Morphological Identification of the Protein Crystal of *Bacillus thuringensis*, Isolated from Various Iraqi Soils

Mohammed, H. A. A.
Plant protection Dept. College of Agriculture/Baghdad University

Received: *5 /6/2014*

Abstract: Samples of *Bacillus thuringensis Berl.* were Isolated from field soil from Baghdad, Sulaimaneya, Deyala, Anbar, Babylon, Maisan and Thi-Qar governorates. The Identification was based on the shape of protein crystal (crystal body) using a 1500 and 2500 X microscope. Three morphological characters of the crystal body were recognized, namely (pure rhombic bi-pyramidal, cubic bi-pyramidal and spherical).

Keywords: *Bacillus thuringensis*, Iraqi field soil, Shape of protein crystal, Morphological identification.

INTRODUCTION

The entomopathogenic bacteria gram positive Bacillus *thuringensis* received a great deal of attention as a microbial agent in biological control of pests. It has been used to produce many types of microbial pesticides in two formulations: 1- Spores and crystal protein formulations, 2- Crystal protein only formulations (the safest formula). *B.T.* commercial formulations of B. *thuringensis* have a number of features: species, low cost production, non-pollutant to environment. Leaving no toxic residues in soil or water, due to its specialization, it has no effect on biological enemies of insect pest, no contact effect and not poisonous to Mammals, reptiles and amphibian. (Bijawa and Kogan, 2001). First identification for the crystal protein in *B.T.* was made by Berliner in 1915 and the accurate description for its structure was made by Hanna in 1953. Crystal protein represents 25% of the sporangia. Rasoli (2013) reported that the spore can be formed in 7 hours under 37°C and it is heat resistant as it resist U.V. radiation and chemical solvents.

The Varieties differs in numbers and types of genes which leads to a variation in types of proteins produced by each variety where the specialization on the pest take place, add, depending on the type of protein. There are varieties that are specialized on Lepidoptera larvae and others that are specialized on dipterans'. This specialization resulted the expansion in searching for, and testing pathogenicity of thousands of isolates from all over the world in order to discover and record new types with a high effect in controlling and a wide range of various hosts. Due to the lack of classification studies for this bacteria in Iraq, this study was conducted and aimed to place the bacteria in groups depending on the crystal shape that it produces using the light microscope.

MATERIALS AND METHODS

Soil samples were collected from 7 Iraqi governorates (Baghdad, Sulaimaneya, Deyala, Anbar, Babylon, Maisan and Thi-Qar). Samples were collected from 3-5 cm. depth using a sterilized knife, (Targeted bacteria is U.V. sensitive and not expected to be found on top soil). Samples weighted 20 gm. from each soil were collected in sealed plastic bags labeled with the name of the area, topography and date of collection and kept in the fridge Thiery and Farnchon (1997).

Identification and morphological description of the isolates:

Bacillus thuringensis samples, 39, were isolated and incubated for 72 hours on 30°c. on nutrient agar broth and the isolates were taken from the bacterial colonies that matches the typical description of *B.T.* colonies (Rough, white, random unorganized rims and grow fast to cover all the empty areas in the plate).Nutrient broth yeast extract (NBY) constant of beef extract 8gm. Yeast extract 3gm. Agar 15gm. and PH 7.2 were used as purification and identification for the bacteria Thiery and Farnchon (1979).

Slides were made by simple staining with phoxin alkaline stain. Slides were examined by light microscope (objective oil lens with 100 X magnification and Eyepieces with 15, 25 X). The presence of *B.T.* was clear due to the presence of the crystal protein which the bacteria is characterized by producing. The protein stained the crystals color but the spores remained transparent.

Isolates were purified and strained multiple times to make sure that it is *B.T.* and also by examination after 48 hours of incubation on 30° C to identify the crystal protein shape produced by each isolate were grown later on a slant surfaced nutrient agar and kept in fridge till harvest Stanley 1992.

Preparing the nutrient agar:

Ingredients: 5 gm. meat extract, 15gm. peptone, 5gm. sodium chloride, 15 gm. agar.

Ingredients were mixed in a flask, distilled water was added to make a 1 lt. volume pH was adjusted at 7.2 by adding buffer solution (PBS) and shaking. Each 200 ml. were poured in a 250 ml. flask and all 5 flasks were sterilized in autoclave on 120°C. for 15 minutes (Jamil 2007).

Crystal protein was purified and sent to Vietnam (Research center) for photography by scanning electron microscope.

RESULTS AND DISCUSSION

Table 1 shows and figure 1) bi-pyramidal is the most present as compared to other isolates and it was also found in 22 samples out of 39 with 56.4 percentage followed by the cubic bi-pyramidal mixture which was found in 10 out of 39 samples with (%25.6) and the spherical shape at the minimum presence (%18) (7 out of 39 samples). The highest percentage for the bi-

pyramidal was in the samples taken from Sulaimaneya (77.7%) but it was not found in Anbar samples. Both of the shapes were found in their highest level in Anbar and Thi-Qar samples with 66.6% and it was not found in Sulaimaneya, Deyala and Maisan samples. The spherical shape was not found in a high levels although its highest levels was recorded in Diyala at the ratio of 33% of total samples however it was not found in Baghdad.

These results show a variation in B.T. the shapes of crystals in the Iraqi soils and alssome variation from Jordanian soil based on the results obtained by Obeidat *et al.* (2004) in Jordan the spherical shape crystal produced by the *Bacillus thuringensis israelensis* was the most common there. The common type over there can be referred to the vicinity to Palestine the frequent use of *B.t.* formulations is common. Ohba *et al* (2000) noted that spherical shape in Japan soil had a ration 53% but Kumar et al. (1996)view that the pure rhombic bi-pyramidal was the most common type amongst other types produced by the *B. thuringensis*. The same conclusion was mentioned by Vega and Kaya (2012) who also explained that the shape of the crystal plays a role in the toxicity to the target pest.

