J. Agric. Chem. and Biotechn., Mansoura Univ. Vol. 9(8):169 -173 , 2018

Morphological and Pcr Differentiation between some Phages from *Bacillus thuringiensis* Strain C18

Osman, Y. A.; A. A. El-Morsi and Amany E. Atiya
Botany Department, Faculty of Science, Mansoura University, Egypt

This article was CHECKED against plagiarism using TurnitIn software

ABSTRACT

Bacillus thuringiensis (Bt)-based pesticides hold the biggest share of the biopesticides market. This industry suffers huge economic losses due to the activation of their lysogenic phages during fermentation process. The locally isolated wide-spectrum BtaC18 is currently used for commercial production and found to harbors many of these integrated prophages. Many phages were isolated from a laboratory-grown BtaC18; from amongst them three belonged to the order *Caudovirales* were characterized. Traditional and morphological examination found that one of the three viruses has tail: ACTP-01 and two are non-tailed phages: ACTP-02 and ACTP-03. The ACTP-01 phage showed an isometric head (92.6 nm) with long non-contractile, flexible tail (321.33 x 12.5 nm). While, the ACTP-02 phage has a hexagonal head (50.82 nm) and the ACTP-03 phage possesses an icosahedral head (61.3 nm). Data suggested that ACTP-01 phage belongs to *Siphoviridae* family, while ACTP-02 and ACTP-03 belonged to the *Tectiviridae* family. Random Amplified Polymorphic DNA polymerase chain reaction (RAPD-PCR) showed a strong differentiating power between the isolated phages. In conclusion, the numerous phages hosted by this bacterium complicate the derivatization of phage resistant BtaC18 strain.

Keywords: Bacteriophages, *Bacillus thuringiensis*, *Bacillus thuringiensis* subsp. *aegypti* strain C18, RAPD-PCR.

INTRODUCTION

The abundance and diversity of *Bacillus thuringiensis* (Bt) strains (more than 50,000) with different insecticidal activities are documented in the numbers of isolated being recorded in the literatures and patents (Martin and Travers, 1989; Sadder *et al.*, 2006; Xavier *et al.*, 2007; Raymond, 2010; Renganathan *et al.*, 2011 and Argôlo-Filho and Loguercio, 2013). The parasporal insecticidal crystal proteins (ICP) distinguish the Bt from the closely related *Bacillus anthracis* and *Bacillus subtilis*. The ICPs produced by the isolates of Bt are being developed as an ecofriendly biopesticide (Glare and Callaghan, 2000; Aronson, 2002; Jensen, 2003; Devi *et al*., 2005; Roh *et al*., 2007; Sanahuja *et al.*, 2011; Valicente *et al.*, 2010 and Yang and Wang, 1998).

However, the industrial fermentation process is frequently interrupted by either by exogenous phage infection or activation of its own lysogenic phages. Ackermann *et al*., (1994) had reported that 83 % of the examined Bt subspecies carries lysogenic phages in their chromosomes and these integrated prophages had led to a frequent failures in about 15–30 % (may reach 50–80 %) of fermentation production process (Yu, 1990 and Liao *et al.*, 2007). Many of the phages isolated from the different industrial strains of Bt were characterized at different levels and have been classified according to the standard morphological and molecular techniques (Yuan *et al.*, 2012a and El-Didamony, 2014).

The current investigation was carried out for the aim of isolation and identification of the Bt phages represent a step forward solving this industrial problem.

MATERIALS AND METHODS

Bacterial growth media and conditions

Bacillus thuringensis subsp. *aegypti* strain C18 (BtaC18) used for bacteriophage isolation was grown on Luria Bertani (LB) broth (10 g tryptone, 5 g yeast extract and 10 g sodium chloride per liter) at 30^{o}C in a shaker incubator (250 rpm) for 18-24 hr (Sauder *et al.*, 2016). For sporulation, the bacterium was culture in T_3 broth medium contain (5 g Peptone, 1.5 g yeast extract, 0.005 g $MnCl_2$, 50 mM sodium phosphate buffer and was adjusted to pH 6.8).incubated shaking at 30^{o}C for 72-96 hr (Osman and Madkour, 1999).

Preparation of phages-free Bt strain

To inactivate any prophage inside the BtaC18, endospore suspension was boiled at for 30 min, and then samples were streaked onto LB agar plates and examined after 24 hr for lysis (Osman and Madkour, 1999).

