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HISTOPATHOLOGICAL EFFECTS OF AN ALGERIAN STRAIN OF *BACILLUS* THURINGIENSIS ON THE GUT OF ANACRIDIUM AEGYPTIUM (ORTHOPTERA, ACRIDIDAE). INTEREST FOR BIOLOGICAL CONTROL

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ABSTRACT

Locust's biocontrol requires identification of efficient bacterial strains able to be used against these ravagers. In this study, we analyzed the impact of an algerian strain of *B. thuringiensis* called TIM14 on the gut of *Anacridium aegyptium.* We observed that *A.aegyptium* infestation with TIM14 induced a lytic activity on the tegument and the cuticle followed by insect's demise. In conclusion, our results suggest that the local *B.thuringiensis* strain TIM14 would be active through different enzymatic activities involving the chitinase that induces death of this orthoptera.

KEYWORDS: *Bacillus thuringiensis*, Anacridium, *a*egyptiu; Gut, Biocontrol, Histopathology, Bacterium Strains, Biopesticides

INTRODUCTION

With the reduction of farmland in the world, insects are an important source in the competition for plant food resources [Kumar, 1991]. In particular, locusts and grasshoppers are a threat to human agricultural activities because of their ability to ingest the value of their own weight daily. Locusts and grasshoppers can make incredibly dense migratory swarms containing 40 to 80 million locuts [Steedman,1988]. In Algeria, many locusts and grasshoppers can cause important damages that affect crops [Doumandji et Doumandji-Mitiche, 1994]. The current approach to fight against these pests, based in particular on using chemical insecticides. However, this approach seems to be badly managed because of their non-use streamlined and systematic and represents a serious danger to the environment as well as for the user and the consumer [Launois-Luong et al, 1988]. In the context of alternative approaches to pest control, the development of biopesticides is a research priority. However this requires a good command of the pathogenicity of biopesticides and technical applications. Moreover, it is necessary to isolate strains capable of overcoming the constraints specific climate regionsconsidered.

Bacillus thuringiensis is a spore-forming bacterium of the soil, its main characteristic is synthesizing during the sporulation a crystalline inclusion composed of proteins with insecticidal properties. The large diversity of these toxins,

their effectiveness and relatively low production cost make the biopesticide Bacillus the most widely used in the world. In agriculture, it helps to fight against many pests, mainly Lepidoptera larvae and beetles. In human health, it can effectively control the population of several dipteran disease vectors [Sanchis et al, 1995]. Many results about biological control against locusts have been published [Bissad et al, 2010; Halouane et al, 2001] however the specific action of B. Thuringiensis against locusts remains poorly studied [Boufersaoui et al, 2009; Saidi-Touati.et.al,2008].

In our study, we were interested in identifying indigenous strains of B. thuringiensis with character against locust: Aiolopus strepens, widespread species in Algeria and subject of several studies [Hamadi, 1998, Saidi-Touati,1996] and Anacridium aegyptium large species preferring trees [Chopard,19943] . In addition to the entomopathogenic toxins present in B. thuringiensis, recent studies have revealed the presence of chitinase in this bacterium [Barboza et al, 2009; Driss et al, 2005; Ghasemi et al, 2011; Gomaa et al, 2012 Liu et al, 2002]. The identification of enzyme activity affecting especially the anterior part of the digestive tract, muscles, cuticle and the intestinal epithelium of locusts) would reveal the toxicity of the strain tested.

MATERIAL AND METHODS

Sampling of Locusts

The locusts were collected randomly. The capture of *Anacridium aegyptium* was performed using sweep nets. The sampling has been done in the coastal region of Algiers. Catches were also made only with hands in cold weather (below 15 °C).

Isolation of Bacterial Strains

A local strain of *Bacillus thuringiensis* called TIM14 was isolated in the Laboratory of Cellular and Molecular Biology by the team of Microbiology Faculty of Biological Sciences (USTHB), from wheat and soils of different regions in Algeria.

