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Antimicrobial activity of bacteriocins produced by different strains of *Bacillus Thurigiensis* (B*T*) against some indictors bacteria, under different experimental conditions

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Abstract
The project objective was to scrutinize the effect of bacteriocins fashioned by *Bacillus Thuringiensis* (B*t*) strains have gotten from Middle Tennessee (USA) on three bacterial strains, *Pseudomonas aeruginosa, Bacillus cereus* and *Staphylococcus aureus,* under different experimental conditions which were temperature, pH, and incubation time. The clear inhibition zone on plates (20mm, 12mm) indicated that 32 B*t* strains out of 66 tested B*t* strains had produced bacteriocin. It was apparent that no bacteriocin was produced at 15 hours of incubation, while a maximum production was noted at incubation time of 24 h. Our results had generally declared the effect of the pH and the temperature on the B*t* antimicrobial activity through bactriocince production, that is, almost all bacteriocins' strains of B*t* exhibited antibacterial activity at pH between 7-9 against all bacteria used as indicators, and bacteriocins production was sensitive to higher temperatures.

Keywords: *Bacillus Thuringiensis;* Bacteriocins; Antimicrobial activity, pH, temperature

Introduction
The placement of *Bacillus thuringiensis* (B*t*) bacteria as a separate species within the genus Bacillus has been controversial up to 1973, other similar species such as B*a* and B*c* share many common phenotypic and genotypic properties with B*t*, to the extent that the three species have been placed under one group called the Bacillus cereus (B*C*) group (Gordon RL, 1973) [1].

(B*t*) is a unique bacterium in its similarity to some chemical compounds used as a pesticide. The antimicrobial activity spectrum of B*t* is wider than many other bacteria used for that purpose (Mohamed A. Ibrahim, 2010) [2].

By nature, *Bacillus thuringiensis* (B*t*) comprising of gram-positive bacteria, it undergoes protein synthesis to generate two kinds of toxins known as *Cyt* and *Cry* occur in the form of parasporal crystals and comprise at least one protein christened *Cry* proteins (Bravo *et al*., 2018) [3]. The *Cry* proteins are specific to target insects, thus incorporated in various crops to mitigate insect attack (Ujváry, 2010) [4]. These proteins were antimicrobial peptides that classify under bacteriocins (Dobson *et al*., 2012) [5].

Bacteriocins are a group of antimicrobial compounds, which are ribosomally synthesized peptides produced by bacteria to inhibit the growth of similar or closely related bacterial strains either in the same species, or across genera (Bowdish *et al*., 2005; Cotter *et al*., 2005) [6, 7].

Bacteriocins exist in four classes, namely those manufactured by gram-negative bacteria and gram-positive species (Chen & Hoover, 2003) [8].

Studies have shown that bacteriocins can be utilized for food preservation due to its safe nature. It has proved to be safer than antibiotics and chemical preservatives mostly used by various industrial sectors (Ramu *et al*., 2015) [9].

Bacteriocins considered as alternatives to antibiotics or other chemicals regarding food preservation. The inherent ability to inhibit microorganisms contamination makes it the most reliable to use for preservation purposes (Chen & Hoover, 2003) [8].

Bacteriocin production can be influenced by culture conditions, such as incubation atmosphere, pH, temperature and microbial growth phase (Zhou *et al*., 2015; Turgis *et al*., 2016) [10, 11]. Bacteriocin production by LAB has been reported as a temperature-sensitive process (Leroy and De Vuyst 1999) [12].

However, it is necessary to study the impact of microbiological culture circumstances such as pH, and temperature, on the bacteriocin activity and viability of bacterial strains. In this study Antimicrobial activity of bacteriocins produced by different strains of *Bacillus thurigiensis* (B*t*) against some indictors bacteria (*Staphylococcus, P. aeruginosa, and Bacillus cereus*). Under different microbiological culture circumstances which were pH, and temperature.

2. Methodology
A number of 66 *Bacillus thurigiensis* (B*t*) strains isolations from Middle Tennessee, USA, were tested for the presence of bacteriocin.

Agar well diffusion technique was used to determine the activity of bacteriocin strains (Ogubanwo *et al*., 2003) [13]. The agar well diffusing assay involved the usage of three bacterial types which were *Bacillus cereus (CB154870A), Staphylococcus aureus (CB155554A),* and *Pseudomonas aeruginosa (CB155250A)* as indicator organisms. The culturing process lasted between 24 and 48 hours of incubation for the three bacteria using Luria-Bertin (LB) agar (Ejiofor & Johnson, 2002) [14]. First, the temperature was maintained at 30ºC, and then set at 60°C, 70°C, 80°C, 90°C, and 100°C, it was then kept at room temperature for a few hours (4hrs).