Phage propagation and isolation

Prophages were isolated from the wild-type BtaC18 as described by Lu *et al.*, (2003) and Carey-Smith *et al.*, *(*2006). A 5 ml LB broth culture was inoculated with an actively growing overnight culture and incubated at 30^{o}C in shaking incubator at 250 rpm. Bacterial growth was visually monitored (turbidity) every 2hrs; where most of BtaC18 cells were lysed after 8 hrs. Successive culture transfer increased the activity of phages and efficiency of bacterial cell lysis (bacterial lysate).

Chloroform (5%) was used to purify virus from the bacterial lysate. The mixture was gently shacked for few minutes, centrifuged at 5,000 rpm for 15 min and the supernatant was transfer to a new sterile tube. After phase separation, the supernatant was filtered through 0.22 μl pore size filter and the phage filtrate was stored at 4°C for subsequent experiments (Bonilla *et al.*, 2016).

Phage precipitation

Phage filtrate from 5 ml culture was mixed with 3.6 ml of polyethylene glycol (PEG 8000) and incubated in ice for 2 hrs and centrifuged at 6,500 rpm for 30 min. The pellet was resuspended in 1 ml sodium-magnesium buffer (SM buffer: 50 mM Tris-HCl, pH 7.5; 100 mM NaCl; 8 mM MgSO4), and then kept stirring at 4^{o}C for overnight. Two stages removal of PEG was done by centrifugation at 6,500 rpm for 30 min at 4 ^{o}C and the supernatant was clean centrifuge again at 16,000 rpm for 30 min. The precipitated pellet was then resuspended in 250 μl SM buffer and store at 4 ^{o}C for further studies (Gill and Abedon, 2003; Colombet *et al.*, 2007;Carlson, 2005).

Phage titration

The concentration of obtained purified phage was measured as an absorbance at two wave lengths 269 nm and 320 nm according to Wiseman and Day (1987):

$$\text{Virions / ml} = \frac{(\text{A269 nm} - \text{A320 nm}) \times 6 \times 10^{16}}{\text{Number of bases /phage*} \times \text{Volume used (ml)}} \times 100 \times \text{dilution}$$

*** Number of bases/ phage= 15,000 (Strömsten *et al.*, 2003 and Verheust *et al.*, 2005).**

Phage susceptibility tests (Detection of phage activity)

Spot (Drop) test was performed as described by Carlson (2005). A 50 µl drop of virus solution was spotted onto a BtaC18 lawn (agar plate) and incubated overnight at 30°C.

Electron microscopy of phages

Morphological feature assessments of the purified phages were examined by transmission electron microscope (TEM). Negative staining purified phage by adding a drop of phage suspension on a copper carbon coated grid (400 meshes) for 3 min and the grid was stained with 2% (w/v) uranyl-acetate as described by Roh *et al*., (2013). Excess stain was removed by washing with d.H_2O, left to dry for 5 min and then examined with TEM (JEOL JEM-2100, Japan) at the Electron Microscope Unit, Faculty of Agriculture, Mansoura University, Mansoura, Egypt.

DNA extraction from phages

Phages were precipitation using zinc chloride, with minor modifications (Santos, 1991 and Verheust *et al.*, 2003). To each 1 ml of concentrated phage filtrate a 20 µl of a filter-sterilized $ZnCl_2$ (2 M) was added and incubated for 5 min at 37°C. The mixture was centrifuged for 1 min at 10,000 rpm, the supernatant removed by aspiration and the pellet was resuspended in 500 µl TES buffer (100 mM Tris- HCl pH 8; 100 mM EDTA and 0.3% SDS). The virus solution was incubated at 60°C for 15 min and 60 µl of 3 M potassium acetate solution (pH 5.2) was added, mixed thoroughly and left on ice for 15 min. The white dense precipitate was collected by centrifugation at 12,000 rpm for 1 min and collect supernatant to a new tube. 0.6 volume of isopropanol was added, mixed and left on ice for 5 min. precipitation of the DNA pellet was carried out by Centrifugation at 13,000 rpm for 15 min, DNA pellet was washed with 70% ethanol, dried on air at room temperature and resuspended in 20-100 µl TE (10 mM Tris and 1 mM EDTA; pH 8.0). or distilled sterilized water. The DNA left overnight to relax then examined by gel agarose electrophoresis and determine the concentration and purity of DNA spectrophotometrically as recommended by (Bron, 1990) to estimate the volume needed for RAPD-PCR.

