well as some leguminous seeds and agro-industrial by-products. The cultures were grown under shaking conditions for two days at 30 °C

Media used	*Bacillus sphaericus* strain			
	2362		NRC 69	
	Final pH	APA (OD units)	Final pH	APA (OD units)
Standard				
Minimal medium	7.18	0.00	7.08	0.06
NB	8.90	0.09	8.96	0.00
NYSM	9.30	3.06	9.20	2.36
Legumes seeds				
Soy beans	9.15	4.74	8.40	3.01
Whole beans	8.97	6.62	8.90	5.63
Split beans	9.15	3.28	8.63	3.68
Butter beans	8.47	4.77	8.08	4.89
Black eye beans	7.56	0.68	6.96	1.24
Chick pea	8.41	4.77	7.28	4.74
Lupine seeds	9.07	4.31	8.97	2.65
Whole lentils	9.01	5.60	8.92	7.32
Red split lentils	7.45	3.20	8.93	2.70
Agro-industrial by-products				
Fodder yeast	8.95	.7.15	8.67	7.93
Baking yeast	8.97	4.00	8.58	5.02
Offals meal	9.15	4.04	8.91	4.55
Feather meal	8.61	1.75	8.51	1.06
Cottonseed flour	9.25	4.46	9.31	2.90
Sweet cheese whey	8.82	3.35	8.72	2.99

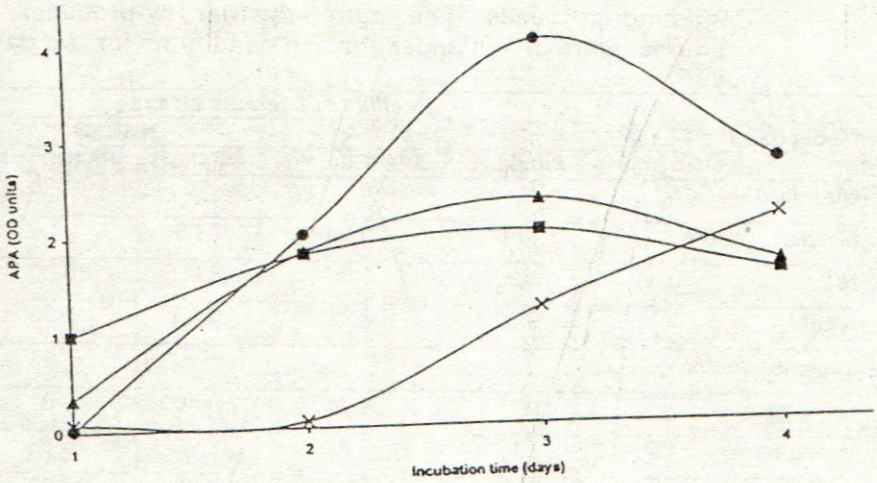

Figure 1 (A). Effect of fodder yeast concentration and incubation time on AP production by *Bs* 2362. Fodder yeast concentrations { (■), 1%; (▲), 2%; (●), 4% & (×), 8%}

