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# Isolation and Characterization of *Bacillus thuringiensis* Isolated

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Six isolates of *Bacillus thuringiensis* named BTA1, BTA2, BTA3, BTA4, BTA5 and BTA6 were adopted from ten isolates obtained from 15 different agriculture habitat samples, using special spore-enhancement method. It was found that these isolates produce very large-sized crystalline protein, which showing differences in shape under phase-contrast microscopy. Total genomic DNA of such isolates showed presence of mega plasmids, ringing from 70 to 125 MD, on fractionation by conventional agarose electrophoreses. The insecticidal activity (Bioassay) of these isolates against *Ephestia larvae* showed the isolates BTA1, BTA4, BTA5 and BTA6 have no pathogenic effects, and no hemothytic activity against human and sheep erythrocyte.

عزل وتوصيف سلالات من بكتيريا الباسلس ثرنجنسس

جبار فرحان المعاضيدي كلية مدينة العلم الجامعة، قسم علوم الحياة، بغداد – العراق

تم اعتماد سنة عزلات من بكتيريا BTA3, BTA4, BTA3, BTA1:*Bacillus thuringiensis*, BTA4, BTA5, BTA5، BTA6 من أصل عشرة عزلات عزلت من عينات جمعت من 15 منطقة زراعية، باستخدام طريقة خاصة لتعزيز نمو السبورات. وجد ان هذه العزلات تنتج بلورات بروتينية كبيرة الحجم مختلفة الأشكال عند فحصها باستخدام مكيروسكوب الطور المتباين. تم عزل الدنا المجيني الكلي للعزلات تحت البحث حيث أظهرت النتائج احتوائها على بلازميدات كبيرة يتراوح حجمها بين 70- 125 ميكادالتون عند فحصها بطريقة الترحيل الكهربائي بالأكاروز.

جرى اختبار فعالية هذه العزلات في قتل يرقات حشرة عثة التمور Ephestia حيث وجد ان العزلات BTA5, . و BTA4 BTA6 و BTA4 ليس لديها القدرة على قتل اليرقات ضمن التراكيز المستخدمة كما انها غير قابلة على تحليل كريات الدم الحمراء للانسان والحيوان باستثناء العزلة BTA4 حيث اظهرت فعالية ضعيفة جداً.

Introduction:

*Bacillus thuringiensis* is a grampositive, spore forming bacterium. It seems indigenous to many environments

(1), and isolated worldwide from many habitats including soil, insects and storedproduct dust (2, 3). There are number of subspecies which synthesize insecticidal toxin that vary in insect host specificity. the toxic activity is primarily associated with a crystalline toxin parasporal produced during the sporulation cycle(4). The entomocidal activity is associated with glycoprotein subunit of the crystal that controlled by single or small number of genetic loci and consequently should be relatively easy to genetically engineer compared to more complex multigene systems(5). The genome size of B. thuringiensis strains is 2.4 to 5.7 milion bp (6). Physical maps had been constructed for two strains of B. thuringiensis and compared with that of *B.cereus*, suggests that all of these chromosomes have similar half near replication origin while display great variability in the terminal half (6). A feature common to *B. thuringiensis* subspecies is the presence of plasmids, the number and size (1.4 to 180 MD) vary considerably between strains (7, 8) but independent of serotype and pathotype. Most plasmids are cryptic, the main function assigned to them is the production of entzomocidal toxin. Evidence for a correlation between crystal protein and the presence of a given plasmid was provided by curving experiment leading to loose of large plasmids(9). investigation Farther demonstratated the transfer of such plasmids between *B. thuringiensis* strains through conjugation-like process (10). The aim of this work is to isolate В. *thuringiensis* strains from different sources, characterization of isolates and comparison of their bioactivity (parsporal proteins) against Ephestia larvae.

### Materials and Methods

### Isolation of Bacillus thuringiensis:

According to the procedure of (11), sixty five samples from 15 agricultural sites in Baghdad, Wasit and Al-Qadesyia governorates were collected and used for isolation of B. thuringiensis. Soil sample (2cm below the surface) collected from grain stores and agricultural field. One gram of each sample was suspended in 10 ml sterile distilled water and pasteurized at 80C° for 30 minutes. One ml of suspension transferred to 9ml of LB broth-sodium acetate medium and incubated at 30C° with shaking (150rpm) 4hrs. Small volume (2ml)from for incubated samples were incubated then at 60C° for1hr. Aliquots of serial dilution were prepared and then subcultured on petridishes containing T3 medium for three days at 28c. Colonies with more than 2mm in diameter were picked for subsequent biochemical lecithenase and catalase tests. Colonies that are positive for both tests were selected and then checked by phase contrast for the presence of crystals.