Bacterial Infestation of locusts

A group of 27 individuals of *Anacridium aegyptium* were infested by topical application of a suspension (0.1 ml to 10^6 cells / ml) of *B. thuringiensis* TIM14. Locusts were kept in isolation in a kennel with food infected with the same suspension previously applied. The insects were kept under observation for 24-48h in the laboratory at room temperature. Controls were exposed to a solution of sterile water.

Microscopic Observations

Adult locusts controls of *Anacridium aegyptium* and those treated with bacterial strains were dissected under binocular. The gut was removed in its entirety and fixed, dehydrated, cut and stained by the methods of Heidenhain azan and Mallory [Gabe,1968 Martoja et Martoja- Pierson, 1967]. Transverse sections of the digestive tract were observed under a light microscope (magnification X40 and X100).

RESULTS

The Infestation of *A. aegyptium* with TIM14 Strain Induces Total Disintegration of the Stomodeum

To check whether local strain of *B. thuringiensis* showed toxicity towards the digestive tissues of locusts, TIM14 was tested on *Anacridium aegyptium*; an arboreal species widespread large. We noted with interest that the cross section of the

stomodeum of *A. aegyptium* witness had a highly developed muscles and that large and complex villi were arranged on a regular basis. The intestinal lumen containing the food bowl is narrow. The epithelium consists of a regular cell layer topped by a thick cuticle (Figure 2A). The infestation with TIM14 induced on the locust a disorder in behavior a few hours later which was manifested by excitement, followed by death 12h after exposure to TIM14. Dead individuals had been disintegrated and had a perforation in the body wall of the abdomen (Figure 1). Histological analysis of the digestive tract of treated insects showed a stomodeum completely disintegrated, muscles totally destroyed, an irregular lumen, epithelium and cuticle also totally destroyed (Figure 2B). We noted with interest a total deterioration of gastric caecas. From these observations, we concluded that the strain TIM14 induced the total disintegration of the stomodeum.

DISCUSSIONS

In our investigation we were interested to study indigenous algerian strains of *Bacillus thuringiensis* on the degradation of the digestive tract of the locusts. For this purpose, one specie of locusts was infested with a local strain of *B. thuringiensis* isolated from algerian soil; *Anacridium aegyptium* treated withTIM14 proved that this strain was extremely deleterious, as demonstrated by the total destruction of the digestive tract and the integument of the abdomen. These results show that the bacterial strain TIM14 killed the locust 12 hours after the microbial treatment. The entomopathogenicity of *Bacillus thuringiensis* is demonstrated by the fact that this bacterium is a widespread species in the soil but also in the bodies of insects [Kaelin et al1974]. *Bacillus thuringiensis* is the most studied bacteria and programs of strain's selections have identified thousands of species each one limited to a specific insect order: Lepidoptera, Coleoptera, Diptera, and Orthoptera [Sanchis et al, 1995] and [Feitelson,1993] which confirms the acridicide power of TIM14 against the orthoptera *Anacridium aegyptium*. TIM14 has proved the deleterious power of the intestinal epithelium which is explained in several studies [Butko, 2003; Gill et al1992; Yu et al, 1997] confirming that toxins from *Bacillus thuringiensis* act as poisons causing intestinal paralysis of the gut followed by the death of the insect. Elsewhere in Algeria, several species of *Bacillus* were isolated from pupae causing mortality rates according to different *Bacillus* species [Saiah et al, 2010].