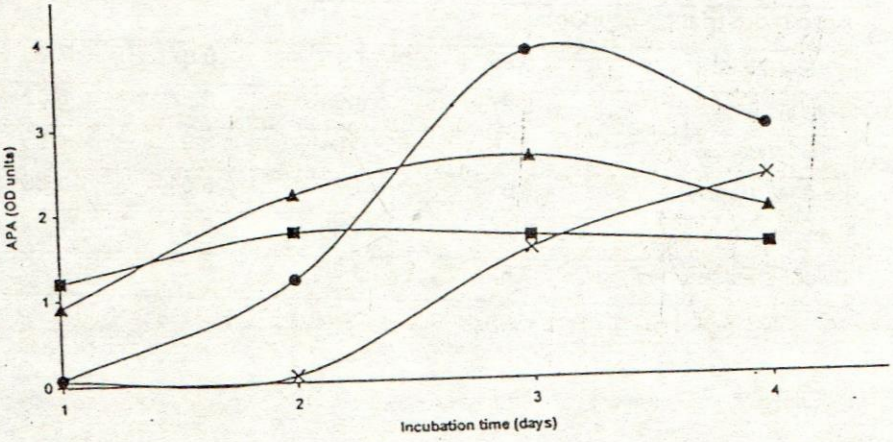

Figure 1 (B). Effect of fodder yeast concentration and incubation time on AP production by *Bs* NRC 69. Fodder yeast concentrations { (■), 1%; (▲), 2%; (●), 4% & (×), 8%}

Physiological factors affecting AP production by *Bs* 2362 and NRC 69. Effect of initial pH.

The data of Table 3 showed that buffering the medium at different pH values did not enhance the AP production by the two organisms under study.

Table 3: Effect of initial pH of the fodder yeast buffered medium on AP production by *Bacillus sphaericus* strain 2362 and NRC 69. The media were buffered using 0.05 M phosphate buffer at indicated pH values.

Initial pH of buffered medium		*Bacillus sphaericus* strain			
		2362		NRC69	
Before autoclaving	After autoclaving	Final pH	APA (OD units)	Final pH	APA (OD units)
unbuffered	5.80	8.54	5.34	8.71	5.24
5.50	5.53	7.65	4.34	6.42	4.28
6.00	5.64	8.00	4.90	7.10	4.75
7.00	6.40	8.43	5.53	7.93	4.81
8.00	6.97	8.60	5.45	8.49	3.84

Table 4: Effect of aeration level on AP production by *Bacillus sphaericus* 2362 and NRC 69 grown in 4% fodder yeast medium. Growth conditions are reported in the text.

Aeration level		*Bacillus sphaericus* strain							
		2362				NRC 69			
Volume of medium (ml/250ml flask)	Air:medium ratio	Static conditions		Shake conditions		Static conditions		Shake conditions	
		Final pH	APA (OD units)	Final pH	APA (OD units)	Final pH	APA (OD units)	Final pH	APA (OD units)
6	41:1	8.78	5.67	8.53	5.78	8.77	5.46	8.56	6.57
12.5	19:1	8.57	3.45	8.98	7.02	8.63	4.16	9.05	6.79
25	9:1	7.69	1.56	8.73	5.63	7.94	1.86	8.75	6.00
50	4:1	7.28	1.05	8.10	2.04	7.21	1.34	8.12	2.85
100	1.5:1	6.93	0.78	7.20	0.39	6.54	0.90	7.38	0.51

Table 5: Effect of Inoculum Size on ap Production IN Fodder Yeast Medium by *Bacillus sphaericus* 2362 and NRC 69.

Approximate CFU/ml medium	*Bacillus sphaericus* strain			
	2362		NRC 69	
	Final pH	APA (OD units)	Final pH	APA (OD units)
$4x10^5$	8.95	6.20	8.89	6.78
$8x10^5$	8.90	6.20	8.85	6.86
$2x10^7$	8.88	5.32	8.80	5.60
$4x10^7$	8.86	5.30	8.84	5.22
$8x10^7$	8.84	4.82	8.76	5.16
$16x10^7$	8.82	4.63	8.76	4.20
$32x10^7$	8.80	4.50	8.72	4.00

Effect of aeration level.

AP produced by *Bs* 2362 and NRC 69 at different ratios of air: medium in shaken and static cultures are shown in Table 4. The highest APA was obtained in shaken cultures when the medium volume occupied 5% of the flask volume for both tested organisms.

Effect of inoculum size.

As shown in Table 5, the decrease of inoculum size has led to the increase of the levels of alkaline protease produced by the two organisms under study.

Properties of crude AP produced by *Bs* 2362 and NRC 69.

Effect of pH.