For comparing bacteriocin sensitivity in different pH values, the cultures had been set at pH ranges of 3 to 9. The next

step involved the determination of the antimicrobial activity against the indicator organism as outlined by Larsen *et al*. (1993) [15]. The dynamic inhibition zones were exploited to weigh the impact of bacteriocins on the bacterial strains, and consequently, the bacteriocins production by B*t* strains.

3. Results & Discussion

In general, our experimentation has indicated that 32 out of 66 strains of the *Bacillus thuringiensis* (B*t*) validated as bacteriocin producers. In literature, *Bacillus thuringiensis* has been shown to exhibit antimicrobial properties via its aptitude to synthesize bacteriocins against specific strains of bacteria. The production of bacteriocins varies between B*t* varieties and was impacted by the pH and temperature variations (Dobson *et al*., 2012; Ugras *et al*., 2013) [5, 16].

Some bacteria types other than B*t* were studied and reported in the international literature as bactriocine producers, such as *Bacillus cereus*, *B. Weihenstephanensis*, and *L. monocytogenes* (Djenane *et al*., 2017; Salazar-Marroquín *et al*., 2016) [17, 18].

Figures 1, 2, and 3 were showing zones of inhibition on lawns of the three bacterial indicators (*Staphylococcus, P. Aeruginosa, and Bacillus cereus*) inhibited by *Bacillus Thurigiensis* (B*t*) after 24 hours incubation. The inhibition zone was (20mm) by B*t* 71 against *Staphylococcus*, (12mm) by B*t* k and B*t* 71 against *P. Aeruginosa*, and (12mm) by B*t* 70 and B*t* 71 against *Bacillus cereus*.

It was apparent that no bacteriocin was produced at 15 hours of incubation; while the maximum production of bacteriocin was noted when the incubation time was 24 hours.

Results showed the resistance of *Bacillus cereus* to almost all bacteriocins at 60, 70, and 80 degrees Celsius, and pH range of 7-9. And showed the resistance of *Staphylococcus aureus* to most of bacteriocins other than B*t* 3, B*t* 4, B*t* 5, B*t* 10, B*t* 18, B*t* 27, B*t* 33, and B*t* 38 at 60, 70, 80, 90, and 100 ^{o}C, and pH range of 7-9 (Table 1).

Our results of *Bacillus cereus* resistance to bacteriocin were similar to those results reported by Raymond *et al*. (2010) [19], and Sharma *et al*. (2006) [20]. In general, we found that almost all bacteriocins' strains of B*t* exhibited antibacterial activity at a pH between 7-9 against all bacterial indicators (Table 1 & Table 2). Additionally, it was noticed that bacteriocins production is sensitive to low and high pH values and higher temperatures, the result was similar to the result reported by Yang *et al*. (2014) [21].

Being the most sensitive bacteria, *Staphylococcus aureus* responded to swift temperature changes (Table 2) thus may enhance the production of bacteriocins by B*t*.

These results were supported by some studies cited in the literature, which assessed the impact of varying temperatures and a pH on bacteriocins produced by B*t* (Salazar-Marroquín *et al*., 2016) [18], besides some other experimental factors such as lack of nutrients. Chehimi *et al*. (2007) [22] had reported that the bacteriocins isolated from *Bacillus thuringiensis* exhibited antibacterial traits at pH between 3-9 (Chehimi *et al*., 2007) [22]. Some other studies indicated that bacteriocin strains were resistant to a pH between 4.5 and 7 but sensitive to a pH above 9 (Mohankumar & Murugalatha, 2011) [23].

Studying the effect of factors such as a pH, temperature, and the impact of incubation on bacteriocins production may be used as characterization criteria for these bacteriocins (Mohankumar & Murugalatha, 2011) [23].

Our results had generally declared the effect of the pH and temperature on the B*t* antimicrobial activity through bactriocince production. These results are supporting the theory of using bacteriocince as an alternative of some chemical antibiotics, and that is crucial to humanity and other living organisms (Lee & Kim, 2011) [24], reminding that B*t* is applied widely as an environmentally compatible and safe bio pesticide (Ujváry, 2010) [4], as they are harmless to the environment and the human body (Ramu *et al*., 2015) [9].

Fig 1: Zone of inhibition on a lawn of *Staphylococcus* by *Bt* after 24 hour of incubation The inhibition zone was (20mm) by *Bt* 71. There was no bacteriocin activity detected on the other *Bt* strains.