## DNA analysis of BTA isolates:

Each isolates was subcultured in 20ml modified SCGY medium overnight at 30 C° with shaking (200rpm). Grown culture was transferred to one little flask containing 25ml SCGY medium and incubated at the same conditions. The culture was monitored for lag-phase optimization at 0.D.600 every hour, until 0.7-0.9 reading was obtained. The culture was centrifuged for 15 min. at 5000rpm with SW-15 rotor. The pellet was washed

with TE buffer and centerfugeed again, resuspended in 6 ml then TE25S 1mg/ml lysozyme freshly containing prepared (100µL lysozyme solution), and incubated at 37C° for 30min. with gentle agitation, followed by adding 0.18 mg/ml proteinase K (50µL proteins K) to the mixture, then 300µL SDS was added and inqupated for 60min. at 55C°, one ml. of 5M NaCl was added to the mixture and mixed by inversion followed by adding 0.65ml. CTAB. This mixture has been cooled to 37C° and mixed with 5ml. Chloroform/isoamyl alcohol by inversion for 30min. and centrifuged at 10000 rpm in SW-60i rotor for 15min. at -20C°. The supernatant was transferred to a new tube, DNA precipitation was performed by adding 0.6 volume cold isopropanol, mixed by inversion and kept in ice for 30min., precipitated DNA were spooled on to Pasteur pipette, rinsed in 70% ethanol, air dried then dissolved in 1ml. TE buffer at 55C°, and stored at -20C°. Total genomic DNA analysis of *B. thuringiensis* isolates were performed according to Kiesr, 1984(12).

### Bioassay against Ephestia Larvae:

Serial dilutions of collected spores for each isolate were prepared and assayed against *Ephestia larvae* as recommended by WHO scientific group on biological control (13).

Third to fourth instars larvae were tested using series of dilutions of NaBr purified spores and cell suspension of each isolate. Bioassay was conducted at 25C° (room temperature). Topically applying 100µL of each dilution upon each cube of the agar base artificial diet in the plastic feeding cup. One larva was placed in each cup. Four replicates were made per concentration; mortality was recorded after 24 hours.

### **Results and discussion**

Of the 65 collected samples 10 (15%) showed positive test for *Bacillus thuringiensis* (table 1), with an average of 6.5 isolates per sample. The richest inhabitant was grain dust from grain stores, which yield 2.5 isolates per positive sample, followed by vegetable-cultivated soil, garden soil and animal contaminated soil.

The viable count of *B. thuringiensis* was scored after acetate selection. A total of 10 B. thuringiensis isolates were according the selected to colonial morphology differences (table1). These isolates were highly abundance (8.32 ± CFU/g) in soil cultivated with 0.27 vegetable. Whereas, animal contaminated soil has (0.97 ± 2.57 CFU/g). Numerous studies have found that grain dust and soils are suitable environmental habitats for *B. thuringiensis*(14,15), 70<sup>7</sup>/ of soil samples collected from 30 countries were found positive in *B. thuringiensis* (16).

Six isolates out of ten were adopted for farther experiment according to large different shape-crystal exists. All or isolates were grow at almost the same rate. sporulation was completed (beginning of lysis) after 10 hours, parasporal-crystals formed at late stage of sporulation, the PH of the culture medium during exponential growth was decreased from 7 to 6.6 at the beginning of sporulation, and then increased to 8 and remained constant during the subsequent stages of sporulation. Crystalline protein, spores and sporangium of the six isolates were shown in the figures (1.1 - 1.10).

In order to show the difference between the six isolates, total genomic DNA was studied , *B. thuringiensis* possesses a chromosomal DNA sized of 2.4 - 5.7 million bp(17), and numerous plasmid from 1.4 to 180Md., with 2 to17 plasmid in each isolate, depending upon subspecies(7).

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Two procedures have been adapted to isolate total genomic DNA content CTAB (cetyl trimethyl ammonium bromide) and salting out. Total genomic DNA of BTA isolates by CTAB method is show in Fig.2. All BTA isolates have large plasmid, which differ greatly in size, ranged from 70MD (in BTA2 isolate) to 125 MD (in BTA6 isolate). Molecular weight estimated by using photo Capt-Bio profile program. Most of B. thuringiensis plasmids are cryptic; the main function that has been assigned to them is the production of crystalline protein. Evidence for these correlations was provided by curing experiments leading to loss the large plasmid (9). There are many studies on plasmid-curing experiments; showed loss of crystal production was associated with loss of 75MD plasmid in В. thuringiensis subspecies thuringiensis HD-2, 29MD plasmid in subspecies alsti HD-4, and130 MD plasmid in subspecies galleria HD-8. These results indicates that only single, large plasmid (size depend on subspecies) are involved in crystalline protein production(18).