The results in Figure 2 Showed that the optimum pH value (at which maximal APA was observed) was pH 8.5. The observation suggested that the enzyme is an alkaline protease. Furthermore, about 59% of maximum activity could be recorded at pH 10.0 for both organisms. Dumusois and Priest (1993) found that the optimum pH of alkaline protease produced by *Bacillus sphaericus* BSE 18 was about 10.2.

Reaction temperature.

The enzyme exhibited its maximum activity at incubation temp 55 °C (Figure 3), for both tested organisms followed by fast decline in enzyme activity at higher incubation temperature.

Thermal stability.

The enzyme was not thermo-stable (Figures 4, 5(A), 5(B) & 6(A), 6(B)). Incubation at temperature 60 °C for 40 min resulted in the loss of about 75% of enzyme activity. However the enzyme was stabilized in the presence of 2 mM $CaCl_2$, about 55% of activity remained after incubation for 40 min at the same temperature (60 °C). Dumusois and Priest (1993) found the same effect of $CaCl_2$ in their study on alkaline protease produced by *Bacillus sphaericus* BSE 18.

pH stability.

AP produced by *Bs* 2362 and NRC 69 is pH stable up to pH 9.0 (Figure 7). However, Their activities were decreased about 15% at pH 9.5 and about 50% at pH 10.0 after incubation 24 hours at room temperature.

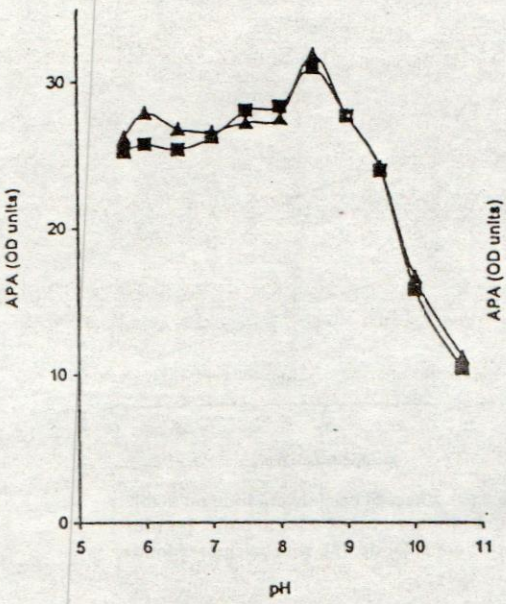

Figure 2. Effect of pH on APA of *Bs* 2362 (■) and NRC 69 (▲)

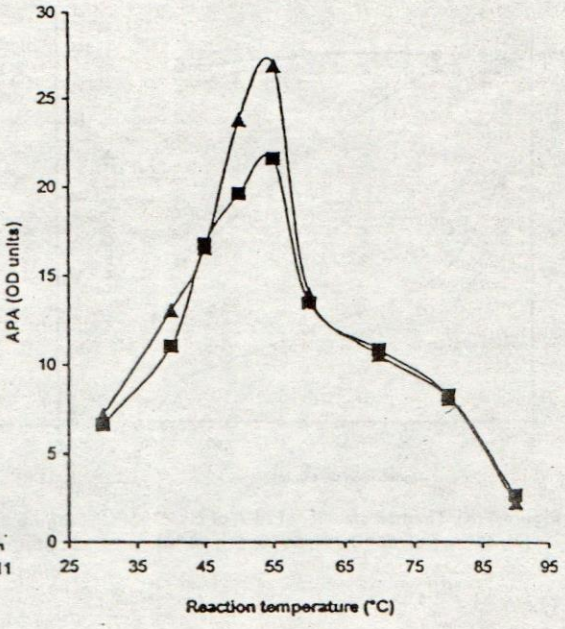

Figure 3. Reaction temperature of APA of *Bs* 2362 (■) and NRC 69 (▲)

