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Research article

Detection the presence of *Bdellovibrio bacteriovorus* in the bacterial DNA extracts of *Culex pipiens* intestine using polymerase chain reaction

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Abstract

Mosquitoes are tiny creatures but could do a lot of damages to the people life and especially by transmitting dangerous diseases such as dengue fever transported by Aedes aegypti. The control of these bugs is hostorically challenging and difficult. This difficulty increases after the emerging problems of insecticide resistance. The gut flora(GF) in different animals has been detected to place an important role on the physiology of the intestine. This study was conducted to discover if the Bdellovibrio bacteriovorus is a member of the GF of the mosquitoes. B. bacteriovorus is a bacterium that has been found in the intestine of animals and healthy and patient humans but not in mosquitoes. Seventy adult mosquitos of Culex pipiens were used for extraction of the bacterial DNA from their intestine. The 70 mosquitoes were assigned randomly to 7 groups, 10 mosquitoes for each group to pool the amount of DNA extracted. Each mosquito was dissected under the microscope to isolate the intestine and use it later for DNA extraction. After the DNA had been extracted using a phenol/chloroform method, I subjected the extracted DNA to polymerase chain reaction (PCR) to detect the presence of this bacterium among the bacteria of the intestine using the following specific primers: Bd529F (5'-GGTAAGACGAGGGATCCT-3') and Bd1007R (5'-TCTTCCAGTACATGTCAAG-3') that amplify a 481-bp of the 16S rRNA gene. By the amplification that happened in all 7 groups, the result indicates the presence of Bdellovibrio bacteriovorus in the intestine of the Culex pipiens. Because this bacterium preys on Gram negative bacteria, our results help to use this bacterium to fight insecticide resistance that caused by the degradation of these chemicals by gram negative bacteria in the ingestion of these mosquitoes.

Keywords: *Bdellovibrio bacteriovorus*, *Culex pipiens*, Mosquitoes, PCR, Bacteria

Introduction

Stolp *et al*. (1962) (1) were the first to find out about *Bdellovibrio bacteriovorus*. A Gram negative, tiny, and motile bacterium preys other Gram-negative bacteria. *Bd. bacteriovorus* belongs to *Deltaproteobacteria* group of bacteria (2; 3; 4) and can be found in soil, rivers, seas, sewer, feces of animals and humans and their digestive tracts, sells of oyster, and crab gills (5, 6). These bacteria have been found in the gut of healthy and patient human (7). *Hit* locus was recognized to affect the capability to prey bacteria (8). Due to its predatory behavior, scientists have used them in experimental cures of some diseases that caused by Gram-negative bacteria such as pink eye in cattle (9) and dental diseases (10). Explanation of the lifestyle of this bacterium is shown in figure 1 (2). PCR is the method of choice to determine this bacterium using the 16S rRNA gene (11; 12). I used PCR with specific primers to this bacterium, which are Bd529F (5'-GGTAAGACGAGGGATCCT-3') and Bd1007R (5'-TCTTCCAGTACATGTCAAG-3') that amplify a 481-bp of the 16S rRNA gene (7). My current study result has shown the

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presence of these bacteria in DNA samples of 70 *Culex pipiens* mosquitoes. This mosquito often develops in water polluted with human waste where fecal bacteria thrive. This result leads us to hypothesize that this predatory bacterium may modify the degree of interaction of mosquitoes and their gut microbes with fecal bacteria.

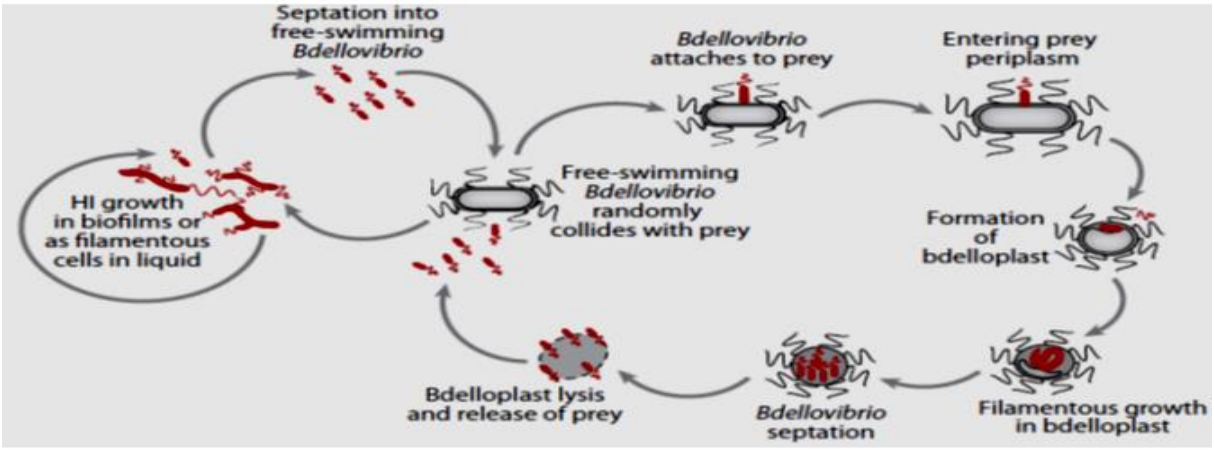

Figure (1): The predatory process of *Bd. bacteriovorus* (2)

Materials and Methods

Ethical approval

The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study under permission No: **369**

Sampling

Seventy adult mosquitos of *Culex pipiens* were used for extraction of the bacterial DNA from their intestine. The 70 mosquitoes were assigned randomly to 7 groups, 10 mosquitoes for each group to pool the amount of DNA extracted. Each mosquito was dissected under the microscope to isolate the intestine and use it later for DNA extraction. The DNA was extracted using a phenol/chloroform method (13).