RAPD-PCR amplification

Random amplification of phages DNA was carried out by using four Operon oligonucleotide primers available at our laboratory: OPA-11, OPB-11, OPD-03 and OPD-07 (Rychlik *et al.*, 1990). Nucleotide sequences of the primers and their annealing temperatures are listed in table 1.

Table 1. Nucleotide sequences of the Operon random primers

Primers	Nucleotide Sequence (5´ to 3´)	Annealing temp.
OPA-11	CAA TCG CCG T	35ºC
OPB-11	GTA GAC CCG T	35ºC
OPD-03	GTC GCC GTC A	35ºC
OPD-07	TTG GCA CGG G	37ºC

The 25 µl reaction mixture contained 15.5 µl water (ddH_2O), 5 µl Taq buffer, (5 Mm dNTPs, 15 Mm $MgCl_2$ stabilizers and enhancers), 2 µl of each selected primer (20 µM), 2 µl DNA template was added and 0.5 µl Taq polymerase. The reaction mixtures were overlaid with a drop of light mineral oil per sample. Amplification was carried out for 35 cycles in a ProFlexPCR System with an initial DNA denaturation step at 94 ºC for 5 min. Each cycle consisted of denaturation step at 94 for 1 min, annealing as shown in table 1 for 45 sec and extension at 72 ºC for 3 min, at the end of the 35 cycles a final extension was done at 72 ºC for 7 min. PCR products were analyzed by agarose gel DNA electrophoresis and documented using Nippon Genetics Imaging System. Gel extraction of PCR product by Zymoclean Gel DNA Recovery Kit for further studies (Rychlik *et al.*, 1990).

RESULTS

Phages were isolated from *B. thuringensis* subsp. *aegypti* strain C18 by induction of self-propagated prophages. A phage-free-Bt strain was used to test the phage sensitivity assay, by boiling the BtaC18 strain (cultured on T3 broth medium at 30°C for 72 h) to eliminate potential prophages. Filtrate of BtaC18 was incubated with phage free exponential bacterial culture and incubated at 30°C and after 24 hrs clarity of media obtained and compared with control without filtrate (figure 1a).

Lytic clear area was formed in the lawn of phage free bacterial culture when a drop of filtrate was added on BtaC18 strain; this indicates presence of phages in the filtrate (figure 1b).

The culture filtrate of BtaC18 revealed the presence of two different morphotypes of phages when examined with transmission electron microscope named: ACTP-01, ACTP-02 and ACTP-03 shown in figure (2a-c). -01 has isometric head of 92.6 nm diameter and long non-contractile flexible tail of 12.5 nm wide and 321.33 nm length. ACTP-02 showed hexagonal head of 50.82 nm diameter and without tail. ACTP-03 has icosahedral head of 61.3 nm. The general morphology of the three phages is summarized in table 2.

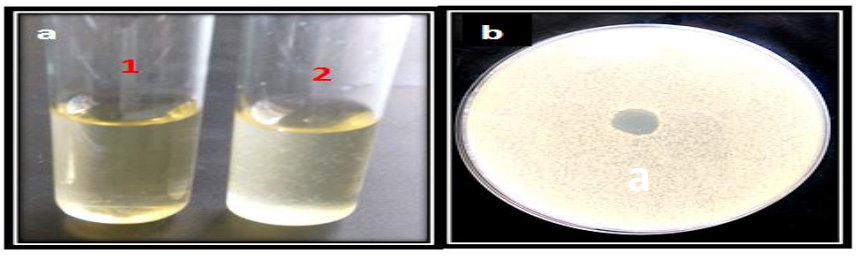

Fig .1. Phage susceptibility tests note. Panel a-1) Complete lysis of wild-type BtaC18 strain due to phages infection. 2) Control turbid bacterial culture without phage infection. Panel b) BtaC18 wild-type strain showing clear zone in the position of spotted phage filtrate.