The action of *Bacillus thuringiensis* is explained by the presence of delta-endotoxin that is released following the dissolution of the crystal in an alkaline pH [Butko,2003] and then activated receptor recognizes the one that is already on the surface of the epithelium of intestinal membrane cells [Hoffman et al,1988; Van Rie et al,1989], because this toxin pore formation leading to an imbalance of ion exchange between the cell and the intestinal lumen [Gill et all,1992; Hôfte et Whitley, 1989; Knowles,1994 ; Knowles et al,1987] responsible of cell lysis. This was particularly well observed in TM14. Besides it has been shown in previous work that a culture of myotubes muscles of rats subjected to *Bacillus thuringiensis israelensis* strain were completely destroyed [Cahan et al, 1994], as observed on fly tested with other strains of *B. thuringiensis* [Singh et al, 1986]. This explains clearly the muscle damage also found at caecas gastric followed by epithelial alteration as noticed previously on the stomodeum. TIM14 seems to have a toxin ready to be activated in an acid locust gut. Moreover TIM14 has also totally affected the body wall and the cuticle of *A.aegyptium*, this strain possesses, in addition to destroying toxins muscles and epithelium, a chitinase similar to that found in some strains of *Bacillus thuringiensis* as mentioned in some studies [Barboza et al, 2009 ; Driss et al, 2005; Ghasemi et al, 2011; Gomaa et al, 2012; Liu et al, 2002] . This chitinase has been isolated from high levels of *Bacillus thuringiensis* NM101-19 [Gomaa et al, 2012]. TIM14 strain completely destroyed the locust by altering its integument, muscles, the cuticle and epithelium causing its death within hours after the bacterial treatment.

CONCLUSIONS

Our study showed that the TIM14 strain has been effective on Anacridium aegyptium, indeed the gut was totally destroyed and the impact was noticed on all parts of the digestive tract: stomodeum, mesenteron and proctodeum, The cuticle has also been perforated, wich would leave the suggestion that this strain contains Kitinase enzyme. All this was followed by the death of the insect within 24 hours after treatment. Our results would suggest that this strain is very interesting, more deep studies should be done in order to study the possibility to be used in biological control against locusts.

REFERENCES

1. Barboza-Corona JE, Ortiz-Rodríguez T, de la Fuente-Salcido N. Bideshi DK, Ibarra JE et Salcedo-Hernández R, [2009]: Hyperproduction of chitinase influences crystal toxin synthesis and sporulation of *Bacillus thuringiensis*. Antonie Van Leeuwenhoek. 96(1), 31-42.
2. Bissad F.Z, Youcef M. et Doumandji-Mitiche B. [2010]: Effet d'un champignon entomopathogène *Metarhizium anisopliae* Var.*Acridum* sur la cuticule du criquet pèlerin (*Schistocerca gregaria*, Forskäl, 1775). European Journal of scientific research, 45(1) 55-33
3. Boufersaoui A, Allilech S., Garici Y, Chikhi A, Metiaz- Nateche, et Abderahmani A, [2009] : Microbiologie et lutte : action de *Bacillus thuringiensis* sur des paramètres histologiques d'*Acrida Turrita*, Biotechnologie microbienne au service du developpement Congrès . International. Maroc
4. Butko P, [2003] Cytolytic Toxin Cyt1A and Its Mechanism of Membrane Damage: Data and Hypotheses, Appl. Environ. Microbiol. 69,(5),2415-2422
5. Cahan R, Shainberg A, Malik Z, Nitzan Y, [1994]. Biochemical and morphological changes in rat muscle cultures caused by 28,000 mol. Wt toxin of *Bacillus thuringiensis israelensis* . Toxicon. 32(9), 1125-36
6. Chopard L, [1943] : Faune de l'empire français, Orthoptéroides de l'Afrique du nord. Librairie Larose, Paris, 395-396
7. Doumandji S. et Doumandji-Mitiche B, [1994] Criquets et sauterelles (Acridologie) O.P.U., 99P.
8. Driss F., Kallassy-Awad M, Zouari N, and Jaoua S, [2005] Molecular characterization of a novel chitinase from *Bacillus thuringiensis* subp. KURSTAKI. Journal of Applied Microbiology.99, 945-953
9. Feitelson J.S, [1993]. The *Bacillus thuringiensis* family tree. Advanced engineered pesticides. Marcel Dekker, New York, PP: 63-72
10. Gabe M, [1968] Techniques histologigues, Masson, Paris.
11. Ghasemi S, Ahmadian G, Sadeghi M, Zeigler DR, Rahimian H, Ghandili S, Naghibzadeh N, Dehestani A, [2011] First report of a bifunctional chitinase lysozyme produced by *Bacillus pumilus* SG2. Enzyme and microbial technology. 48 (3) 225-31.
12. Gill S.S, Cowles E.A et Pietrantonio P.V, [1992] The Mode of action of *Bacillus thuringiensis* endotoxins. Ann. Rev. Entomol . 37, 615-636

