ACTA SCIENTIFIC MICROBIOLOGY (ISSN: 2581-3226)

Volume 3 Issue 3 March 2020

Research Article

Determination of the Biochemical Profile of *Bacillus thuringiensis* Strains Isolated from Middle Tennessee

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DOI: 10.31080/ASMI.2020.03.0525

Received: January 22, 2020

Published: February 14, 2020

Abstract

There is an ever growing need for control of insects of agricultural and health importance. The practice of using chemicals for this purpose has been flawed due to many untoward fall outs including insect resistance, non-target toxicity and non-biodegradability. A promising and viable practice is the use of biological agents such as *Bacillus thuringiensis* (Bt) that are efficacious as well as safe. *Bacillus thuringiensis* (Bt) was first isolated in the early 1900s, and interest in it and its used have soared as a result of its insecticidal capability. Bt is a Gram-positive, endospore-forming organism that produces crystal proteins that serve as toxins to select insects. Thus, its use as a bioinsecticide has exploded prompting the search for newer isolates and the establishment of culture collections of Bt strains in many laboratories. The purpose of the work presented here was to assess the biochemical profile of 72 strains collected in Middle Tennessee as well as those of two standard strains, *B. t. israelensis* (BtI) and *B. t. kurstaki* (BtK) as a method for their characterization. The approach was to determine the biochemical profile of each strain using the Biolog Identification System, which allowed for the assessment of the metabolic activity of each strain in the presence of 95 different substrates. It was found that the strains clustered into two major groups having common substrates, and these groups gave two and three sub-clusters. Thus, it was concluded that the strains of this study had varying metabolic ability for the substrates provided in the Biolog Gen III Microplates; however, there were certain substrates that were commonly used by the strains used in this study.

Keywords: *Bacillus thuringiensis*; Biolog Identification System; Gen III Microplates; Biochemical Profile

Introduction

Bacillus thuringiensis (Bt) is naturally occurring, soil-dwelling, Gram-positive bacterium used as a biological pesticide because it typically produces a fatal toxin. The toxin targets particular herbivorous insects. The bacterium is also harmful to other moths such as *Cadra calidella*, as observed when the moths interact with the bacterium in a laboratory setting. Apart from its natural occurrence in the soil, *Bacillus Thuringiensis* is also naturally produced in the gut of caterpillars of different species of moths and butterflies [17,20]. The bacterium also exists in water, facilities for storing grain, animal dung, surfaces of a leaf surfaces, and flour mills. The natural toxin elicited by *Bacillus thuringiensis* (Bt) has facilitated the fight with insects since the 1910s. It is used to manufacture insecticide spray for controlling insect infestation [18,21]. Endotoxins are a crystal protein with insecticidal action generated during sporulation [19]. It enhances the use of the bacterium as an insecticide. A more in-depth understanding of the bacterium occurs with research undertakings that use particular techniques to find results that promote discussion on the topic.

In 1901, a Japanese Biologist, Ishiwatari Shigetane, revealed the presence of bacterium in silkworms. Again, in 1911, a German microbiologist, Ernst Berliner, re-established the presence of the bacterium when he isolated it in flour moth caterpillars [1,19]. A strain of Bt containing a plasmid that participates in endospore and

crystal formation was reported in 1976 by Robert A. Zakharyan. The bacterium and other members of the genus *Bacillus*, such as *Bacillus cereus* and *Bacillus anthracis*, produce endospores [9,12]. The genes also enhance the genetic modification of various food crops to trigger the production of *Bacillus thuringiensis* as an initiative to deter different moths and insects [13]. The toxin is lethal to separate orders of insects, including Diptera, Lepidoptera, and Coleoptera.

