

## **ACTIVATION OF BACTERIAL CYTOCHROME P450 IN THE INTESTINE OF LARVAL MOSQUITOES AN INDICATION OF ITS ROLE IN THE INSECTICIDE RESISTANCE**

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### **ABSTRACT**

Mosquitoes apply very important impact on people life, and their control, day by day, is getting harder and harder. This difficulty comes from different issues such as insecticide resistance. The gut microbiome (GM) in different animals has been discovered to add principle influence on the gastrointestinal tract physiology. For example, GM participates in degradation of some poisonous chemicals into safer molecules that could be handled by the animal host. Eukaryotic cytochrome P450 has been detected to insert some degradation processes in other animal models such as zebra fish. However, these effects are not yet known in mosquitos. In order to stand on these problems and find if the prokaryotic P450 is involved, this study had been done to find out if the GM has any role in developing such resistance in mosquitos. Larvae were used in this experiment which exposed to benzothiazole (BZT) to trigger the hypothetical role of prokaryotic P450. By using polymerase chain reaction (PCR), the result indicates that the bacterial P450 was initiated and might be involved in the BZT exposure handling.

### **INTRODUCTION**

Mosquitoes affect people life by inducing big impact through disease transmission such as Rift Valley Fever and West Nile virus (1, 2), and their eradication difficulties are devastatingly increasing. These problems induced by different issues such as insecticide resistance (3, 4). The gut microbiome (GM) in different animals has been discovered to add principle influence on the gastrointestinal tract physiology and pathology such as Alzheimer's Disease and entambiiasis (5,

6). For example, GM participates in degradation of some poisonous chemicals into safer molecules that could be handled by the animal host (7). Eukaryotic cytochrome P450 has been detected to insert some degradation processes in other animal models such as zebra fish (8). In a study by (9) that has shown up-regulation of the host P450 in larval mosquitoes inside tires after exposure to benzothiazole (BZT), a chemical leachate that released by the tires (9, 10). BZT is degraded by some bacteria such as *Rhodococcus pyridinovorans* strain PA that uses BZT as source of energy (11). So Mosquito control is an evolving field where often concerns about environmental pollution resulting from chemical insecticides and the possible development of insecticide resistance in the mosquitoes. In particular, the development of resistance against pyrethroid insecticides such as permethrin by mosquitoes is often mediated by the up-regulation of the host cytochrome P450 (12). To explore if the prokaryotic P450 is involved in developing resistance against insecticides, I hypothesized that shifts in the microbial composition and the possible role of bacterial P450 in the mosquito gut may have synergistic effects on the development of insecticide resistance, and that is why this study was performed.

## **MATERIALS AND METHODS**

### **Experimental Design**

The experiment used 3 treatment groups (BZTG) plus 3 control groups (CG), with 10 mosquito larvae (*Aedes albopictus*) in each group. Larvae were reared in close to aseptic conditions, in which larvae were placed in disposable sterile petri-dishes (PDs) contained autoclaved double deionized distilled water (AddH<sub>2</sub>O) and autoclaved larval food; moreover; larvae were transferred to new PDs using sterile pipettes every 24 hours that were subjected to the same above aseptic condition procedures to the end of experiment, to minimize contamination from microbes present in the water and/or in the larval food. The experiment was run for 3 days to allow time for bacterial turnover. BZT 20 ppm has been applied to each BZTG every day.

### **DNA extraction and polymerase chain reaction**

After the experiment was terminated, larvae were collected, dried in a sterile paper towel and placed in 1.5 ml tubes, each group in a tube, and frozen at -80°C. DNA was extracted using a Phenol-Chloroform extraction method and we obtained approximately 114.5 ng/ul of DNA

evaluated using a Nanodrop. P450 was subjected to PCR using the primers, P450F GTSGGCGGCAACGACACSAC and P450R GCASCGGTGGATGCCGAAGCCRAA. P450 PCR reaction mixture of total 20 ul contained: buffer 10X 2ul, dNTPs 10 mM 0.4 ul, P450F 10 uM 2 and P450R 10uM 2 ul, Amp Taq. 5U/ul 0.2, MgCl<sub>2</sub> 25 mM 2 ul, BSA 0.2 ul, DNA 2 ul. PCR protocol was: Initial denaturation 95 C for 5 min, denaturing 95 C for 45 sec, annealing 58 C 45 sec., elongation 72 C 1 min, and elongation stop 72 C for 4 min. Amplification cycles were 35 cycles. 5 ul of the PCR products were loaded in the wells using loading dye. Agarose gel 1.5% was run on 110 mA, 85 Vol for 35 min.

### RESULT

The result showed the amplification of the bacterial P450 in the larval mosquitoes after the exposure to the BZT, figure, 1. The product size was at 350-bp and was positive in the BZTGs. Moreover, the CGs have revealed no amplifications for the bacterial P450. There are constant positive results in the BZTGs and constant negative results in the CGs.

