Global Journal of Science Frontier Research
Biological Sciences
Volume 12 Issue 8 Version 1.0 Year 2012
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Antibiotic Susceptibility Testing of Isolated *Bacillus thuringiensis* from Three Soil Types Around Iligan City, Philippines

By Jing R. Bautista, Faith B. Amorado, Maria Luisa S. Orbita & Franco G. Teves

MSU-Iligan Institute of Technology

Abstract - This study was conducted with the aim of isolating and identifying novel strains of *Bacillus thuringiensis* (Bt) from the three different kinds of soils and to determine its antibiotic susceptibility. Soil samples were taken from three different uncultivated sites that have no history of treatment with Bt products. Isolation of the Bt strain was conducted according to the method as described by Travers *et al* (1987) and the phenotypic characteristics of the isolated Bt were determined by making use of standard methods. Twenty presumptively identified strains of Bt were subjected to antibiotic testing by using ampicillin, amoxicillin, tetracycline, streptomycin, and ofloxacin. Results showed that the isolated strain of Bt were resistant to β-lactams (amoxicillin and ampicillin).

Keywords : *antibiotic susceptibility, β-lactams, vermicast.*

GJSFR-C Classification : *FOR Code: 050399*

Strictly as per the compliance and regulations of :

Antibiotic Susceptibility Testing of Isolated *Bacillus thuringiensis* from Three Soil Types Around Iligan City, Philippines

Jing R. Bautista [α], Faith B. Amorado [σ], Maria Luisa S. Orbita [ρ] & Franco G. Teves [ω]

Abstract - This study was conducted with the aim of isolating and identifying novel strains of *Bacillus thuringiensis* (Bt) from the three different kinds of soils and to determine its antibiotic susceptibility. Soil samples were taken from three different uncultivated sites that have no history of treatment with Bt products. Isolation of the Bt strain was conducted according to the method as described by Travers *et al* (1987) and the phenotypic characteristics of the isolated Bt were determined by making use of standard methods. Twenty presumptively identified strains of Bt were subjected to antibiotic testing by using ampicillin, amoxicillin, tetracycline, streptomycin, and ofloxacin. Results showed that the isolated strain of Bt were resistant to β-lactams (amoxicillin and ampicillin).

Keywords : antibiotic susceptibility, β-lactams, vermicast.

I. Introduction

Bacillus thuringiensis (Bt) is considered to be the most widely used entomopathogenic microorganisms used as a biological control against agricultural insects and pests (Samsonov et al., 1997). The said bacterium is a member of a group of crystalliferous spore-forming aerobic Gram-positive, rod-shaped of the genus *Bacillus* that is uniquely characterized by the ability to form endospores that produces one or more proteinaceous parasoporal crystal that are resistant to inactivation by heat, desiccation and organic solvents.

B.thuringiensis is largely used in agriculture especially in organic farming, in urban aerial spraying programs, and in transgenic crops. Its proteins have been used in many organic farms for over 50 years as a microbial pest control agent. Previous researches and investigators have reported that natural environments of tropics and subtropics of Southeast Asia are a good reservoir of *B.thuringiensis* populations with a great diversity of serological and biological characteristics (Attathom, et al., 1995). Moreover, *B.thuringiensis* can also be found among insect cadavers, stored product dust, leaves of plants, aquatic environments and from the marine sediments (Maeda et al., 2000). Bt, like other *Bacillus* species, has been classified on the basis of its cellular, cultural, biochemical and genetic characteristics with a width approximately one μm and 5 μm in length (Madigan and Martinko, 2005; Sakai et al., 2007).

This study was conducted to isolate potential *B.thuringiensis* by using standard methods and testing its antibiotic susceptibility with β-lactam antibiotics amoxicillin and ampicillin.

II. Materials and Methods

a) Soil Collection and Characterization

Soil samples were taken from three (3) different uncultivated sites that have no history of treatment with *B.thuringiensis* products. The sampling sites were the following: a residential house with a backyard garden at Carbide Village; a corn plantation at Luinab; and a vegetable garden at Tibanga, Iligan City.

