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The Effects of Bacillus Thuringiensis Kurstaki (Btk) on Sheep Blood

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Abstract: **Bacillus Thuringiensis Kurstaki (Btk) is widely used as an insecticide which is effective against lepidopterans, but is it safe for animals to ingest? What consequence does it have on animal blood? Through my research I had the desire to find the answers to these questions, which is why I exposed numerous sheep blood samples inoculated with Btk at different fixed temperatures. Once exposed to these temperatures, I observed the visual characteristics of the blood samples and used microscopy to view past what the naked eye could see. My findings to this research returned quite fascinating results which could question whether the use of Bacillus Thuringiensis Kurstaki is a safe choice of insecticide if used on plants and crops which are intended for animal consumption. Although the Bacilli bacterium does not directly implicate the function of blood, it does introduce the presence of micro-organisms too large to be bacterial which can have a negative impact on the blood function.**

Keywords: **Bacillus Thuringiensis Kurstaki (Btk), animals, sheep blood.**

1. INTRODUCTION

Bacillus is a genus of gram-positive, rod-shaped bacteria and a member of the phylum Firmicutes which contains an innumerable amount of unique species. Some of these known species are pathogenic and cause disease in human hosts which as a result, explains why Bacilli bacterium is so well known throughout the Microbiology community.

One particular species of Bacilli; Bacillus Thuringiensis (Bt) possesses very effective insecticidal properties when it undergoes sporulation. Through this process, many Bt strains produce crystal proteins called delta-endotoxins (E.Schnepf et al. 1998) which are deadly to insects which is why it is used so widely on crops and plants as an insecticide.

As a result of this popular choice for pest control, I wanted to monitor and observe the effects that Bacillus Thuringiensis had on animal blood after an animal had digested an item which was infected by this species of Bacilli. The strain that I isolated in particular was Bacillus Thuringiensis Kurstaki (Btk) EG 2348 which is effective against a wide range of lepidopterans.

Warning:

Live strains of bacteria are being used throughout this method, so care should be taken. Only fully trained personnel should perform the following method.

Personal protective equipment should be worn at all times, including safety glasses, laboratory coat and sterile gloves at a minimum.

A full risk assessment should be performed prior to following this method.

Materials:

Bacillus Thuringiensis Kurstaki (Strain EG 2348) pure culture.

Pre-made blood agar base (SHEEP) plates with relevant certificates of analysis and certificates of conformance. (Quantity: 2)

Defibrinated sheep blood with relevant certificates of analysis and certificates of conformance. (Quantity: 6 x 10ml)

Sterile 1μL inoculation loops. (Quantity: 4)

Pasteur pipettes. (Quantity: 6)

Microscope slides. (Quantity: 6)

Microscope with 40x magnification.

37.0°C incubator with a tolerance of +/- 1.0°C.

7.0°C chiller with a tolerance of +/- 0.5°C.

2. METHOD

Initially I sub-cultured a 1μL loop of Bacillus Thuringiensis Kurstaki culture onto a pre-made blood agar base so that I could isolate individual colonies. In order to do this, I incubated the inoculated blood plate along side a non-inoculated blood plate at 37.0°C for 24 hours +/- 2 hours.

After 24 hours had passed I retrieved the two plates from the incubator where I found the inoculated plate to contain white colonies with a diameter of > 0.9μm and a wide haemolytic zone around these colonies. The non-inoculated plate had no growth, which acted as my sterile control.

To confirm these colonies were Bacillus Thuringiensis Kurstaki isolates, typical confirmation stages should be followed prior to following the rest of this method. For a fast confirmation, the use of Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) may speed up this process.

Now that I have isolated individual confirmed colonies, I aseptically used a 1μL loop to transfer 5 different colonies to 10ml of defibrinated sheep blood. I did this three times, so that I had 3 vials of 10ml inoculated sheep blood and 3 vials of non-inoculated sheep blood.

Once inoculation had been achieved to a point where I was confident that contamination had not taken place, I individually labelled the 6 vials of sheep blood in the following way: 1 vial of inoculated sheep blood and 1 vial of non-inoculated sheep blood were labelled with (A) 37.0°C.

Secondly, 1 vial of inoculated sheep blood and 1 vial of non-inoculated sheep blood were labelled with (B) 7.0°C.

Finally, 1 vial of inoculated sheep blood and 1 vial of non-inoculated sheep blood were labelled with (C) 37.0°C and 7.0°C.

Vials (A) were then incubated at 37.0°C for 48 hours +/- 4 hours.

Vials (B) were chilled at 7.0°C for 48 hours +/- 4 hours.

Vials (C) were incubated at 37.0°C for 24 hours +/- 2 hours followed by 7.0°C for 24 hours +/- 2 hours.

Following the incubation of Vials (A):

After Vials (A) had been incubated at 37.0°C for 48 hours +/- 4 hours, the non-inoculated vial of sheep blood had <u>no visible signs</u> of change (acting as my sterile control) and the inoculated vial of sheep blood had a <u>colour change</u> from a blood red, to a darker red / purple.

Using a Pasteur pipette I transferred a small volume of the inoculated sheep blood to a microscope slide and allowed to air dry. I also did the same for the non-inoculated sheep blood.

Under 40x magnification I counted a <u>high amount of circular micro-organisms</u> in the inoculated blood sample. These unidentified organisms were too big to be bacterial, but fit the profile more towards being protozoa.

Under 40x magnification I did not count any micro-organisms within the non-inoculated blood sample.

Following the incubation of Vials (B):

After Vials (B) had been chilled at 7.0°C for 48 hours +/- 4 hours, the non-inoculated vial of sheep blood had <u>no visible signs</u> of change (acting as my sterile control) and the inoculated vial of sheep blood had <u>no visible signs</u> of change.