Biological activities of six BTA isolates have been summarized in Table (2). The isolates BTA1, BTA5 and BTA6 have no ability to lyse blood cells (human or sheep).wile the rest isolates show hemolytic activity with different extent, from weak (+) to strong (+++), this phenomenon was studded previously, the hemolytic activity due to various virulence factors that are secreted into the culture medium, many of these factors could be the lyses agent like exotoxin (20), or production phospholipids(21), blood lyses can not be assigned to crystalline toxin only when Cry toxin in soluble phase. Also *B. thuringiensis* isolates can produce Cyt toxin, which has the ability to lyses erythrocytes under solublization conditions of such toxin(22).

The second activity of BTA isolates assayed against insects larvae, was results revealed showed the four isolates BTA1, BTA4, BTA5 and BTA6 have no pathogenic effect against Ephestia larvae (Table2), it was reported previously that the noninsecticidal B. thuringiensis widely distributed in nature than the insecticidal once(3,14). Also the crystalline protein of noninsecticidal strains and producing has another application as anti-tumor. It was reported that the protein associated with parasporal inclusions named "parasporin" has anti-tumor activity against human leukemic cell(24,25). Therefore activated parasporal proteins produced by these isolates will be examined for cytopathic effect on CLL and HeP2 cells in next studies.

# Table 1: Abundance of local *Bacillus thuringiensis* isolates obtained from five habitats

Habitat	No. of samples	No. of B. thuringiensis isolates	Average viable count of B. thuringiensis (CFU×107/g)
Grain dust	5	2	4.65 ± 1.32.
Palm cultivated soil	15	1	5.61 ± .98.
Vegetable cultivated soil	15	3	8.32 ± 0.27
Animal contaminated soil	10	1	0.97 ± 2.57
Garden soil	20	3	7.53 ± 1.03
Total	65	10	

# Table 2: Bioactivities of Bacillus thuringiensis isolates on blood erythrocyte and Ephestia larvae

B. thuringiensis isolates	a Hemolytic activity Sheep erythrocyte Human erythrocyte		b Activity against Ephestia larvae
BTA1	-	-	-
BTA2	++	++	+
BTA3	+	+++	+
BTA4	+	+	-
BTA5	-	-	-
BTA6	-	-	-

- a- Hemolytic activity carried out on blood agar base plates.
- b- Bioassay carried out against lepidopteran third instar larvae of *Ephestia*.

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# Approximation Solution of Fredholm Integral Equation Using Adomian Decomposition Method

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### Abstract

In this paper, Adomian Decomposition method has been used to find the approximation solution for the linear Fredholm integral equation of the second kind. In this method the solution of a functional equation is considered as the sum of an infinite series usually converging to the solution, and the Adomian decomposition method is also used to solve linear integral equation. Finally, numerical example are prepared to illustrate these considerations.

# حل تقريبي لمعادلة فريدهوم التكاملية باستخدام طريقة ادموند الانحلالية

أمنة قاسم حسين ماجستير في الرياضيات التطبيقية كلية الهندسة - الجامعة المستنصرية

الخلاصة

في هذا البحث استخدمت طريقة الانحلال لإيجاد حل تقريبي لمعادلة فريدهوم التكاملية من النوع الثاني. والحل في هذه الطريقة يكون دالة تمثل مجموع متسلسلة غير محددة تتقارب إلى الحل. كما أن طريقة أدموند الانحلالية هي طريقة لحل المعادلات التكاملية الخطية وغير الخطية. والمثال المطبق يوضح هذه الطريقة.

# 1. Introduction

The integral equation is generally defined as an equation which involves the integral of an unknown function u(x) appears under the integral sign.

Therefore the general form of integral equation is given by the following form, [1]:

$$a(x)u(x) - \int_{\Omega} k(x,t)u(t)dt = f(x) \qquad \dots (1)$$

where *a*, *f* and *k* are known functions; k(x,t) is called the kernel of the integral equation, *u* is the unknown function to be determined and  $\Omega$  be a measurable set in a measurable space E.