About 100g of soil samples were collected by scraping off 2-5 cm below the surface with a sterile spatula. All samples were placed in a sterile autoclavable plastic ware aseptically and were brought to the laboratory immediately for processing.

The soil texture was determined and pH was determined by suspending 50 grams of the soil in 100 ml of distilled deionized water. The solution was stirred for 1 hour at 800 rpm on a rotary shaker. The pH of the supernatants was recorded using a pH meter.

b) Presumptive Identification

Isolation of the Bt strain was conducted according to the method described by Travers et al (1987). The samples were processed by acetate selective method in concentrations of sodium acetate (pH= 6.3). In this procedure, acetate inhibits germination of *B.thuringiensis* spores, so other spore germinates and non-spore forming bacteria are eliminated by heat treatment (7 min at 80^{0}C). The surviving spores were plated and grown on a suitable medium and incubated at 30^{0}C for 24 hours to obtain colonies.Bt-like colonies, which are usually described as cream-colored and have the appearance of a fried egg on a plate, were purified and cultured. Smears were Gram-stained and with malachite green method, and were examined under phase-contrast light microscope for the observation and determination of the presence of spores and parasporal bodies (delta-endotoxin crystals) of the bacterium at 24-hours intervals.

Author α σ ρ ω : Department of Biological Sciences, College of Science and Mathematics, MSU-Iligan Institute of Technology, Iligan City, Philippines 9200. E-mail : jngbautista@gmail.com

c) Cultural Method of Characterization of Bacterial Isolates

The following differential tests were performed following the identification flow charts on Bergey's Manual of Determinative Bacteriology: determination of oxygen requirement, starch hydrolysis, catalase test, Voges-Proskauer Test, and test on Triple Sugar Iron agar.

d) Antibiotic Susceptibility Testing

The antibiotic resistance of the isolated *B.thuringiensis* was tested against specified antibiotic discs common against the said species using the disc diffusion method (Bauer et al., 1966). A 16 h broth cultures of the isolated strains were grown at 37^{0}C was transferred using sterile cotton swabs into the Mueller Hinton Agar (MHA) plates by aseptically dipping the swabs into the tubes and streaked on the plate. Then ampicillin (10μg/disc), amoxicillin (30μg/disc), tetracycline (30μg/disc, streptomycin (10μg/disc), and ofloxacin (30μg/disc) antibiotics were distributed on plate. The plates were incubated at room temperature for 16 hours and the growth of the bacteria was observed.

III. Results and Discussion

a) Soil Characteristics

Sampled soil from Carbide Village has a pH of 7.06 and with a dark –loamy description (Table 1); this is because the soil is composed of degraded organic materials such as banana peels, rice, etc. Accordingly, soil pH influences the solubility of nutrients and affects the activities of many microorganisms responsible for breaking down organic matter and most chemical transformations in the soil (USDA, 1998). The type and population densities of these microorganisms change with respect to the pH of the soil. A pH of 6.6 to 7.3 (a neutral soil) is favorable for microbial activities that contribute to availability of nitrogen, sulphur, and phosphorus in soils. Thus, soil sample #1 is on its range which may harbor a number of microorganisms unlike the other two samples which has a pH above the neutral line.

b) Presumptive Identification

The isolated colony that was identified as Gram-positive was allowed to grow on sporulation medium to induce endospore formation and to identify bacterial isolates belonging to the genus *Bacillus.* The most distinguishing feature of *B.thuringiensis* from closely related bacillus species (e.g. *B.cereus, B. anthracis*) is the presence of a parasporal crystal body that is near to the spore, outside the exosporangium during endospore formation (Andrews et al., 1985, 1987; Bulla et al., 1995). Bt is a member of the genus *Bacillus* and like the other members of the taxon, it has the ability to form endospores that are resistant to inactivation by heat, desiccation and organic solvents. Another distinguishing feature is the production of endospores, which are highly refractile resting structures formed within the bacterial cells (Todar, 2005).