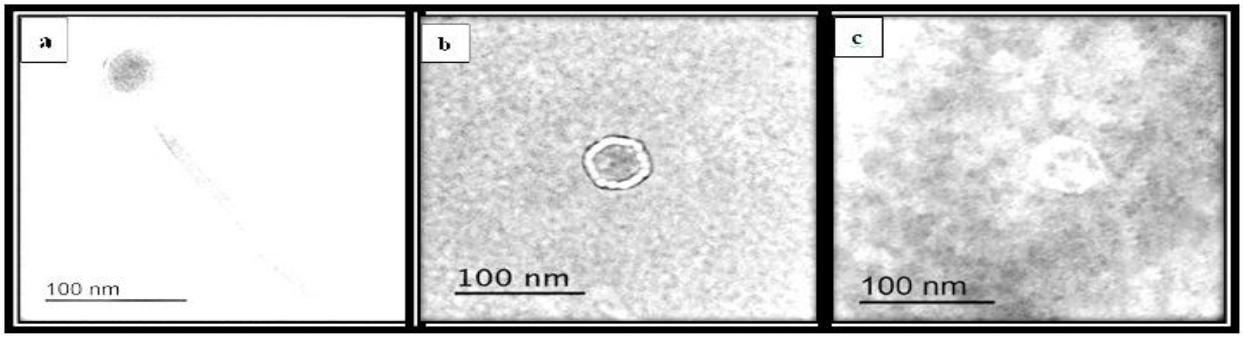

Fig. 2 a-c. Transmission electron micrographs of the three different morphotypes of the viruses isolated from BtaC18. Panels: (a) ACTP-01 phage, (b) ACTP-02 and (c) ACTP-03.

Table 2. Morphological features of the three phage particles isolated from BtaC18

phage	Family	Head		Tail		
		Shape	Diameter (nm)	type	Width (nm)	Length (nm)
ACTP-01	*Siphoviridae*	isometric	63	Long non- contractile, flexible	12.5	217.39
ACTP-02	*Tectiviridae*	hexagonal	50.82	-	-	-
ACTP-03	*Tectiviridae*	Icosahedral	46.15	-	-	-

RAPD-PCR amplification

The phages isolated from the wild-type BtaC18 bacterium were characterized at molecular level by RAPD-PCR amplification. The four RAPD primers resulted in four distinctive DNA bands molecular weights ranged from 440, 933 bp, 1230 bp, and 1548 bp for primers OPB-11, OPA-11, OPD-03 and OPD-07, respectively (Fig. 3).

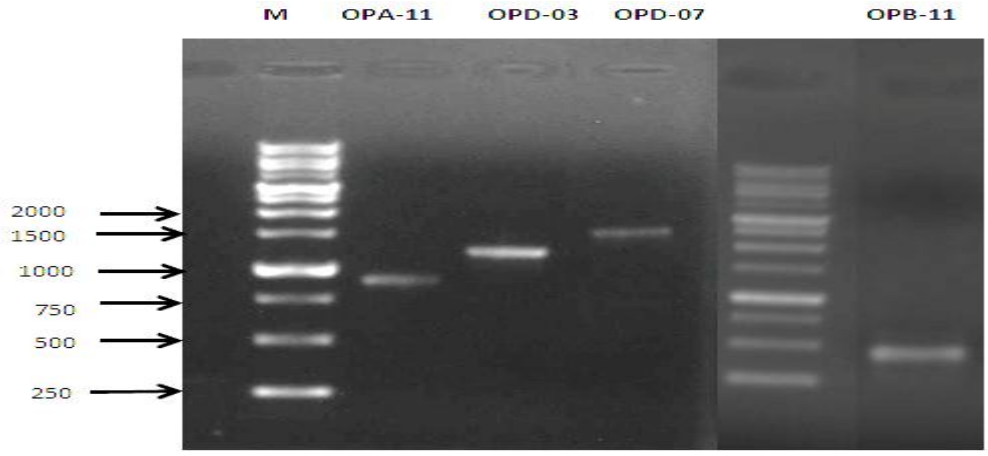

Fig. 3. RAPD-PCR amplified DNA fragments using phages genomes as template and four primers OPA-11, OPB-11, OPD-03, OPD-07.

