SHORT TERM SCIENTIFIC MISSION (STSM)
SCIENTIFIC REPORT

This report is submitted for approval by the STSM applicant to the STSM coordinator

Action number:
STSM title:
STSM start and end date: 22/02/2021 to 20/03/2021
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PURPOSE OF THE STSM:

Recently, multiple contiguous cytochrome P450 (CYP6 genes) showing elevated gene copy number in association with deltamethrin resistance were identified in *Ae. aegypti*. Further NGS data from an isofemale line from French Guiana deprived from Kdr by controlled cross and still showing significant resistance to deltamethrin confirmed the importance of this large genomic duplication spanning five CYP6 genes.
In this context, this STSM project aimed at validating through RNAi-mediated gene silencing the role of these CYP6 genes in deltamethrin resistance.. Such validation constitutes a prerequisite for validating the role of these P450 in the resistance phenotype and designing a novel DNA-based assays to track down P450-mediated deltamethrin resistance in mosquitoes in conjunction with bioassays and Kdr mutations genotyping.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

During this STSM an RNAi experiment for the P450s included in the duplication was performed, in order be used as a tool for silencing each P450 of interest and their implication in the resistant phenotype aftter deltamethrin exposure.
More specifically this project included:
A (IMBB)Preparation of dsRNAs for duplicated *Ae. aegypti* CYP6 genes was carried out: Two to three distinct dsRNA were prepared for each target-gene following standard procedure (in vitro transcription) in order to optimize RNAi specificity. dsRNAs were prepared using cDNA extracted from adult females of the kdr-free resistance line.

B (LECA). Preliminary RNAi experiment:
b1) injection of each dsRNA in adult females of the kdr-free resistant line
b2) 3 days post-injection, gene expression study by RT-qPCR for assessing gene knock down efficiency . A total of 30 adult females across replicates was be used for each dsRNA injection and RT-qPCR. Silencing efficiencies were estimated and constructs with higher than 40% were used for downstream analysis

C (LECA). Constructs for which the best silencing efficiency was observed were tested for off-target effects against the other P450s as they share high sequence similarity. RT-QPCR analysis was carried out to asses this question.

D (LECA). Full RNAi experiment:
d1)for each target gene, the dsRNA showing the best efficiency/specificity profile was injected in adult females of the kdr-free resistant line. Threedays post-injection, comparative bioassays with deltamethrin were performed between mosquitoes injected with
each CYP6 dsRNA versus controls injected with GFP-dsRNA (100-150 mosquitoes per condition). This experiment is still ongoing at LECA as replicationsare needed to obtain reliable data on the effect of dsRNA injection on the resistance phenotype.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

During this STSM action we managed to successfully perform RNAi-mediated silencing of five P450s included in the duplication of *Ae. aegypti* resistant line associated with insecticide resistance. More specifically we created dsRNAs against all targeted P450s and we tested their silencing efficiency. According to our resluts, we identified at least one dsRNA against each P450 of interest that results in adequate silencing. Estimated silencing efficiencies were acceptable (45-70 % knock-down of gene expression), in order to proceed to bioassays following ds RNA injection.

We also tested the off-targets effects of these dsRNAs against the other P450s of the targeted gene cluster which share high sequence similarity. This gene study revealed that 3 out of the 5 candidate dsRNAs indeed perform a specific silencing, yet two of them show a cross-reactivity to one more (highly similar) P450. This information is very useful for future correlation of the knock-down expression effect to the phenotype that will be observed from bioassays. A first run of ds RNA injections followed by bioassays with deltamethrin was performed. Preliminary results obtained show that at least two P450s out of five are involved in the deltamethrin resistance phenotype. this experiment will be reapeated at LECA in order to consolidate these results. All together, the data obtained through this STMS project will likely contribute to the production of a high level joint publication between LECA and IMBB related to metabolic resistance to pyrethroids in the mosquito Ae. aegypti (expected submission by end of 2021).

FUTURE COLLABORATIONS (if applicable)

The new collaboration created through this STSM action will be continued to the future. More precisely, similar collabrative studies might be conduct in the future on the tiger mosquito *Ae. albopictus* for which pyrethroid resistance is rising in several regions (resistance mechanisms under study at both LECA and IMBB). In addition, this very fruitful STSM project contributed to enhance the communication between IMBB and LECA on other subject (insecticide resistance mechanisms in *An. gambiae*, cuticular resistance mechanisms, …) which will likely lead to further collabrative links on these aspects.