The three sampling sites harbor a number of microorganisms that are able to tolerate extreme conditions. Out of the number of isolates, only twenty (20) were randomly chosen and were successfully purified and characterized. The identification of bacteria was based on cultural, cellular, and biochemical characteristics exhibited by each respective species and strain (Figure 1).

c) Antibiotic Susceptibility

The twenty (20) purified isolates were subjected to antibiotic assay to determine the isolates' sensitivity to a set of antibiotics available. The isolates were tested for resistance to amoxicillin, ofloxacin, ampicilin, tetracycline, and streptomycin (Figure 2).

Identified *B.thuringiensis* from verrmicast was susceptible to three out of five broad spectrum antibiotics (Figure 3). Figure 4 shows the sensitivity of the isolates from the two other soil samples. Isolates show amoxicillin and ampicillin resistance (Figure 2). Ampicillin and amoxicillin are closely related belonging to a class of antibiotics called penicillins that are used for treating bacterial infections. These antibiotics all have a similar mechanism of action; stopping bacteria from multiplying by preventing it from forming the walls that surround them. Bt which has been long considered as non-pathogenic and used extensively for pest control were found out to be resistant to the β-lactams (amoxicillin and ampicillin). This characteristic is essential in the identification of *B.thuringiensis* species for most of the said species are resistant to the said antibiotics while susceptible to the remaining antimicrobials (Luna et al., 2007).

Based on the conventional method of characterization and antibiotic resistance of the microorganisms isolated, out of the twenty (20) isolates, only ten (50%) were presumptively identified as *B. thuringiensis* and the ten isolates are still identified as belonging to the genus *Bacillus* spp. (Table 2).

IV. Summary and Conclusion

The purpose of this first paper was to isolate *B. thuringiensis* strains that are collected from three soil samples around Iligan City by Sodium Acetate method by Travers et al (1987), in order to select the target organism. As a result, there is a number of different *Bacillus* sp. that was isolated. Vermicast type of soil which has a pH of 7.1 harbors the most number of microorganisms. Out of the numerous counts of isolated colony, only twenty colonies were purified and characterized by conventional method of identification according to the Bergey's manual of bacteriology and its antibiotic resistance against β-lactam antibiotics

namely ampicillin and amoxicillin. From the twenty characterized colonies, ten were identified as *B.thuringiensis* based on colonial morphology, gram stain, presence of endospore, cell diameter and its resistance to ampicillin and amoxicillin.

References Références Referencias

1. Attathom TW, Chongrattanameteekul, JC, Siriyan R. (1995). Morphological diversity and toxicity of delta-endotoxin produced by various strains of *Bacillus thuringiensis*. Bull.Entomol.Res. 85:167-173.
2. Bauer, AW, Kirby, WMM, Sherris, JC (1966). Antibiotic susceptibility testing by a standard sigle disc method.American Journal of Clinical Pathology. 45: 493-496.
3. Bulla, LA Jr, Bechtel DB, Kramer KJ, Shethna YI, Aronson AI, Fitz-James PC (1980). Ultrastructure, physiology, and biochemistry of *Bacillus thuringiensis*. Crit. Rev. Microbiol. 8:147-204.
4. Luna, AV, King, SD, Gulledge, J, Cannons, CA, Amuso, TP, Cattani, J (2007). Susceptibility of *Bacillus anthracis, Bacillus cereus, Bacillus mycoides, Bacillus pseudomycoides and Bacillus thuringiensis* to 24 antimicrobials using Sensititre automated microbroth dilution and Etest agar gradient diffusion methods. Journal of Antimicrobial Chemotherapy. 60(3), 555-567.
5. Madigan, M, Martinko, J (2005). Brock Biology of Microorganisms.11th EditionPrentice Hall, USA. pp. 545-572.
6. USDA Natural Resources Conservation Service. (1998). Soil Quality Indicators:pH. http://soils.usda.gov/ sqi/publications/files/indicate.pdf
7. Samsonov P, Padron RI, Pardo C, Cabrera J, Deal GA (1997). *Bacillus thuringiensis* from biodiversity to biotechnology. J. Ind. Microbiol. Biotech. 19: 202-219.
8. Travers R, Martin P, Relchelderfer C (1987). Selective Process for Efficient Isolation of *Bacillus* spp. Appl. Environ. Microbiol. 53(6):1263-1266.