13. Gill S.S, Cowles E.A et Pietrantonio P.V, [1992] The mode of action of *Bacillus thuringiensis* endotoxins . Annu. Rev. Entomol. 615-36

14. Gomaa E.Z, [2012] Chitinase production by *Bacillus thuringiensis* and *Bacillus licheniformis*: Their potential in antifungal biocontrol. The journal of Microbiology. 50 (1) 103-11.

15. Halouane F, Benzara A, Doumandji-Mitiche B, and Bouhacein M., [2001] Effet de deux entomopathogènes, *Beauveria bassiana* et *Metarhizium flavoviride* (Hyphomycètes, *Deuteromycotina*) sur l'hémogramme des larves de 5ème stade et des adultes de *Locusta migratoria migratorioides* (Orthoptera: Acrididae). Journal of Orthoptera Research. 10(2) 331-334.

16. Hamadi K [1998] : Bioécologie de la faune orthoptérologique en Mitidja. Etude de l'activité biologique de plantes acridifuges sur *Aiolopus strepens* (Latreille 1804) (Orthoptera, Acrididae), Thèse de Magister. INA, Alger, 197P.

17. Hoffman H, Van der bruggen H, Höft H, Van Rie J, Jansens S, et Van H, [1988] : Specificity of *Bacillus thuringiensis* delta endotoxins is correlated with the presence of high affinity binding sites in the brush border membrane of target insect midgets. Proc. Natl. Acad. Sci.USA, 85 7844-7848

18. Hôfte H. et Whiteley H.R,[1989]: Insecticidal crystal proteins of *Bacillus thuringiensis* . Microbiol.mol.biol rev. 53, 242-255

19. Kaelin P, Morel P, Gadani F. [1974]: Isolation of *Bacillus thuringiensis* stored tobacco and *Lasioderma serriocorne* (F.). Appl. Environ. Microbiol. 60, 19-25

20. Knowles B.H, [1994]: Mechanism of action of *Bacillus thuringiensis* insecticidal delta endotoxins. Adv.Insect.Physiol.24, 275-308

21. Knowles B.H.et Ellar D.J. [1987]: Colloid osmotic lysis is a general feature of the mechanism of action of *Bacillus thuringiensis* delta endotoxines with different insect specificity. Biochim. Biophys. Acta. 924, 509-518

22. Kumar R. [1991] : Les insectes ravageurs des cultures edition Karthala et CTA, 6-7

23. Launois –Luong M.H, Launois M. et Rachadi T, [1988] : Lutte chimique contre les criquets du sahel. CIRAD, coll Acrid.Operat 3, 83P.

24. Liu M, Cai Q.X, Liu H.Z,.Zhang B.H, Yan J.P and Yuan Z.M, [2002] : Chitinilytic activities in *Bacillus thuringiensis* and their synergitic effects on larvicidal activity. Journal. of applied Microbiology, 93, 374-379

25. Martoja R, et Martoja-Pierson M, [1967] : Initiation aux techniques de l'histologie animale. Masson, Paris.

26. Saiah F, Bendahmane BS, Youcef Benkada M, Berkani A. et Gacemi A. [2010] : Isolement et identification de bactéries entomopathogènes à partir de *Phyllocnistis citrella* Stainton 1856 dans l'Ouest algérien *Faunistic Entomology* 2011. 63 (3) 121-123

27. Saidi-Touati M. [1996] : Bioecologie des Caelifères dans trois types de milieux à Birkhadem. Utilisation de *Melia azedarach* contre le genre *Aiolopus* (Fieber, 1853) (*Orthoptera*, *Acrididae*), thèse de Magister, INA, Alger, 134P.