Material and Methods

GEN III Micro Plate™ system is an essential technique in bacterial analysis in microbiology. The method uses the Biolog Microbial Identification System software (e.g., OmniLog® Data Collection), a powerful technology that uses a harmonized 94 biochemical tests to determine the purity of bacteria [30]. The technology uses 71 carbon source utilization assays and 23 chemical sensitivity assays in phenotypic analyses. The simplicity and appropriateness of the machine enhance performance; thus, it increases the identification of 4 times species. It uses a combination of biochemical and nutrients, Micro Plate, and Tetrazolium redox dyes. The process involves the growing of an isolated bacterium in the agar medium before its suspension in inoculating fluid [11,29]. During incubation, an increase in the respiratory rates of the cells observed with increased utilization of the carbon and a reduction of the tetrazolium redox dye. Thus, the formation of purple coloration. Negative wells remain colorless, whereas positive wells turn light purple to streaky purple to dark purple. The coloration results in a "Phenotypic Fingerprint" of the microorganism. It enables the identification of the organism at the species level. Coloration determines the presence or lack thereof of the bacterium. Coloration enhances the identification of the microorganism.

Isolation of *Bacillus thuringiensis*

Bacillus thuringiensis strains isolated from middle Tennessee by Ejiofor and Johnson [10]. The collections were cultured on Luria-Bertani (LB) agar plate for 24 to 48 hours at 30oC. All the samples were transferred into LB media incubated at 30oC for 24 hours.

The isolation of a pure bacterial sample involves a scientific analysis of *Bacillus thuringiensis* in controlled environmental conditions. It also follows specific protocols in the methodologies and components used in the study [26,27]. Few changes in methods and use of materials enhance the study. The steps in the study include.

Step 1. Culture of organism on agar media

The isolation of a pure culture in LB agar media. The speed of growth of a bacterial colony should be high in the LB agar media selected [5]. The viability of the cells for the study is guaranteed when care applied in the selection of cells. The cells are also freshly grown for better outcomes. Mitigation, where insufficient growth occurs, involves the re-streaking and incubation of the plates.

Step 2. Preparation of inoculum

A balance of Inoculation Fluid (IF) and cell density monitored during the bacterial release into the IF. The uniform cell suspension achieved by stirring the IF in the tube. Where the cell density dropped, additional cell components added. However, adding IF controls advanced cell density. Care ensures the removal of highly clumpy bacteria with the preparation of an inoculator swab that helps remove the clumpy bacteria. Growth on the surface of the agar plate was removed using cotton-tipped Inoculator swabs [2,24,25]. After the preparation of a favorable environment, a single colony of the bacteria released into the IF. The procedure encompasses rubbing the tip of the swab against the lowermost section of the IF holding tube. After releasing the bacteria, cells slowly dispersed into the fluid. The cell suspension remains still for about 5 minutes to settle the clumps at the bottom of the testing tube. Uniform suspension of cells forms when the IF fluid stirs. It also enhances vision and taking turbidity readings in the spectrophotometer.

Step 3. Inoculation of the microPlate

The inoculation of the MicroPlate involves the pouring of suspension into a multichannel pipet reservoir. The pipettor draws the suspension based on the tips of sterile 8 [6,7]. The wells of the Micro Plate were filled with 100 microliters to form a soft gel. The MicroPlates openings blocked with lids, and the pipet tips discarded.

Step 4. Incubation of micro plate

The Micro Plate was inserted in an incubator for 24 hours, with monitored temperatures kept at 30°C [3]. Biolog's Microbial Identification Systems software enhanced the reading of the results of the Micro Plates after 5 to 10 minutes, shaking on a rocker shaker.

Statistical analysis

Recorded data were statistically analyzed using Dendrogram output for hierarchical clustering.