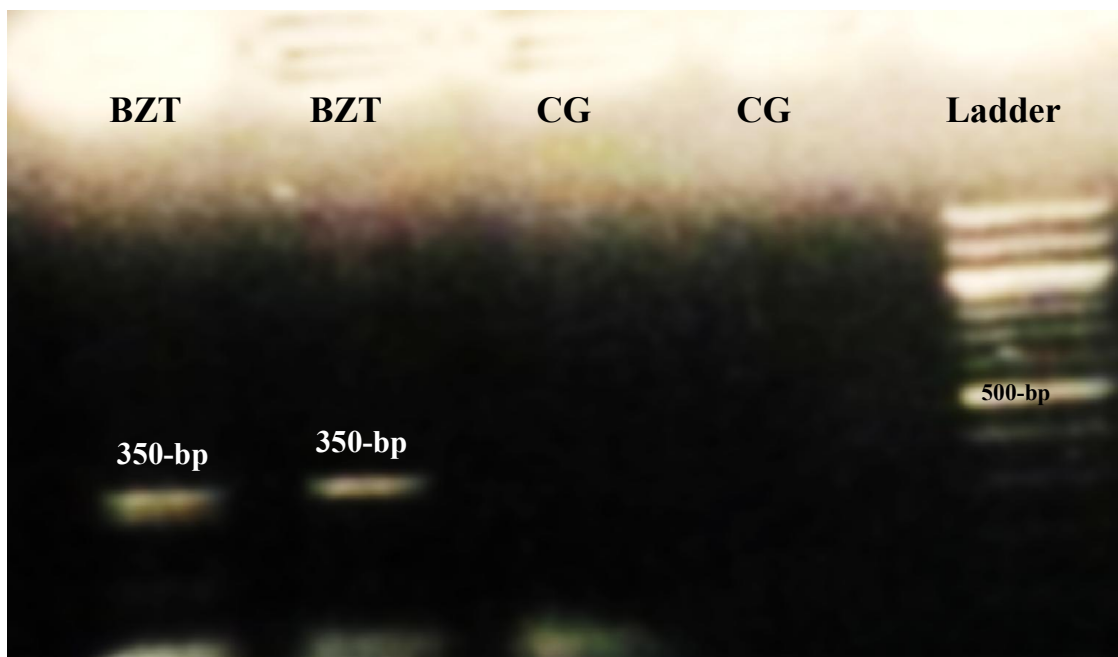


Fig 1: PCR amplification of P450 in the gut bacteria of larval mosquitoes after exposure to the BZT

## DISCUSSION

Mosquitoes influence the whole world by enhancing disease transmission such as Rift Valley Fever and West Nile virus (1, 2). To date, the globe countries face huge obstacles in controlling these creatures. Overcoming these problems is fulfilled by defeating insecticide resistance in these mosquitoes (3, 4). GM might insert important bacterial players such as P450 to increase resistance in these bugs (5, 6, 7, 8, 9, 10). The current study results showed the amplification of the bacterial P450 in the mosquito larvae after the exposure to the BZT, figure, 1. The results agree with (13) who found that some bacteria isolated from the intestine of the moth, *Spodoptera frugiperda*, used some insecticides as sources of food to grow up in media. In the current findings, the activation of P450 in the BZTGs was probably enhanced by the changing in the GM profile of the larvae after the exposure to BZT, and this agrees with (14) that showed the GM of the resistant strains of diamondback moth have different bacterial profile than the susceptible strains. The present experiment results are highly confirmed when exploring the findings of (15) who examined soil contaminated with insecticides and found that some P450-bacteria have biodegradation activities. This shows that bacteria of the mosquito intestine might play very important role in the degradation of insecticide chemicals using some enzymatic components such as the bacterial P450, and this increases the chances of developing insecticide resistance in mosquitoes. These results will elevate the attention toward the gut bacteria and the P450 as new targets for developing new procedures to control mosquitoes in the world. This is the first study in the world to show evidence of the roles of intestinal bacteria and their P450 component in providing insecticide resistance to the mosquitoes.

## تفعيل السيبتوكروم البكتيري بـ ٤٥٠ في امعاء يرقات البعوض كدليل على دوره في مقاومة المبيدات الحشرية

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### الخلاصة

يسلط البعوض تأثيرات مهمة على حياة الناس والسيطرة والقضاء عليها يوما بعد يوم يصبح امرأ من صعب الى اصعب. وتأتي هذه الصعوبة من قضايا مختلفة مثل المقاومة التي تولدها الحشرات للمبيدات الحشرية. اكتشف ان مايكروبايوم الامعاء في مختلف الحيوانات يؤثر بشكل اساسي على وظائف الامعاء. مثلا يساهم المايكروبايوم في تحطيم بعض المواد الكيميائية السامة ويحولها الى جزيئات امينة غير سامة والتي يستطيع الجسم التعامل معها. سابقا تم تحديد على ان السابتكروم بـ ٤٥٠ في الكائنات حقيقية النواة يقوم بادخال عمليات تحطيمية لمواد كيميائية كما اكتشف في سمك الحمار الوحشي. هذه الفعاليات للمايكروبايوم و السابتكروم بـ ٤٥٠ البكتيري ليست معروفة في البعوض. لغرض الوقوف على هذه المشاكل ومعرفة فيما اذا كان للسابتكروم بـ ٤٥٠ في الكائنات غير حقيقية النواة، جائت هذه الدراسة الحالية للكشف فيما اذا كان لمايكروبايوم الامعاء دور في تطوير المقاومة في البعوض. في هذه الدراسة استخدمت يرقات البعوض والتي عرضتها لمادة البنزوثيايزول لتفعيل الدور الافتراضي للسابتكروم بـ ٤٥٠ في الكائنات غير حقيقية النواة. استخدم تفاعل البلمرة المتسلسل لغرض الكشف عن تفعيل للسابتكروم بـ ٤٥٠. كانت النتيجة وجود تفعيل لهذا السيبتكروم في البكتريا. تدل النتيجة على دور للسيتوكروم البكتيري في التعامل مع البنزوثيايزول.

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