We can distinguish between two types of integral equations which are,[2]:

**1.** Integral equation of the first kind when a(x) = 0 in equation (1):

$$f(x) = \int_{\Omega} k(x,t)u(t)dt \qquad \dots (2)$$

**2.** Integral equation of the second kind when  $a(x) \neq 0$ , then equation (1) can be written as:

$$u(x) = f(x) + \int_{\Omega} k(x,t)u(t)dt \qquad \dots (3)$$

Now integral equations can be classified into different kinds according to the limits of integration:

**1.** If the limits of equation (1) are constants then this equation is called Fredholm integral equation. In this case, Fredholm integral equations of the first and second kinds will respectively have the following expressions, [3]:

$$f(x) = \int_{a}^{b} k(x,t)u(t)dt \qquad \dots (4)$$

$$u(x) = f(x) + \int_{a}^{b} k(x,t)u(t)dt$$
 ...(5)

where a, b are constants.

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**2.** If the upper limit of the integration in equation (1) is a variable then equation (1) is called Volterra integral equation. They are divided into two groups referred to as the first and second kinds.

Volterra integral equation of the first kind is,[3]:-

$$f(x) = \int_{a}^{x} k(x,t)u(t)dt \qquad \dots (6)$$

and Volterra integral equation of the second kind is:-

$$u(x) = f(x) + \int_{a}^{x} k(x,t)u(t)dt$$
 ...(7)

where a is constant and x is variable.

The integral equation (1) is said to be singular if the range of integration is infinite  $0 < x < \infty$ or  $-\infty < x < \infty$ , or if the kernel k(x, t) is unbounded.

If the kernel k(x, t) in equation (1) depends only on the difference x-t, such a kernel is called a difference kernel and the equation:

$$a(x)u(x) - \int_{\Omega} k(x,t)u(t)dt = f(x) \qquad \dots (8)$$

is called integral equation of convolution type.

# 2. The Decomposition method Applied to Fredholm integral Equation

In this subsection a Decomposition method to find the approximation solution for Fredholm integral equation is discussed [5],[6],[7].

Let us reconsider the Fredholm integral equation of the second kind.

$$f(t) = g(t) + \int_{a}^{b} k(t,s)f(s)ds, \qquad ...(9)$$

where g and the kernel k are known functions and f is the unknown function to be determined.

From equation (9), we obtain canonical form of Adomian's equation by writing

$$f(t) = g(t) + N(t)$$
 ...(10)

where

$$N(t) = \int_{a}^{b} k(t,s)f(s)ds \qquad \dots (11)$$

To solve by Adomian's method, let

 $f(t) = \sum_{m=0}^{\infty} f_m(t)$ , and  $N(t) = \sum_{m=0}^{\infty} A_m$  where  $A_m$ , m = 0,1,..., are polynomials depending on  $f_0, f_1,..., f_m$  and they are called Adomian polynomials. Hence, (10) can be rewritten as:

$$\sum_{m=0}^{\infty} f_m(t) = g(t) + \sum_{m=0}^{\infty} A_m(f_0, f_1, \dots, f_m).$$
 (12)

From (11) we define:

$$\begin{cases} f_0(t) = g(t), \\ f_{m+1}(t) = A_m(f_0, f_1, \dots, f_m), \\ m = 0, 1, 2, \dots \end{cases}$$
(13)

In practice, all terms of the series  $f(t) = \sum_{m=0}^{\infty} f_m(t)$  can not be determined and so we use an

approximation of the solution by the following truncated series:

$$\varphi_k(t) = \sum_{m=0}^{k-1} f_m(t), \quad \text{with } \lim_{k \to \infty} \varphi_k(t) = f(t). \quad \dots (14)$$

To determine Adomian polynomials, we consider the expansions:

$$f_{\lambda}(t) = \sum_{m=0}^{\infty} \lambda^m f_m(t), \qquad \dots (15)$$

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$$N_{\lambda}(f) = \sum_{m=0}^{\infty} \lambda^m A_m, \qquad \dots (16)$$

Where,  $\lambda$  is a parameter introduced for convenience. From (16) we obtain:

$$A_m = \frac{1}{m!} \left[ \frac{d^m}{d\lambda^m} N_\lambda(f) \right]_{\lambda=0}, \qquad \dots (17)$$

and from (11), (15) and (17) we have:

$$A_m(f_0, f_1, \dots, f_m) = \int_a^b v(s, t) \left[ \frac{1}{m!} \frac{d^m}{d\lambda^m} \sum_{l=0}^\infty \lambda^l f_l \right]_{\lambda=0} ds$$
$$= \int_a^b v(s, t) f_m ds. \qquad \dots (18)$$

So, (13) for the solution of linear Fredholm integral equation will be as follow:

$$\begin{cases} f_0(t) = g(t), \\ f_{m+1}(t) = \int_a^b v(s,t) f_m(t) ds, \\ m = 0,1,2,... \end{cases}$$
 ...(19)