Results and Discussion

Biolog's Microbial Identification Systems software is used to analyze *Bacillus thuringiensis*. The readings show a difference in the patterns formed by the pink shaded wells. However, in other cases, a result of no identification of any organism is recorded [28]. The result does not dispute the role of Bt2, a Gram-positive centralized endospore in the formation of aerobic. The rod produces the typical Bacillus colonies when grown on LB agar plates. Again, the color densities in wells of the carbon source utilization assays showed pink-flecked wells. The wells were visible because of the positive reactions emanated during the analysis. Further, the identification of different organisms produced a high similarity type of bacillus known as *Aneurinibacillus aneurinilyticus* ([22,23]. Test results also show a panel with a Phenotypic Fingerprint of the microorganism. The property is essential in identifying the species level because it gives various similarity levels when in contact with different types of the genus bacillus [4]. For example, it gave a low similarity when exposed to *Bacillus cereus*.

Color densities in wells of the carbon source utilization assays displayed pink-colored small wells. The species portrayed dark, easily noticeable "positive" reactions [8]. The blue coloration in an experiment was negative control well (A-1), and in other "negative" wells revealed presented a false and unreliable positive reaction. *Bacillus thuringiensis* is a glucose-reducing organism. Exposure to the controlled environment results in a 100% negative color in the A-1 well inoculation chambers.

From figure 1, the dendrogram displays phenotypic double division of the strains. The division is founded on their ability to absorb substrates - used for cataloguing. Further division of the two groups is based on the resemblance in substrate activity. The categorization puts the strains with identical substrate together because of the biochemical property's similarity [14]. Strain specificity occurs from the metabolized substrates. A comparison of the result with strains recognized by means of Biolog's Microbial Identification Systems software produce the dendrogram.

The dendrogram demonstrates different strains' genetic design and connection. The BT cluster exposed ~A only three groups lacked heavy huddling because of the variance in physical location. Heavy clustering is also attributed to the general exploration of the *Bacillus* group as opposed to unambiguous study of *Bacillus thuringiensis* strains [15,16]. RAPD DNA facilitates the analysis of the isolates using markers. The markers determine genetic assortment leading to the formation of a dendrogram that displayed genetic interaction. Two main groups were formed with the division of 70 isolates. The two groups were further subdivided into four subgroups. Resulting clusters have varying number of strains. 6 clusters composed of various Bt strains result with further classification of the dendrogram.

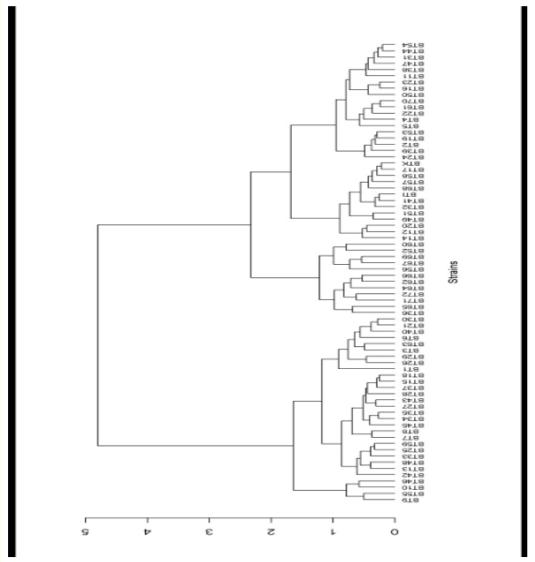

Figure 1: Dendrogram output for hierarchical clustering of the Bt strains based on their biochemical profile.

The outcomes of the study are consistent with other previously conducted analyses where the researchers screened the *Bacillus thuringiensis* bacteria from diverse geo-ecological locations in Ukraine. Most of the reviews based on both biochemical and physiological analyses. They used various approaches and methodologies to analyze the species [14]. The subgroups selected for the study were composed of a cluster of different Bt strains. The composition of the chosen strains differed significantly, where some groups were formed of very few strains, while others had many. These results indicate that there is a possibility of the existence of 6 subgroups of *Bacillus thuringiensis* based on the biochemical and genetic capability of various strains. The study differs significantly

from other similar studies in the field. The difference was linked to the use of different strains of bacillus species for the analysis [15,16]. The analyses also used various techniques and biochemical structures such as the sequencing and plasmid outline of 16S rRNA.