Table (1): The presence of protein crystal of *Bacillus thuringensis* in accordance to the Iraqi soils collected from different governorates

Governorates	Protein Crystal Shape						Total no. of samples
	Pure rhombic bi-pyramidal	% of Samples per location	Cubic bi-pyramidal	% of Samples per location	Spherical	% of Samples per location	
Sulaimaneya	7	77.7	0	0	2	22.2	9
Deyala	4	66.6	0	0	2	33.3	6
Anbar	0	0	4	66.6	2	33.3	6
Baghdad	4	66.6	2	33.3	0	0	6
Babylon	4	66.6	2	33.3	0	0	6
Maisan	2	66.6	0	0	1	33.3	3
Thi-Qar	1	33.3	2	66.6	0	0	3
Total	22	56.4	10	25.6	7	18	**39**

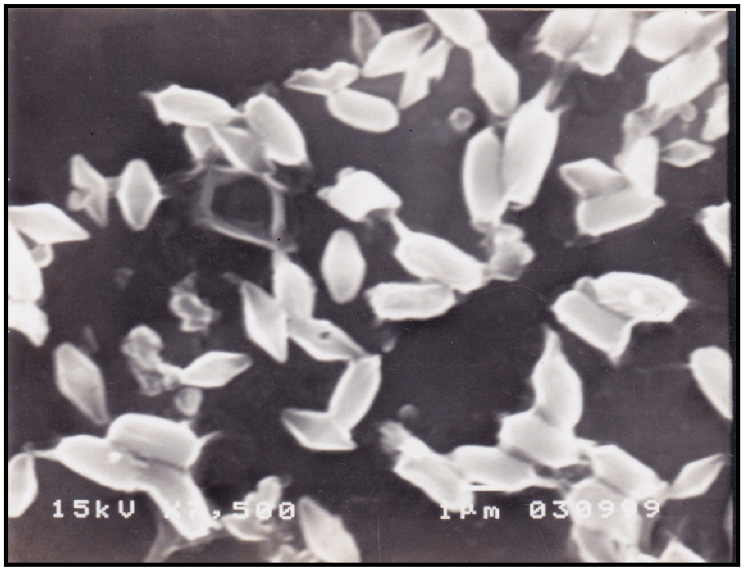

Fig.(1): Pure rhombic bi-pyramidal for *Bacillus thuringensis* (scanning electron microscope)

REFERENCES

Bajaw W. I. and Kogan. M. (2001). *Bacillus thuringensis* based on biological control of insect pests. National IPM network htm bajwa@bcc.orst.edu .

Berliner E (1915). About the sleep sickness of the *Ephestia kuhniella* Zell and its Pathogen *Bacillus thuringensis*. (In German) Cited by Franken Huyzen 1993 Z .Angew. Entomol. 2:29-56.

Hanna C.L (1953). Crystalline inclusions in aerobic spore-forming bacteria. Nature (Lond.), 172:1004.

Jamil, Jasser Mohamad (2007). Evaluating the efficiency of locally isolated *Bacillus thuringensis* as a bioinsecticide. A thesis submitted to the council of the college of Agriculture, University of Baghdad for Ph.D. degree.

Kumar, P.A. Sharma, R.P. and Malik V.S. (1996). The insecticidal proteins of *Bacillus thuringensis* in: Advances in applied microbiology, Neideleman, S. L. and Las Kin A. l. (eds.) Academic press Inc. Vol. 42:1-43.

Obeidat, M; Hassawi, D. and Ghabeish, I. (2004). Characterization of *Bacillus thuringensis* strains from Jordan and their toxicity to the Lepidoptera, *Ephestia kuheniella* Zeller. African Jornal of Biotechnology. Vol. 3(11):222-226.

Ohba, M.i; Wasano. N and Mizuki.E (2000). *Bacillus thuringensis* soil population naturally occurring. In: The Microbial Res. 155 (1):17-22.

Rasoli, Said Mustafa, 2013. Biofilm formation in *Bacillus thuringensis.* Ph.D. in University of Oslo. PP.147.

Stanly, D; Andrew, R. and Yousten, A. (1992). The genus *Bacillus* Spp. Insect pathogens. In the *Procaryotes.* Balows, A., Truper, H., Dworkin., M., Harder, W. and Schleifer, K. New York: Springer-Verlag.:1697-1744.

Thiery I. and Franchon E. (1997). Identification, Isolation, culture and preservation of Entomopathogenic bacteria in manual of techniques in insect pathology: Academic press 54-77.

Vega. E. Fernando and Harry K. Kaya (2012) "Insect pathology" second edition, ELSVier pp. 490.

التشخيص المظهري للبلورة البروتينية لبكتريا *Bacillus thuringinesis* المعزولة من ترب مختلفة في العراق

حسام الدين عبد الله محمد

قسم وقاية النبات - كلية الزراعة - جامعة بغداد

جمعت ٣٩ عينة لبكتريا *Bacillus thuringinesis* من ترب محافظات مختلفة بالعراق، بغداد و السليمانية و ديالى و الانبار، وبابل و ميسان و ذي قار. التشخيص اعتمد على شكل البلورة البروتينية (الجسم البلوري) باستعمال مجهر ضوئي بتكبير 1500 و 2500 X. ظهرت ثلاثة اشكال هي هرمي مضاعف مكعب نقي وهرمي مضاعف وكروي.