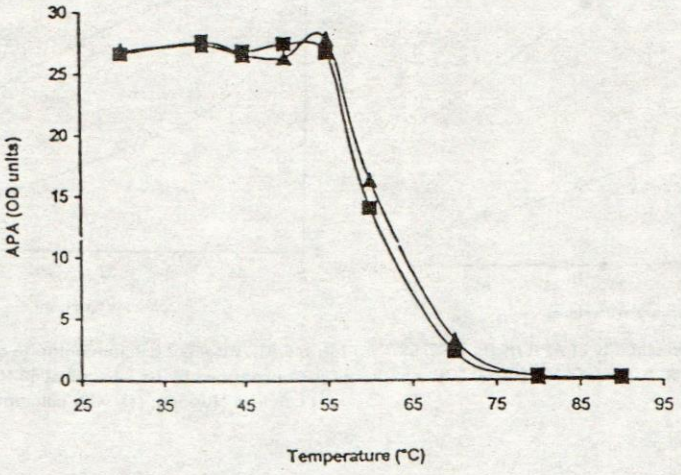

Figure 4. Thermal stability of APA of *Bs* 2362 (■) and NRC 69 (▲)

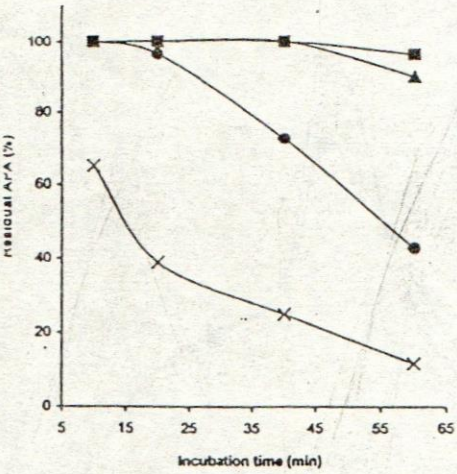

Figure 5 (A). Thermal stability of APA of *Bs* 2362. (■), 45 °C; (▲), 50 °C; (●), 55 °C; (×), 60 °C).

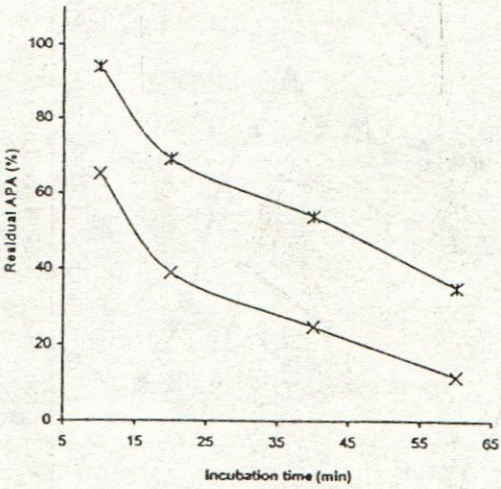

Figure 5 (B). Effect of calcium chloride on stability of APA produced by *Bs* 2362 at 60 °C. (×),with out calcium chloride; (‡), with calcium chloride.

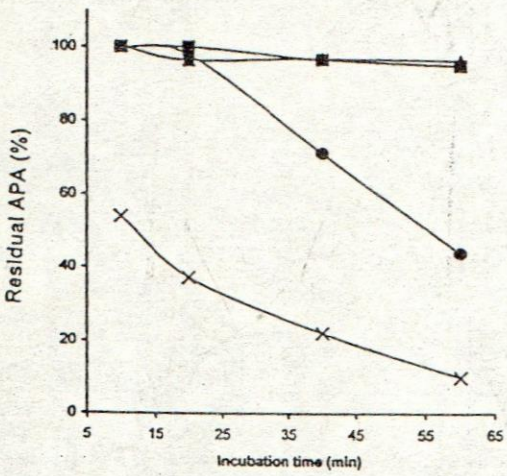

Figure 6 (A). Thermal stability of APA of *Bs* NRC 69. (■),45 °C; (▲), 50 °C; (●), 55 °C; (×), 60 °C).