DISCUSSION

In this study, the number of phages that has been isolated from *Bacillus thuringensis* (Bt) subsp. *aegypti* strain C18 (BtaC18) revealed the necessity for developing of an industrial strain to continue as a source for manufacturing the bioinsecticide. Nevertheless, it is found that infection and lysis of this particular strain by bacteriophages is the main problem limiting their commercial production (Al-Heeti and Osman, 2008 and Osman *et al.*, 2013). Electron microscopy examination of the isolated prophages represented not only the different phages parasitized on BtaC18 but also their morphological diversity. Bradley (1967) and Ackermann (2007) published a dependable system for phages classification based on the morphology of phage. Depending on this classification system the three isolated phages in this study belongs to order *Caudovirales.* However one of them (ACTP-01) is tailed and the others (ACTP-02 and ACTP-03) have no tail. Therefore, they further classified as members of families: *Siphoviridae* and *Tectivridae*. The phage ACTP-01 of isometric head and Long non- contractile, flexible tail considered as member of the family *Siphoviridae* or Barley's group B1. This phage is similar to phage MZTP02 of Liao *et al.* (2008) isolated from Fermentative strain MZ1 (Bt subsp. *kurstaki*; BtCS33 phage isolated from Bt subsp. *kurstaki* strain CS33 (Yuan *et al.*, 2012b) and ALE(H3a3c) phage (Roh *et al.*, 2013). While ACTP-02 and ACTP-03 have hexagonal and icosahedral head respectively and no tail so it considered to be in the family *tectivridae* or Bardley group D4. This is in agreement with Bam35 phage (Ravantti *et al.*, 2003).

RAPD-PCR of extracted and purified phage DNA help to characterize the purified phages. RAPD-PCR also used to assess the genetic diversity of phages infecting *Pseudomonas aeruginosa* (Li *et al.*, 2010) and *Escherichia*

coli (Dini and de Urraza, 2010) and analysis of DNA of *B. subtilis* phage isolate (Marie, 2013).

CONCLUSION

Three distinctive prophages were successfully isolated from the Egyptian Bt isolate (BtaC18) by induction. The transmission electron microscopy of the partially purified phages show that one of them belongs to *Siphoviridae* and the two others belong to *Tectiviridae*. Moreover, RAPD-PCR showed the genome differences among the three isolated phages.

REFERENCES

Ackermann, H. ; Azizbekyan, R.; Emadi Konjin, H.; Lecadet, M.; Seldin, L. and Yu, M. (1994). New *Bacillus* bacteriophage species. Archives of virology, 135(3): 333-344.

Ackermann, H. W. (2007). 5500 phages examined in the electron microscope. Archives of virology, 152: 227-243.

Al-Heeti, A. and Osman,Y. A. (2008). Biological Pest Control: Advantages and Challenges in the Arab World. Journal of Arab Organization for Agricultural Investment And Development, 20: 31-42.

Argôlo-Filho, R. C. and Loguercio, L. L. (2013). *Bacillus thuringiensis* is an environmental pathogen and host-specificity has developed as an adaptation to human-generated ecological niches. Insects, 5(1): 62–91.

Aronson, A. (2002). Sporulation and delta-endotoxin synthesis by *Bacillus thuringiensis.* Cellular and Molecular Life Sciences, 59: 417–425.

Bonilla, N.; Rojas, M.; Cruz, G.; Hung, S.; Rohwer, F. and Barr, J. (2016). Phage on tap–a quick and efficient protocol for the preparation of bacteriophage laboratory stocks. PeerJ, 4: e2261.

Bradley, D. E. (1967). Ultrastructure of bacteriophage and bacteriocins. Bacteriological reviews, 31(4): 230.

Bron, S. (1990). Plasmids. In: Molecular Biology methods for Bacillus. C.R. Harwood and S.M. Cutting (ed.), John Wiley and sons. NY.

Carey-Smith, G.; Billington, C.; Cornelius, A.; Hudson, J. and Heinemann, J. (2006). Isolation and characterization of bacteriophages infecting Salmonella spp. FEMS microbiology letters, 258(2): 182-186.

Carlson, K. (2005). Appendix: Working with Bacteriophages: Common techniques and methodological approaches. In: Bacteriophages: biology and applications. Kutter, E. and Sulakvelidze, A. (Eds.). CRC PressBoca Raton London New York Washington, D.C.1st Ed. 428 – 458.