PCR technique

The DNA samples were used in PCR, (Life Technology, USA), to detect the presence of *Bd. bacteriovorus* using the following specific primers for the bacterium: Bd529F (5'-GGTAAGACGAGGGATCCT-3') and Bd1007R (5'-TCTTCCAGTACATGTCAAG-3') that amplify a 481-bp of the 16S rRNA gene (7). PCR reaction mixture of total 25 ul contained: buffer 1X 2.5 ul, dNTPs 200 uM 0.5 ul, 0.5 uM 1.25 ul of each primer, Amp Taq. 1.25 U/ul 0.3 ul, MgCl2 2.5 mM 2.5 ul, BSA 300 ug/ml 0.75ul, and DNA 100 ng 2 ul. For *hit* locus was 95 C for 5 minutes, 35 cycles of 95 C for 1 min, 60 C for 1 min, 72 C for 1 min, and a final step of 72 C for 10 minutes. Five microliters of the PCR products were loaded in the wells using loading dye. Agarose gel 2% was run on 110 mA, 80 Vol for 1 hour. The ladder was 1500-100 bp.

Results

DNA samples of *Culex pipiens*, a mosquito that often develops in water polluted with human waste where fecal bacteria thrive have revealed the presence of *Bdellovibrio bacteriovorus* as a member of the intestinal bacteria of this mosquito in all groups used in this study. All 70 mosquitoes (100%) were positive and were amplified in the agarose gel indicating the presence of this bacterium in the intestine of these bugs. Furthermore, the amplified products were 481-bp which confirms these results, Figure (2).

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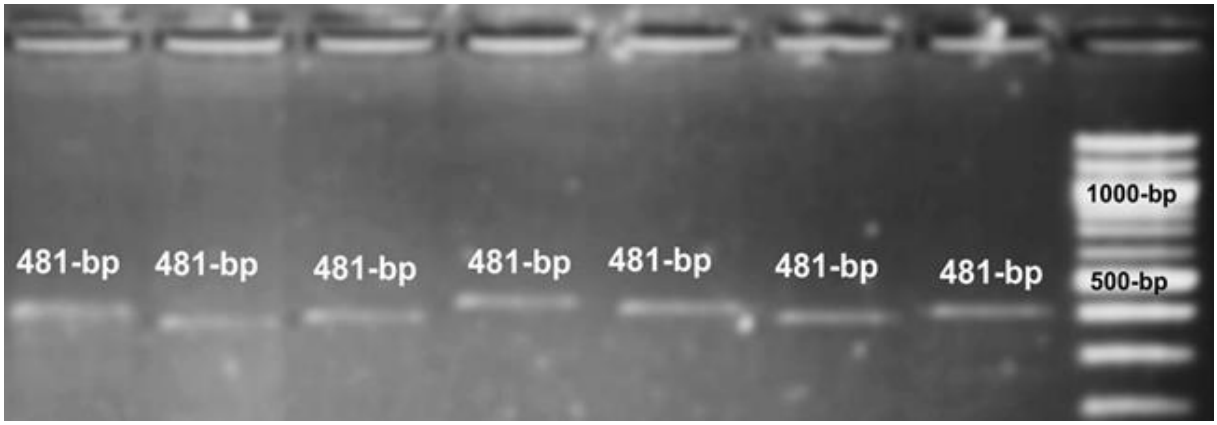

Figure. (2): amplification of the 481-bp of the 16S rRNA gene of the *Bdellovibrio bacteriovorus*

Discussion

This is the first study that shows the presence of *Bdellovibrio bacteriovorus* in the intestine of *Culex pipiens*. I have revealed the presence of *Bdellovibrio bacteriovorus* as a member of the intestinal bacteria of this mosquito in all groups used in this study. All 70 mosquitoes (100%) were positive and were amplified in the agarose gel indicating the presence of this bacterium in the intestine of these bugs. Furthermore, the amplified products were 481-bp which confirms these results, Figure (2). The current result agrees with (7) who had recognized the presence of *Bdellovibrio bacteriovorus* in high numbers in the intestine of healthy humans. Moreover, the current study result show agreement with (14), when they had detected the presence of this bacterium in the shrimp. In addition, my result also matches (15) who mentioned the presence of *Bdellovibrio bacteriovorus* in the intestine of vertebrates. Because (16) had isolated *Bdellovibrio* from and used it as a treatment to a certain disease in fish, my result refers to the presence of this bacterium in the intestine of mosquitoes. The current result is important because intestinal bacteria in mosquitos might degrade the insecticide chemicals to non-harmful substances on the host mosquitoes, and this is similar to what (17) had found that intestinal bacteria degrade polyphenol in humans. To kill and eliminate these bacteria, we need a natural system, a predator, that is able to do this job, and *Bdellovibrio bacteriovorus* is the bacterium of choice to take the mission. This agrees with the many researches that had been done to find the therapeutic activities of this bacterium (18) who had found that *Bdellovibrio bacteriovorus* preys on *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Moreover, the present study result is important because it had been found that this bacterium could be used to treat *Shigella* infection in Zebra fish (19), and to treat the lung of rats infected with *Klebsiella pneumoniae* (20). The current study might open the doors to control mosquitoes using predator bacteria especially *Bdellovibrio bacteriovorus.*

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