Conclusion

Thoughtful analysis of Bt facilitates a more in-depth understanding of the bacterium and the proper application in mitigating insect infestation. The study resulted in the achievement of the aim of the study - to illustrate the phenotypic relationship of the different *Bacillus thuringiensis* strains. The data obtained from this analysis will help in improving further comprehension of the phenotypic diversity of Bt. It will act as a guide for other scholars and researchers who venture to scrutinize data in the same field. Therefore, future examinations should aim establishing the relationship between the phenotypic classification and genetic assortment of the *Bacillus thuringiensis* strains. It creates a better comprehension of group existence.

Acknowledgements

This work is supported in part by Tennessee State University (TSU). The authors are thankful to the Saudi Arabian Cultural Mission (SACM) and Northern border university(NBU).

Bibliography

1. Alper Mehlika., *et al*. "Distribution, occurrence of cry genes, and lepidopteran toxicity of native Bacillus thuringiensis isolated from fig tree environments in Aydın Province". *Turkish Journal of Agriculture and Forestry* 38.6 (2014): 898-907.

2. Armada E., *et al*. "Native plant growth promoting bacteria Bacillus thuringiensis and mixed or individual mycorrhizal species improved drought tolerance and oxidative metabolism in Lavandula dentata plants". *Journal of Plant Physiology* 192 (2016): 1-12.

3. Badran, Ahmed H., *et al*. "Continuous evolution of Bacillus thuringiensis toxins overcomes insect resistance". *Nature* 533.7601 (2016): 58.

4. Bassi, Daniela., *et al*. "Transcriptome analysis of Bacillus thuringiensis spore life, germination and cell outgrowth in a vegetable-based food model". *Food Microbiology* 55 (2016): 73-85.

5. Ben-Dov Eitan. "Bacillus thuringiensis subsp. israelensis and its dipteran-specific toxins". *Toxins* 6.4 (2014): 1222-1243.

6. Blackburn, Michael B., *et al*. "Phylogenetic distribution of phenotypic traits in Bacillus thuringiensis determined by multilocus sequence analysis". *PloS one* 8.6 (2013): e66061.

7. Buentello-Wong S., *et al*. "Characterization of Cry Proteins in Native Strains of Bacillus thuringiensis and Activity Against Anastrepha ludens1". *Southwestern Entomologist* 40.1 (2015): 15-25.

8. Chouikha Iman and B Joseph Hinnebusch. "Silencing urease: a key evolutionary step that facilitated the adaptation of Yersinia pestis to the flea-borne transmission route". *Proceedings of the National Academy of Sciences* 111.52 (2014): 18709-18714.

9. Deng, Chao., *et al*. "Division of labour and terminal differentiation in a novel Bacillus thuringiensis strain". *The ISME Journal* 9.2 (2015): 286.

10. Ejiofor AO and T Johnson. "Physiological and molecular detection of crystalliferous Bacillus thuringiensis strains from habitats in the SouthCentral United States". *Journal of Industrial Microbiology and Biotechnology* 28.5 (2002): 284-290.

11. El-Ghany., *et al*. "Molluscicidal activity of Bacillus thuringiensis strains against Biomphalaria alexandrina snails". *Beni-Suef University Journal of Basic and Applied Sciences* 6.4 (2017): 391-393.

12. Fagerlund Annette., *et al*. "Sin R controls enterotoxin expression in Bacillus thuringiensis biofilms". *PLoS One* 9.1 (2014): e87532.

13. Gassmann Aaron J., *et al*. "Field-evolved resistance by western corn rootworm to multiple Bacillus thuringiensis toxins in transgenic maize". *Proceedings of the National Academy of Sciences* 111.14 (2014): 5141-5146.