Table 1 : The three (3) soil description based on its physical properties and pH.

Sample Number	Soil Description	Texture	pH
1	dark gray loam, high humus content	loamy	7.06
2	grayish brown loamy sand, low humus content	sandy loam	7.64
3	grayish light-brown sandy loam, low humus content	sandy loam	7.37

Legend: (1) Carbide Village; (2) Barangay Luinab; (3)Barangay Tibanga

Table 2 : Colonial morphological characteristics of bacterial isolates.

Isolates	Colony Size (mm)	Edge/Margin	Elevation	Surface	Shape	Identification
1A	3	Entire	Flat	Glistening	Round	*Bacillus* spp.
1B,1D	2.5	Entire	Raised	Dull	Round	*B.thuringiensis*
1C,1G	2	Entire	Raised	Dull	Round	*B.thuringiensis*
1E,1F	3	Entire	Raised	Dull	Round	*B.thuringiensis*
1H	1.5	Undulate	Raised	Glistening	Irregular	*Bacillus* spp.
1I	3	Entire	Raised	Dull	Round	*Bacillus* spp.
2A	2.5	Entire	Raised	Dull	Round	*Bacillus* spp.
2B	1	Entire	Raised	Glistening	Round	*Bacillus* spp.
2C	<1	Entire	Raised	Dull	Round	*Bacillus* spp.
2D,2F	3	Entire	Raised	Dull	Round	*B.thuringiensis*
2E	1	Entire	Raised	Glistening	Round	*Bacillus* spp.
3A	2	Undulate	Flat	Dull	Irregular	*Bacillus* spp.
3B,3C	4	Entire	Raised	Dull	Round	*B.thuringiensis*
3D	4	Entire	Raised	Dull	Round	*Bacillus* spp.
3E	1.5	Entire	Raised	Glistening	Round	*Bacillus* spp.

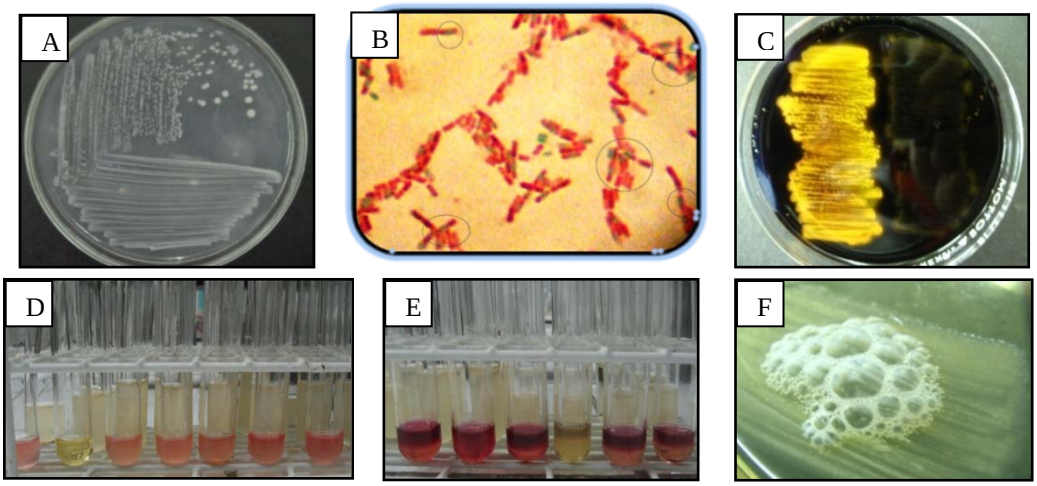


Figure 1 : Conventional method of identification *Bacillus thuringiensis* : (A) purified culture; (B) endospore staining; (C) Starch hydrolysis; (D) Methyl Red; (E) Voges- Proskauer; (F) Catalase test.