Colombet, J.; Robin, A.; Lavie, L.; Bettarel, Y.; Cauchie, H.; Sime-Ngando, T. (2007). Virioplankton 'pegylation': use of PEG (polyethylene glycol) to concentrate and purify viruses in pelagic ecosystems. Journal of microbiological methods, 71(3): 212-219.

Devi, P.; Ravinder, T. and Jaidev, C. (2005). Cost-effective production of *Bacillus thuringiensis* by solid-state fermentation. The Journal of Invertebrate Pathology, 88(2): 163–168.

El-Didamony, G. (2014). Occurrence of *Bacillus thuringiensis* and their phages in Yemen soil. VirusDisease, 25(1): 107-113.

Gill, J. and Abedon, S. (2003). Bacteriophage ecology and plants. APSnet Feature. 1-17.

Glare, T. and O'callaghan, M. (2000). In: *Bacillus thuringiensis*: biology, ecology and safety. John Wiley and Sons Ltd., Chichester, UK, 350.

Jensen, G.; Hansen, B.; Eilenberg, J. and Mahillon, J. (2003). The hidden lifestyles of *Bacillus cereus* and relatives. Environmental Microbiology, 5(8): 631–640.

Liao, W.; Sun, F.; Song, S.; Shi, W. and Pang, Y. (2007). Biology of two lysogenic phages from *Bacillus thuringiensis* MZ1. Wei sheng wu xue bao= Acta microbiologica Sinica, 47(1): 92-97.

Lu, Z.; Breidt, F.; Fleming, H.; Altermann, E. and Klaenhammer, T. (2003). Isolation and characterization of a *Lactobacillus plantarum* bacteriophage, ΦJL-1, from a cucumber fermentation. International journal of food microbiology, 84(2): 225-235.

Martin, P. A. and Travers, R. S. (1989). Worldwide abundance and distribution of *Bacillus thuringiensis* isolates. Applied and Environmental Microbiology, 55(10): 2437-2442.

Osman, Y. A.; Mohamedin, A. H. and Elkafrawy, R .E. (2013). Molecular characterization of *Bacillus thuringiensis* mutant resistant to bacteriophage. Egyptian Journal of Biological Pest Control, 23: 110-123.

Osman, Y.A. and Madkour, M.A. (1999). Discovery and development of a unique new Bacillus thuringiensis subsp. *Aegypti* to control agricultural pests. Arab Agricultural Research Journal, 4: 1-15.

Raymond, B.; Johnston, P.; Nielsen-LeRoux, C.; Lereclus, D. and Crickmore, N. (2010). *Bacillus thuringiensis*: an impotent pathogen. Trends in Microbiology, 18(5): 189–194.

Renganathan, K.; Rathinam, X.; Danial, M. and Subramaniam, S. (2011). Quick isolation and characterization of novel *Bacillus thuringiensis* strains from mosquito breeding sites in Malaysia. Emirates journal of food and agriculture, 23(1): 17-26.

Roh, J. Y.; Choi, J. Y.; Li, M. S.; Jin, B. R. and Je, Y. H. (2007). *Bacillus thuringiensis* as a specific, safe, and effective tool for insect pest control. Journal of Microbiology and Biotechnology, 17(4): 547–559.

Roh, J. Y.; Park, J. B.; Liu, Q.; Kim, S. E.; Tao, X.; Choi, T. W.; Choi, J. Y.; Kim, W. J.; Jin, B. R. and Je, Y. H. (2013). Existence of lysogenic bacteriophages in *Bacillus thuringiensis* type strains. Journal of invertebrate pathology, 113(3): 228-231.

Rychlik, W. J. S.; Spencer, W. J. and Rhoads, R. E. (1990). Optimization of the annealing temperature for DNA amplification in vitro. Nucleic acids research, 18(21): 6409-6412.

Sadder, M.; Khyami-Horani, H. and Al-Banna, L. (2006). Application of RAPD technique to study polymorphism among *Bacillus thuringiensis* isolates from Jordan. World Journal of Microbiology and Biotechnology, 22(12): 1307-1312.

Sanahuja, G.; Banakar, R.; Twyman, R.; Capell, T. and Christou, P. (2011). *Bacillus thuringiensis:* a century of research, development and commercial applications. Plant Biotechnology Journal, 9(3): 283-300.