Using a Pasteur pipette I transferred a small volume of the inoculated sheep blood to a microscope slide and allowed to air dry. I also did the same for the non-inoculated sheep blood.

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Under 40x magnification I counted a very small amount of circular micro-organisms in the inoculated blood sample. These unidentified organisms were too big to be bacterial, but fit the profile more towards being protozoa.

Under 40x magnification I did not count any micro-organisms within the non-inoculated blood sample.

Following the incubation of Vials (C):

After Vials (C) had been incubated at 37.0℃ for 24 hours +/- 2 hours, the non-inoculated vial of sheep blood had no visible signs of change (acting as my sterile control) and the inoculated vial of sheep blood had a slight colour change from a blood red, to a darker red / purple.

Using a Pasteur pipette I transferred a small volume of the inoculated sheep blood to a microscope slide and allowed to air dry. I also did the same for the non-inoculated sheep blood.

Under 40x magnification I counted a considerable amount of circular micro-organisms in the inoculated blood sample. These unidentified organisms were too big to be bacterial, but fit the profile more towards being protozoa.

Under 40x magnification I did not count any micro-organisms within the non-inoculated blood sample.

Both Vials were then put into a chiller 7.0℃ for 24 hours +/- 2 hours.

After Vials (C) had been chilled at 7.0℃ for 24 hours +/- 2 hours, the non-inoculated vial of sheep blood had no visible signs of change (acting as my sterile control) and the inoculated vial of sheep blood had no visible signs of change from its original colour. In other words, its slight colour change from a darker red / purple had revered.

Using a Pasteur pipette I transferred a small volume of the inoculated sheep blood to a microscope slide and allowed to air dry. I also did the same for the non-inoculated sheep blood.

Under 40x magnification I counted a very small amount of circular micro-organisms in the inoculated blood sample. These unidentified organisms were too big to be bacterial, but fit the profile more towards being protozoa.

Under 40x magnification I did not count any micro-organisms within the non-inoculated blood sample.

3. RESULTS

Vial (A) Inoculated Blood: Dark red / purple colour change and high micro-organism count. (Figure 1.)

Vial (A) Non-inoculated Blood: No colour change and no micro-organism count.

Vial (B) Inoculated Blood: No colour change and very low micro-organism count. (Figure 2.)

Vial (B) Non-inoculated Blood: No colour change and no micro-organism count.

Vial (C) Inoculated Blood: Slight dark red / purple colour change with considerable micro-organism count after 24 hours incubation. Colour change reversed and low micro-organism count after further 24 hours being chilled. (Figure 3.)

Vial (C) Non-inoculated Blood: No colour change and no micro-organism count.

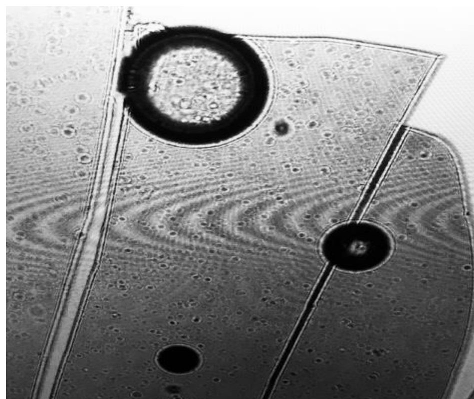

Figure 1: Micro-organisms at 40x magnification

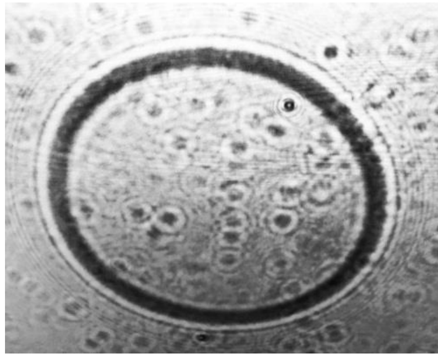

Figure 2: Micro-organism at 40x magnification

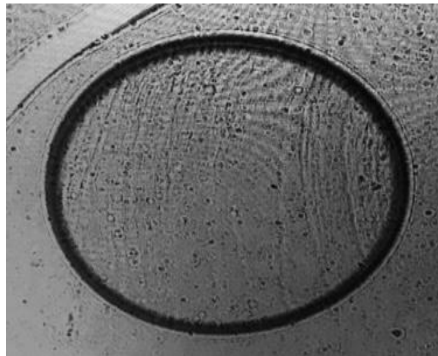

Figure 3: Micro-organism at 40x magnification

4. DISCUSSION

So what do these observations mean? Ultimately I would hypothesis using the evidence from the results that the introduction of Bacillus Thuringiensis Kurstaki (Btk) to animal blood promotes the presence of unidentified micro-organisms, most probable to be protozoa. A higher concentration of this unknown organism is present when they are exposed to body temperature (37.0°C) for a period of time. This causes a colour change in the blood from a blood red, to a darker red / purple, likely due to de-oxygenated blood cells within the sample.

The presence of this unknown organism can be inhibited and the colour change of the blood can be reversed by exposing the blood sample to a chilled state (around 7.0°C).

This observation is only true for sheep blood, and I would like to further research my hypothesis on other animal blood, including but not exclusively; horse blood and human blood. I would also like to observe the effects that other Bacillus Thuringiensis subspecies have on blood, for example Bacillus Thuringiensis Israelensis (Bti).

5. CONCLUSION

As a result of the above method, I can conclude that the presence of Bacillus Thuringiensis Kurstaki (Btk) in sheep blood may not have any direct implications on blood cells, however it does introduce the presence of unidentified micro-organisms which themself may pose a threat to the function of blood cells. This does question whether Bacillus Thuringiensis Kurstaki is a safe insecticide to use on plants and crops which are intended for consumption by animals, including humans.

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