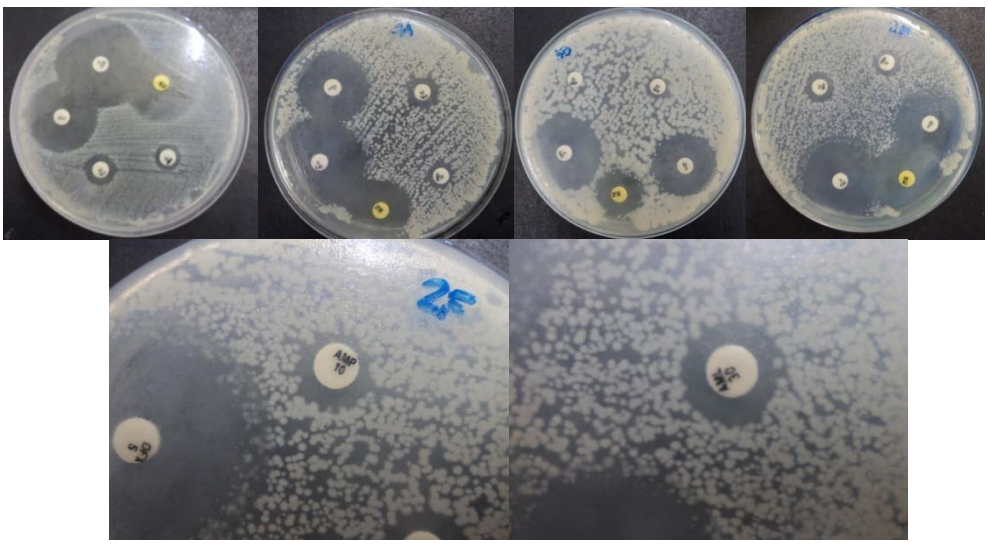
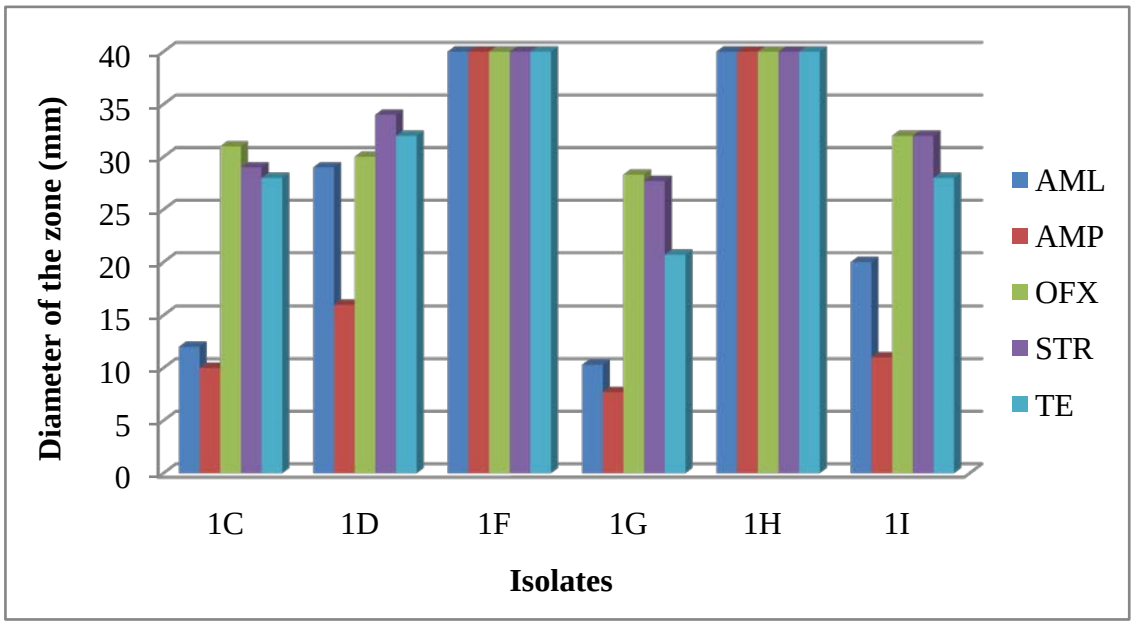
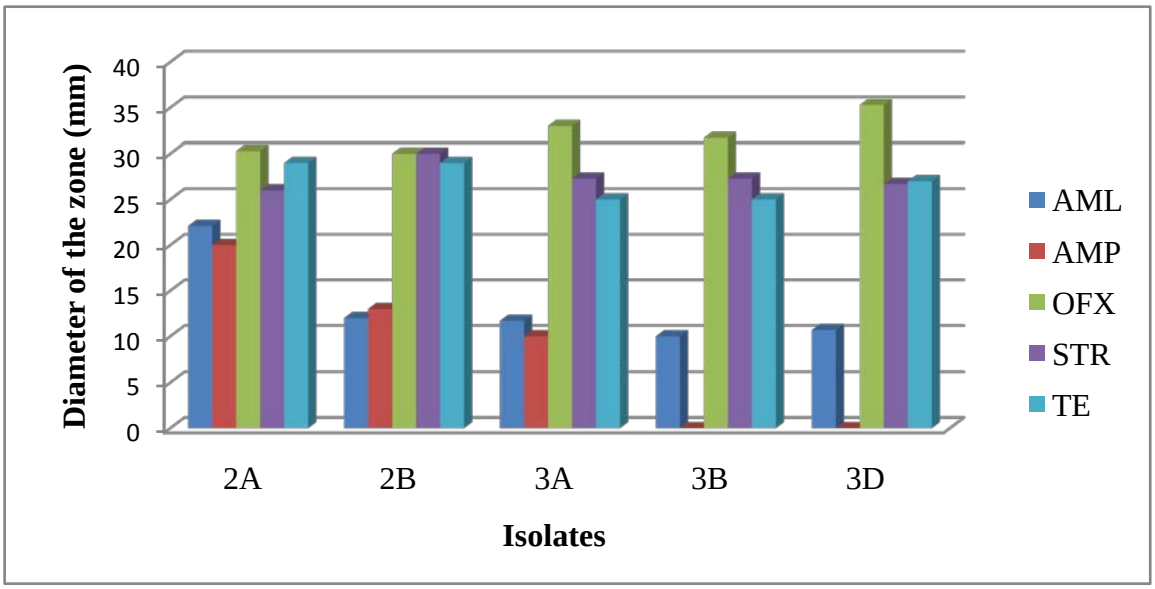


Figure 2 : Ampicillin and Amoxicillin resistant isolates.



Legend: AML – Amoxicillin; AMP – Ampicillin; BT- *Bacillus thuringiensis*; OFX-Ofloxacin; TE – Tetracycline; STR- Streptomycin; BS- *Bacillus subtilis*

Figure 3 : Antibiotic sensitivity of isolates from vermicast type of soil isolated from Carbide Village, Iligan City.



Legend: AML – Amoxicillin; AMP – Ampicillin; BT- *Bacillus thuringiensis*; OFX-Ofloxacin; TE – Tetracycline; STR- Streptomycin; BS- *Bacillus subtilis*

Figure 4 : Antibiotic sensitivity of isolates from Tibanga (2A-2B) and Luinab (3A, 3B, and 3D) soil samples.