14. Guinebretière Marie-Hélène., *et al*. "Bacillus cytotoxicus sp. nov. is a novel thermotolerant species of the Bacillus cereus group occasionally associated with food poisoning". *International Journal of Systematic and Evolutionary Microbiology* 63.1 (2013): 31-40.

15. Hollensteiner Jacqueline., *et al*. "Bacillus thuringiensis and Bacillus weihenstephanensis inhibit the growth of phytopathogenic Verticillium species". *Frontiers in Microbiology* 7 (2017): 2171.

16. Jia Yanhua., *et al*. "A genetically modified broad-spectrum strain of Bacillus thuringiensis toxic against Holotrichia parallela, Anomala corpulenta and Holotrichia oblita". *World Journal of Microbiology and Biotechnology* 30.2 (2014): 595-603.

17. Jiménez Guillermo., *et al*. "Description of Bacillus toyonensis sp. nov., a novel species of the Bacillus cereus group, and pairwise genome comparisons of the species of the group by means of ANI calculations". *Systematic and Applied Microbiology* 36.6 (2013): 383-391.

18. Kamar Rita., *et al*. "Pathogenic potential of Bacillus cereus strains as revealed by phenotypic analysis". *Journal of Clinical Microbiology* 51.1 (2013): 320-323.

19. Kim Min-Ju., *et al*. "Various enterotoxin and other virulence factor genes widespread among Bacillus cereus and Bacillus thuringiensis strains". *Journal of Microbiology and Biotechnology* 25.6 (2015): 872-879.

20. Marchlewicz Ariel., *et al*. "Bacillus thuringiensis B1 (2015b) is a gram-positive bacteria able to degrade naproxen and ibuprofen". *Water, Air, and Soil Pollution* 227.6 (2016): 197.

21. Masri Leila., *et al*. "Host–pathogen coevolution: the selective advantage of Bacillus thuringiensis virulence and its cry toxin genes". *PLoS Biology* 13.6 (2015): e1002169.

22. Mukherjee Krishnendu., *et al*. "Experimental evolution of resistance against Bacillus thuringiensis in the insect model host Galleria mellonella results in epigenetic modifications". *Virulence* 8.8 (2017): 1618-1630.

23. Oves Mohammad., *et al*. "Biosorption of heavy metals by Bacillus thuringiensis strain OSM29 originating from industrial effluent contaminated north Indian soil". *Saudi Journal of Biological Sciences* 20.2 (2013): 121-129.

24. Palma Leopoldo., *et al*. "Bacillus thuringiensis toxins: an overview of their biocidal activity". *Toxins* 6.12 (2014): 3296-3325.

25. Palma Leopoldo., *et al*. "Draft genome sequences of two Bacillus thuringiensis strains and characterization of a putative 41.9-kDa insecticidal toxin". *Toxins* 6.5 (2014): 1490-1504.

26. Pardo-Lopez., *et al*. "Bacillus thuringiensis insecticidal three-domain Cry toxins: mode of action, insect resistance and consequences for crop protection". *FEMS Microbiology Reviews* 37.1 (2013): 3-22.

27. Peng Dong-hai., *et al*. "The expression and crystallization of Cry65Aa require two C-termini, revealing a novel evolutionary strategy of Bacillus thuringiensis Cry proteins". *Scientific Reports* 5 (2015): 8291.

28. Shu Changlong., *et al*. "Use of a pooled clone method to isolate a novel Bacillus thuringiensis Cry2A toxin with activity against Ostrinia furnacalis". *Journal of Invertebrate Pathology* 114.1 (2013): 31-33.

29. Surhio Muhammad Ali., *et al*. "Complete degradation of dimethyl phthalate by biochemical cooperation of the Bacillus thuringiensis strain isolated from cotton field soil". *RSC Advances* 4.99 (2014): 55960-55966.

30. Zhu Lei., *et al*. "Genomic and transcriptomic insights into the efficient entomopathogenicity of Bacillus thuringiensis". *Scientific Reports* 5 (2015): 14129.