28. Saidi-Touati M, Boufersaoui A, Amari K, Kenane S, Metiaz- Natech F, Abderahmani A. [2008] : Impact de *Bacillus thuringiensis* sur *Aiolopus strepens.* Toxicologie Mediterranéenne, Diversité et specificité Perspective de

lutte. Congrès Mixte International. Essaouira.

29. Sanchis V, Chaufaux J, Lereclus D.[1995] : Utilisation de *Bacillus thuringiensis* en protection des cultures et résistance des insectes. Cahier de l'Agriculture. 4,405-406

30. Singh G. J. P, Schouest L.P Jr, Gill S.S. [1986]: Action of *Bacillus thuringiensis subsp. israelensis* δ-endotoxin on the ultrastructure of the housefly larva neuromuscular system in vitro. Journal of Invertebrate Pathology,47, (2) 155-166

31. Steedman A. [1988]: Locust handbook, Overseas Developpement Natural Ressources Institute (O.D.N.I.) London 180P.

32. Van Rie J, Janses S, Höfte H, Degheel D, et Van Mellaert H, [1990] : Receptors on the brush border membrane of the insect midgut as determinants of the specificity of *Bacillus thuringiensis* delta-endotoxins. Appl. Environ. Microbiol. 56, 1378-1385

33. Yu C.G, Mullins M.A, Warren G.W, Koziel M.G et Estruch J.J. [1997]: The *Bacillus thuringiensis* vegetative insecticidal protein Vip3A Lyses midgut epithelium cells of susceptible insects. Appl. Environ. Microbiol 63,532-536

Figure Legend List

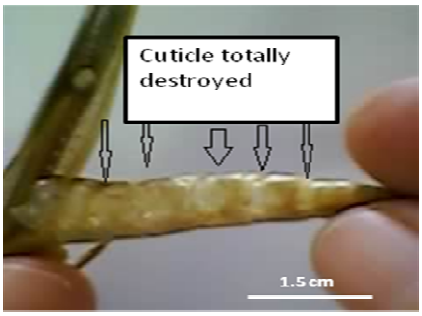

Figure 1: Perforation of the Abdomen of Anacridium Aegyptium after Treatment with TIM14.

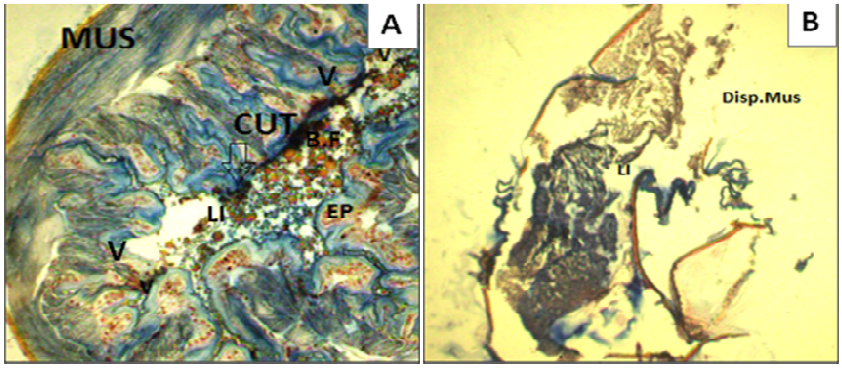

Figure 2: Cross-Section of the stomodeum of *Anacridium aegyptium* Treated with TIM14. A) Control (Magn.X100). LI: Intestinal Lumen, Ep: epithelium. CUT: cuticle, V: villi. B.F: Bowl food. B) Cross Section of the Stomodeum of *A.aegyptium* Treated with TIM 14 (Magn. x40), Disp.Mus. : Disapearance of muscle structure, LI: Intestinal Lumen. (Azan