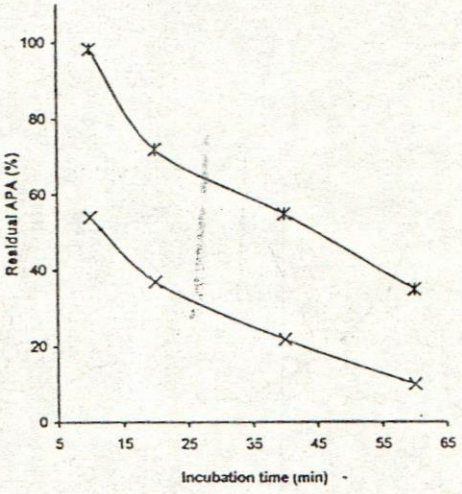

Figure 6 (B). Effect of calcium chloride on stability of APA produced by *Bs* NRC 69 at 60 °C. (×), with out calcium chloride; (‡), with calcium chloride.

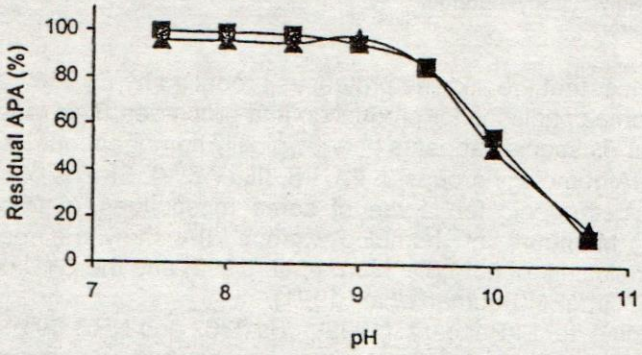

Figure 7. pH stability of APA of *Bs* 2362 (■) and NRC 69 (▲)

Effect of inhibitors.

Table 6 shows that the APA produced by *Bs* 2362 and NRC 69 was inhibited by PMSF, which is a specific inhibitor for serine proteases (about 79 and 77%, respectively). In addition partial inactivation of enzyme activity by EDTA may indicate that some *Bs* strains may also secrete a metal ion dependent neutral protease. Synthesis of both types of enzyme by *Bs* 2362 has previously been noted by Broadwell and Baumann (1986). The production of serine proteases by microorganisms has been reported before. Within the *bacilli* group several members are known to produce serine proteases. Thus subtilisins constitute a family of closely related bacterial serine proteases produced by *Bacillus* species e.g. *B. subtilis*; *B. licheniformis*; *B. amyloliquefaciens* and some alkalophilic *bacilli* (Adler-Nissen, 1993). On the other hand, serine proteases are also formed by entomopathogenic and entomocidal *bacilli* e.g. *B. thuringiensis* (Kunitate *et al.*, 1989) and *B. sphaericus* (Dumusois and Priest, 1993). The presence of these enzymes in entomopathogenic *bacilli* seems to have significant interactions with the insecticidal crystalline endotoxins formed by these bacteria i.e. *B. thuringiensis* (Oppert, 1999) and *B. sphaericus* (Mas *et al.*, 1997). However, the exact role and functions of those enzymes in relation to insecticidal toxins metabolism is yet to be determined.

Table 6: Effect of inhibitors on APA of *Bacillus sphaericus* 2362 and NRC 69.

Inhibitors	Concentration used	Residual APA (%) for *BS*	
		2362	NRC 69
PMSF*	10 (mM)	21	23
EDTA	10 (mM)	32	38
Iodoacetamide	10 (mM)	101	99
Soybean TI**	100 µg/ml	105	107
Cysteine	2 (mM)	103	108

* PMSF, Phenylmethylsulfonyl fluoride.
** TI, trypsin inhibitor.