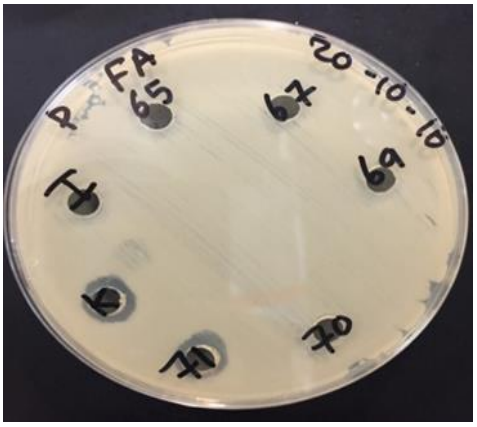

Fig 2: Zone of inhibition on a lawn of *P. Aeruginosa* by *Bt k and Bt* 71 after 24 hour of incubation. The inhibition zone was (12mm) by *Bt k* and there was antimicrobial activity around *Bt* 71 and the size was around (12 mm). There was no bacteriocin activity detected on the other *Bt* strains.

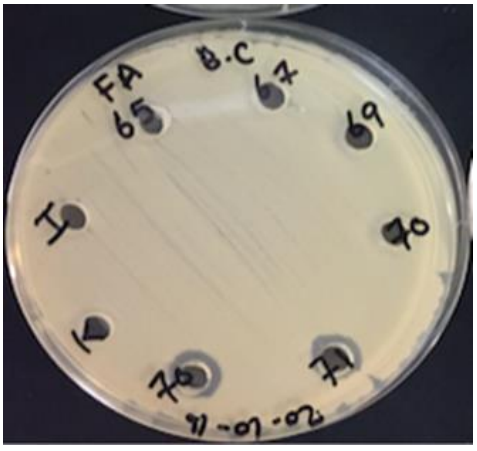

Fig 3: Zone of inhibition on a lawn of *Bacillus cereus* by Bt 70 and Bt 71 after 24 hour of incubation the inhibition zone was (12mm) in both Bt strains.

Table 1: The effect of temperature & PH on the bacteriocins produced by different strains of *B. thuringiensis* against *Bacillus cereus.*

Bt	Heating (10 min)						pH			
	control	60 °C	70 °C	80 °C	90 °C	100℃	3	5	7	9
Bt1	R	R	R	R	R	R	S	S	R	R
Bt3	R	R	R	R	R	S	S	S	R	R
Bt 4	R	R	R	R	R	S	S	S	R	R
Bt 5	R	R	R	R	R	S	S	S	R	R
Bt 10	R	R	R	R	R	R	S	R	R	R
Bt 12	R	R	R	R	S	S	S	S	R	R
Bt 13	R	R	R	R	S	S	S	S	R	S
Bt 18	R	R	R	R	R	S	S	S	R	R
Bt 20	R	R	R	R	S	S	S	S	R	R
Bt 21	R	R	R	R	R	R	S	R	R	R
Bt 22	R	R	R	R	R	R	S	S	R	R
Bt 25	R	R	R	R	S	S	S	S	R	R
Bt 26	R	R	R	R	R	S	S	S	R	R
Bt 27	R	R	R	R	R	R	S	S	R	R
Bt 29	R	R	R	R	R	R	S	S	R	R
Bt 30	R	R	R	R	R	R	S	S	R	R
Bt 32	R	R	R	R	R	R	S	S	R	R
Bt 33	R	R	R	R	R	S	R	R	R	R
Bt 35	R	R	R	R	R	S	S	S	R	R
Bt 36	R	R	R	R	R	R	S	R	R	R
Bt 38	R	R	R	R	R	R	S	S	R	R
Bt 44	R	R	R	R	R	S	S	S	R	R
Bt 57	R	R	R	R	R	R	S	S	R	R
Bt 62	R	R	R	R	R	S	S	S	R	R
Bt 63	R	R	R	R	R	S	S	S	R	R
Bt 65	R	R	R	R	R	R	S	S	R	R
Bt 72	R	R	R	R	R	R	S	S	R	R
Bt K	R	R	R	R	R	R	S	S	R	R

R= Resistance = antimicrobial activity, S= Sensitive = no antimicrobial activity

Table 2: The effect of temperature & PH on the bacteriocin produced by different strains of *B. thuringiensis* against *Staphylococcus aureus.*