Considering (13), we obtain:

$$\varphi_k(t) = g(t) + \int_a^b k(t,s) f_m(s) ds, \qquad m = 0,1,2,...$$
 ...(20)

In fact (15) is exactly the same as the well known successive approximations method for solving of linear Fredholm integral equation defining as, [4]:

$$f_{m+1}(t) = g(t) + \int_{a}^{b} k(t,s) f_m(s) ds,$$
 m = 0,1,2,... ...(21)

The initial approximations for the successive approximation method is usually zero function. In the other words, if the initial approximation in this method is selected that is  $f_0(t) = g(t)$ , then the Adomian decomposition method and the successive approximation method are exactly the same

The following algorithm summarizes the steps for finding the approximation solution for the second kind of Fredholm integral equation.

# 3. Algorithm (ADFI)

**Input:** (g(t),k(t,s),f(s),a,x);

Output: series solution of given equation

Step1:

Put 
$$f_0(t) = g(t)$$

Step2:

Compute 
$$f_{m+1}(t) = \int_{a}^{b} k(x,t) f_m(s) ds$$
  $m = 0,1,...$ 

Step3:

Find the solution 
$$\varphi_k(t) = \sum_{m=0}^{k-1} f_m(t), \qquad m = 0,1,...$$

End

## Example(1):

Consider the following Fredholm integral equation of the second kind with the exact solution f(t) = t.

$$f(t) = \frac{2x}{3} + \int_{0}^{1} xtf(t)dt.$$

where compare the Fredholm integral equation of the second kind, we total

$$g(t) = \frac{2t}{3} \qquad , \quad k(x,t) = xt$$

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To derive the solution by using the decomposition method, we can use the following Adomian scheme:

$$f_0(t) = \frac{2t}{3},$$

And

$$f_{m+1}(t) = \int_{0}^{1} x t f_m(t) dt,$$
 m = 0,1,2,...

For the first iteration, we have:

$$f_1(t) = \int_0^1 x t f_0(t) dt,$$

Then  $f_1(t) = \frac{2t}{9}$ 

Considering (14), the approximated solution with two terms are:

$$\varphi_2(t) = f_0(t) + f_1(t)$$

$$\varphi_2(t) = \frac{2t}{3} + \frac{2t}{9}$$

Next term is:

$$f_2(t) = \int_0^1 x t f_1(t) dt$$

$$f_2(t) = \frac{2t}{27}$$

Solution with three terms are:

$$\varphi_3(t) = f_0(t) + f_1(t) + f_2(t),$$
  
2t 2t 2t 2t

$$\varphi_3(t) = \frac{2l}{3} + \frac{2l}{9} + \frac{2l}{27},$$

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In the same way, the component  $\varphi_k(t)$  can be calculated for k = 3, 4, ... The solution with twelve terms are given as:

$$\begin{split} \varphi_{12}(t) &= f_0(t) + f_1(t) + f_2(t) + \ldots + f_{11}(t). \\ \varphi_{12}(t) &= \frac{2t}{3} + \frac{2t}{9} + \frac{2t}{27} + \frac{2t}{81} + \frac{2t}{729} + \frac{2t}{6561} + \frac{2t}{19683} + \frac{2t}{59049} + \frac{2t}{177147} + \frac{2t}{731441} \\ &+ \frac{2t}{2194323} + \frac{2t}{6582969} + \frac{2t}{59246721} \end{split}$$

Approximated solution for some values of t by using Decomposition method and exact values f(t) = t of Example(1), depending on least square error (L.S.E) are presented in Table(1).

### 4. Conclusion

This paper presents the use of the Adomian decomposition method, for the Fredholm integral equation. As it can be seen, the Adomian decomposition method for Fredholm integral equation is equivalent to successive approximation method. Although, the Adomian decomposition method is a very powerful device for solving the integral equation. From solving a numerical example the following points have been identified:

1- this method can be used to solve the second kind of linear Fredholm integral equation.

It is clear that using the decomposition method basis function to approximate when the m order is increases the error will be decreases.

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t	f(t)	$\varphi_{12}(t)$
	(Exact)	(Approximation)
0	0	0
0.1	0.100	0.0999081
0.2	0.200	0.199999
0.3	0.300	0.299999
0.4	0.400	0.399999
0.5	0.500	0.499999
0.6	0.600	0.599999
0.7	0.700	0.699999
0.8	0.800	0.799999
0.9	0.900	0.899999
1	1	1.0082274
L.S.E		0.000001

Table (1) The results of Example(1) using (ADFI) algorithm.