Santos, M. A. (1991). An improved method for the small scale preparation of bacteriophage DNA based on phage precipitation by zinc chloride. Nucleic acids research, 19(19): 5442-5442.

Strömsten, N. J.; Benson, S. D.; Burnett, R. M.; Bamford, D. H. and Bamford, J. K. (2003). The *Bacillus thuringiensis* linear double-stranded DNA phage Bam35, which is highly similar to the *Bacillus cereus* linear plasmid pBClin15, has a prophage state. Journal of bacteriology, 185(23): 6985-6989.

Valicente, F. H.; Tuelher, E. D.; leite, M. I.; Freire, F. L. and Vieira, C. M. (2010). Production of *Bacillus thuringiensis* biopesticide using commercial lab medium and agricultural by-products as nutrient sources. Revista Brasileira de Milho e Sorgo, 9(1): 1-11.

Verheust, C.; Fornelos, N. and Mahillon, J. (2005). GIL16, a new gram-positive *tectiviral* phage related to the *Bacillus thuringiensis* GIL01 and the *Bacillus cereus* pBClin15 elements. Journal of bacteriology, 187(6): 1966-1973.

Verheust, C.; Jensen, G. and Mahillon, J. (2003). pGIL01, a linear tectiviral plasmid prophage originating from *Bacillus thuringiensis* serovar *israelensis*. Microbiology, 149: 2083–2092.

Wiseman, R. L. and Day, L. A. (1977). Different packaging of DNA in the filamentous viruses Pf1 and Xf. Journal of molecular biology, 116(3): 607-611.

Xavier, R.; Josephine, R. and Sreeramanan, S. (2007). Environmental distribution and diversity of insecticidal proteins of *Bacillus thuringiensis* Berliner. Malaysian Journal of Microbiology, 3(2): 1-6.

Yang, X. M. and Wang, S. S. (1998). Development of *Bacillus thuringiensis* fermentation and process control from a practical perspective. Biotechnology and applied biochemistry, 28(2): 95-98.

Yu, Z. N. (1990). In: *Bacillus thuringiensis*. Yu Z. (ed.), Science Press, Beijing: 113.

Yuan, Y.; Gao, M.; Wu, D.; Liu, P. and Wu, Y. (2012a). Genome characteristics of a novel phage from *Bacillus thuringiensis* showing high similarity with phage from Bacillus cereus. PLoS One, 7(5): e37557.

Yuan, Y.; Peng, Q. and Gao, M. (2012b). Characteristics of a broad lytic spectrum endolysin from phage BtCS33 of *Bacillus thuringiensis*. BMC microbiology, 12(1): 297-307.

التفرقة بين بعض الفاجات التي تصيب بكتيريا*Bacillus thuringiensis* العزلة C18 بستخدام PCR و الشكل الظاهري

يحيى عبد المنعم عثمان ، عادل احمد المرسي و اماني السيد عطيه

قسم النبات كلية العلوم جامعة المنصورة

يعتبر استخدام الميكروبات لمكافحة الامراض احد التطبيقات الهامة لوقاية النبات والتي يمكن ان تمثل بديل آمن للمكافحة الكيميائية السائدة الآن، وفي هذا الصدد استخدمت *Bacillus thuringiensis* علي نطاق واسع كمصدر لمبيدات الآفات البيولوجية. ولكن يشكل تحلل هذه السلالة من البكتيريا نتيجة اصابتها بالفاجات مشكلة رئيسية لإنتاج المبيدات الحيوية علي نطاق واسع، وهذا يؤدي بدوره إلي خسائر اقتصادية كبيرة وبالتالي فإن عزل و تعريف هذه اللاقمات وايضا دراسة الحامض النووي لها تمثل خطوة هامه السيطرة علي الاضرار الاقتصادية لصناعة المبيدات الحيوية. فقد تم عزل ووصف بعض اللاقمات التي تصيب بكتيريا باسيللس ثيرنجنيسيس المعزولة محليا وكان عددها تلات فاجات و قسمت علي حسب الشكل الظاهري لها الي مجموعتين حيث ان ACTP-01 ينتمي الي عائلة *Siphoviridae*، و ACTP-02 و-ACTP 03ينتمو الي مجموعة *Tectiviridae*. وعند عمل *RAPD-PCR كانت تشكل خطوة في التفرقة بين الفاجات المعزولة*.