Bt	Heating (10 min)						pH			
	control	60 °C	70 °C	80 °C	90 °C	100℃	3	5	7	9
Bt1	R	R	R	R	R	S	S	S	R	R
Bt3	S	S	S	S	S	S	S	S	R	R
Bt 4	S	S	S	S	S	S	S	S	R	R
Bt 5	S	S	S	S	S	S	S	S	R	R
Bt 10	S	S	S	S	S	S	S	R	R	R
Bt 12	R	R	R	R	S	S	S	R	R	R
Bt 13	R	R	R	R	S	S	S	S	R	R
Bt 18	S	S	S	S	S	S	S	S	R	R
Bt 20	R	R	R	R	R	S	S	R	R	S
Bt 21	R	R	R	R	R	R	S	S	R	R
Bt 22	R	R	R	R	R	S	S	S	R	R
Bt 25	R	R	R	R	R	R	S	S	R	R
Bt 26	R	R	R	R	R	R	S	S	R	R
Bt 27	S	S	S	S	S	S	S	S	R	R
Bt 29	R	R	R	R	R	R	S	R	R	S
Bt 30	R	R	R	R	R	S	S	S	R	R
Bt 32	R	R	R	R	R	R	S	S	R	R
Bt 33	S	S	S	S	S	S	S	S	R	R
Bt 35	R	R	R	R	R	S	S	S	R	R
Bt 36	R	R	R	R	R	R	S	S	R	R
Bt 38	S	S	S	S	S	S	S	S	R	R
Bt 44	R	R	R	R	S	S	S	S	R	R
Bt 57	R	R	R	R	R	R	S	R	R	S
Bt 62	R	R	R	R	R	R	S	S	R	R
Bt 63	R	R	R	R	R	S	S	S	R	R
Bt 65	R	R	R	R	R	S	S	S	R	R
Bt 72	R	R	R	R	R	R	S	S	R	R
Bt K	R	R	R	R	R	R	R	R	R	R

R= Resistance = antimicrobial activity, S= Sensitive = no antimicrobial activity

Table 3: The effect of temperature & PH on the bacteriocin produced by different strains of *Bt* against *P. aeruginosa*

Bt	Heating (10 min)						pH			
	control	60 °C	70 °C	80 °C	90 °C	100℃	3	5	7	9
Bt1	R	R	R	R	R	R	S	S	R	S
Bt3	R	R	R	R	S	S	S	S	R	S
Bt 4	R	R	R	R	S	S	S	R	R	S
Bt 5	R	R	R	R	S	S	S	S	R	R
Bt 10	R	R	R	R	R	R	S	S	R	R
Bt 12	R	R	R	R	R	S	S	S	R	R
Bt 13	R	R	R	R	R	S	S	S	R	R
Bt 18	R	R	R	R	S	S	S	S	R	R
Bt 20	R	R	R	R	S	S	S	R	R	S
Bt 21	R	R	R	R	R	S	S	S	R	R
Bt 22	R	R	R	R	R	R	S	R	R	R
Bt 25	R	R	R	R	S	S	R	R	R	R
Bt 26	R	R	R	R	S	S	S	S	R	R
Bt 27	R	R	R	R	R	R	S	S	R	R
Bt 29	R	R	R	R	R	R	S	S	R	R
Bt 30	R	R	R	R	R	S	S	S	R	R
Bt 32	R	R	R	R	S	S	S	S	R	R
Bt 33	R	R	R	R	S	S	S	S	R	R
Bt 35	R	R	R	R	S	S	S	S	R	R
Bt 36	R	R	R	R	R	R	S	S	R	R
Bt 38	R	R	R	R	R	R	S	S	R	R
Bt 44	R	R	R	R	S	S	S	S	R	S
Bt 57	R	R	R	R	R	R	S	S	R	R
Bt 62	R	R	R	R	R	S	S	S	R	R
Bt 63	R	R	R	R	R	S	S	R	R	R
Bt 65	R	R	R	R	R	R	S	S	R	R
Bt 72	R	R	R	R	R	R	S	R	R	R
Bt K	R	R	R	R	R	R	S	R	R	R

R= Resistance = antimicrobial activity, S= Sensitive = no antimicrobial activity

4. Conclusion

The experimentation outcome showed that 32 B*t* strains out of 66 tested B*t* strains had produced bacteriocin, this production was evidenced by the presence of a clear inhibition zone on plates, which demonstrated the formation or creation of bacteriocin. Three bacteria types were used as indicators for bacteriocin production, namely *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Staphylococcus aureus.* It was apparent that no bacteriocin was produced at 15 hours of incubation, while a maximum production was noted on incubation time of 24 h. bacteriocins production was sensitive to low and high pH values and higher temperature.

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