It is clear that the alkaline proteases produced by *Bs* 2362 and NRC 69 have the same conditions for production and properties. This may regards to the fact that *Bs* species appears phenotypically homogeneous. It contains at least six DNA homology groups (I, IIA, IIB, III, IV & V). Strains of homology group IIA are pathogenic for larvae of some mosquitoes (Carboulec and Priest, 1989). Members of homology group IIA show the mean DNA sequence homologies of 83% (De Muro *et al.*, 1992) and the G+C content of the DNA is 35-37 mol% (Bauman *et al.*, 1991).

According to El-Bendary (1999), *Bs* 2362 and NRC 69 are toxic to *Culex pipiens* and *Anopheles stephensi*, they do belong to homology group IIA and they have both toxin genes (binary toxin (*btx*) and mosquitocidal toxin (*mtx*)). Moreover, they are placed in serotype 5a5b despite being isolated from geographically different locations (Nigeria and Egypt respectively). Therefore, it is not surprizing that these too strains produce more or less identical alkaline protease enzymes.

In conclusion, the demonstration of extracellular serine alkaline protease synthesis by *Bs* should be helpful in the design of suitable culture conditions for the production of the bacterium for use as a biocontrol agent, since unhydrolyzed proteins generally comprise the major medium component. Moreover, it may be relevant to the ability of organism to recycle in the target insect and increasing the persistence of the organism in the natural habitat. Furthermore, future prospective for the possible production and application of this type of alkaline protease in detergent industry and depilation of hides in leather manufacture remains to investigate.

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دراسات على إنتاج إنزيم سيرين بروتييز القلوى بواسطة باسليس سفيريكس

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أظهرت الدراسات الحصرية على ٧٨ سلالة من *باسليس سفيريكس* أن غالبية المزارع تنتج إنزيم البروتييز القلوى خارج الخلايا بمستويات مختلفة عند نموها على بيئة NYSMتحت ظروف النمو المهتزة.

تم اختيار السلالة رقم ٦٩ والمعزولة محلياً مع السلالة العالمية ٢٣٦٢ واللتان تتميزان بالمقاومة البيولوجية العالية للبعوض للدراسة الفسيولوجية لتكوين الإنزيم .

وجد أن إنزيم البروتييز القلوى ينتج بواسطة هاتين السلالتين على البيئات المختلفة وخاصة تلك المكونة من بذور البقوليات المطحونة والبيئات المكونة من النواتج الثانوية الزراعية الصناعية .

تم الحصول على أعلى إنتاجية للإنزيم تحت الدراسة باستخدام خميرة العلف (بتركيز ٤% وزن/حجم) وهى ناتج ثانوي لصناعة الكحول.

وبدراسة تأثير درجة التهوية على تكوين الإنزيم فقد أعطت المزرعة المهتزة تحت ظروف تهوية عالية أعلى إنتاجية للإنزيم باستخدام حجم لاقحة يتراوح من ٤-٨ × ١٠ ^{٦} خلية / مل بعد ٣ أيام من التحضين عند ٣٠ $^{\circ}$ م.

وبدراسة خواص الإنزيم الخام وجد أن حرارة التفاعل المثلى لنشاطه هي ٥٥ $^{\circ}$ درجة مئوية عند أس هيدروجيني ٨,٥ ، فى حين فقد الإنزيم حوالى ٦٠% من نشاطه عند الأس الهيدروجيني ١٠ لكل من السلالتين .

وقد زاد ثبات الإنزيم الحراري عند ٦٠ $^{\circ}$ م فى وجود كلوريد الكالسيوم.

أوضحت دراسات التثبيط أن المثبط فينيل ميثيل سلفونيل فلوريد (PMSF) وهو متخصص فى تثبيط إنزيمات مجموعة السيرين بروتييز يثبط النشاط الإنزيمي بنسبة ٧٢-٧٩ % مع عدم تأثر النشاط الإنزيمي باستخدام أنواع أخرى من المثبطات المختلفة مما يرجح أن الإنزيم ينتمي إلى مجموعة إنزيمات السيرين بروتييز .