Peer-to-Peer Training Fellowship Report

Jacob Riveron, PhD

[1]Liverpool School of Tropical Medicine Pembroke Place. Liverpool, L35QA, United Kingdom.
[2]OCEAC. Pobox 288. Yaounde. Cameroon.
Email: jacob.riveron@lstmed.ac.uk

Background / Purpose of Visit

Resistance to insecticides in major malaria vectors is spreading rapidly across Africa, threatening the success of malaria control programs. Implementation of evidence-based resistance management strategies against malaria vectors is urgently needed to preserve the effectiveness of current control tools. A good understanding of the molecular, biochemical and functional causes of resistance is key to track and anticipate its course. Previous efforts to characterize the mechanisms of insecticide resistance in *An. funestus s.s.* populations (major malaria vector) have revealed that cytochrome P450 genes such as CYP6P9a, CYP6P9b and CYP6M7 and glutathione S-transferase genes such as GSTe2 confer resistance to pyrethroids and DDT when they are overexpressed. However, the enzymes involved in the subsequent steps of the catabolism reaction in resistant mosquitoes remain unknown. Based on microarray results, other enzymes seem to play a key role in detoxification, but their functions have to be confirmed. In this context, we capitalized on the experience of Dr Venken and Dr Sarrion-Perdigones and the facilities available in the Baylor College of Medicine (Houston, USA) to help achieve our aim: the functional characterization of "second step" detox genes, combining their expression with some of the known genes in *An. funestus s.s.* (P450s and GSTs,) in insect cell culture and transgenic *Drosophila melanogaster*.

Work Carried Out

During my 6 weeks visit to the Baylor College of Medicine (18th March - 4th May 2016), we made excellent progress towards our aim of developing a modified assembly P[acman] vector to express two *An. funestus* genes (first and second "actor" in catabolism), together with the Gal4 binary activators system, a fluorescence marker and the white+ transgenesis marker. In brief, we have assembled almost all the combinations of two genes proposed (13 genes) and the needed P450 partner genes cytochrome p450 reductase (CPR) and cytochrome b5 (Cyt-b5), as shown in Table 1. In order to validate the gene combinations before carrying out the transgenic flies, we co-transfected *Drosophila* S2 cells with some of the generated constructs plus the needed CPR partner. Co-transfected cells were treated with four different insecticides (permethrin, deltamethrin, bendiocarb and DDT), applied independently. The survival percentage was measured using an acoustic flow cytometry. Optimal time for cell co-transformation, concentration of insecticide and time of treatment were obtained for each insecticide. Results with

combinations of the “first step” detox genes show that this system could be used like first screening to know what combinations are potentially increasing resistance in insects (Figure 1). Currently, we continue testing the different gene combinations in Drosophila cell culture before using the genes to transform *Drosophila melanogaster* in my home laboratory.

Table 1. Assembly P[acman] vector to express two *An. funestus* genes: Successful in green; assembly in progress in orange; unsuccessful assembly in red; pending confirmation in white. The number indicates the name of the construct.

		CYP6P 9a	CYP6P 9b	CYP6M 7	CYP6A A4	Gste2	Gst-detox-1	Gst-detox-2	Detox-3	Detox-4	Detox-5	Detox-6	Detox-7	Detox-8	Cyt-b5	
		RIV10	RIV11	RIV12	RIV54	RIV55	RIV13	RIV14	RIV15	RIV16	RIV17	RIV56	RIV57	RIV18	RIV58	ALFA2
CYP6P 9a	RIV1	98	99	101	109	104	123	142	148	155	162	178		185		
CYP6P 9b	RIV2		100	102	110	105	124	143	149	156	163	179		186		
CYP6M 7	RIV3			103	111	108	125	144	150	157	164	180		187		
CYP6A A4	RIV49			111_alt	112//113	106	126	145	151	158	165	181		188		
Gste2	RIV50					107//114	127	146	152	159	168	182		189		
Gst-detox-1	RIV4						115	147	153	160	169	183		190		
Gst-detox-2	RIV5							116	154	161	170	184		191		
Detox-3	RIV6								117	141	139	136	133	128		
Detox-4	RIV7									118	140	137	134	129		
Detox-5	RIV8										119	138	135	130		
Detox-6	RIV51											120		131		
Detox-7	RIV52	171	172	173	174	175	176	177	133_alt	134_alt	135_alt		121	132		
Detox-8	RIV9		186_alt	187_alt	188_alt	189_alt	190_alt	191alt						122		
CPR	RIV53														RIV166	
	ALFA1															

Furthermore I learnt a great deal from Dr Sarrion and Dr Venken about the newly synthetic biology platform for *D. melanogaster* that combines GoldenBraid 2.0 cloning with a high-capacity transgenesis platform, and other aspects of cloning and genome engineering. This short visit has been an excellent opportunity for my personal training in genetic manipulation and, in addition, it enables me to transfer the knowledge acquired to my research groups based in Liverpool and Cameroon.

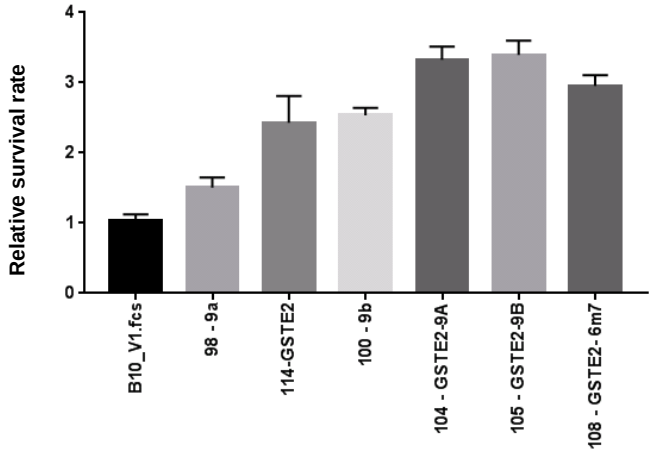

Figure 1. Relative survival rate of S2 Drosophila cells transfected with different single/combined genes against DDT (10^{-3}M) in respect to the empty vector B10_V1