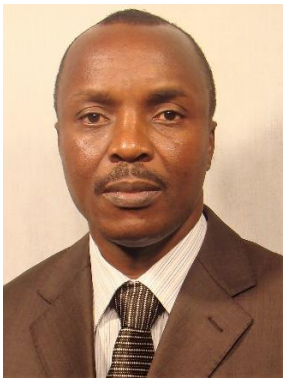
Profile for Web Page-Prof. Z. Ngalo Otieno-Ayayo



Office Contacts:
Rongo University Annex
P.O. Box 103, Rongo-40404
Cell: +254 723203802/752203803
E-mail: zngalo@ruc.ac.ke, zngalo@gmail.com

Prof. Z. Ngalo Otieno-Ayayo is the Ag Coordinator, Rongo Town Learning Centre of Rongo University in Kenya. He is currently serving his second term as the Treasurer of the African Association of Insect Scientists (AAIS) with its headquarters in Nairobi, Kenya (http://aais-africa.org) and Editor-in-Chief of Baraton Interdisciplinary Research Journal www.ueab.ac.ke/BIRJ, hosted by the University of Eastern Africa, Baraton. Prof. Ngalo holds a PhD in Life Sciences from Ben-Gurion University of the Negev (BGU), Israel http://in.bgu.ac.il/en/GlobalImpact/global-impact-2012.pdf . His research interests are in "**Emerging Alternative Systems for Insect Pests and Disease Vectors Control**" with special attention to recombinant *Bacillus thuringiensis* var *israelensis* (Bti) entomocidal proteins. He has had an opportunity to participate in the German Mosquito Control Association's aerial- and hand-, spraying of Bti in the swamps of Waldsee (Germany) and in the meadows of Wroslaw (Poland) respectively. He also belonged to a research team that worked on integrated malaria control with special emphasis on "**Malaria Diagnosis Using Plasma and Combinations of Chemical Reactions**" sponsored by the Bill and Melinda Gates Foundation. His latest Research and Development undertaking is in **"Sericulture for Cottage Industry Development in Rural Kenya"** in collaboration with a team of scientists from Rongo University, the Migori County Government and Wuhan Textile University, China.

Prof. Ngalo previously worked at the International Centre of Insect Physiology and Ecology in various capacities between 1985 and 2000 before moving to Solusi University in Zimbabwe for 5 years and University of Eastern Africa, Baraton (UEAB) for 7 years before joining Rongo University in 2014. He was an International Atomic Energy Agency (IAEA) Research Fellow at

BGU in 1989-90, where he worked on radioisotopes for studying the interactions of Bti toxin proteins and fate of the δ-endotoxin in the mosquito gut under the guidance of Prof. Arieh Zaritsky, who would later supervise his PhD research. In his several visits to Israel, Prof. Ngalo was priviledged to have intellectual interactions with the late Prof. David Chipman, Prof. Zeev Barak, the late Prof. Yoel Margalith (the discoverer of Bti), Dr. Rivka Cahan (of Ariel University) and Prof. Alex Keynan (then advisor to the president of Israel on Science and Technology). At the moment we have a Memorandum of Understanding with Ariel University.

PhD Thesis Abstract

Emerging Alternative Systems for Mosquito Biocontrol Using Recombinant Bacteria Expressing Genes from *Bacillus thuringiensis* subsp. *israelensis*

ABSTRACT

With the increase of mosquito capacity to transmit diseases and the threats of selection for resistance by both parasites and vector, it has become imperative to develop more effective management strategies for disease control. A diversity of biological entomotoxins is one way of averting the possibility of the vectors to select for resistance. Three alternative systems were tested in this study, all based on heterologous expression of four genes from the mosquito larvicidal *Bacillus thuingiensis* subsp. *israelensis* de Barjac (*Bti*).

Toxicity of the same lyophilized powder prepared identically of 16 combinations of four genes, *cry4Aa, cry11Aa, cyt1Aa* and *p20* from *Bti* expressed in *Escherichia coli* were examined against three key mosquito vectors of diseases, namely *Anopheles arabiensis* Patton, *Culex quinquefasciatus* Say and *Aedes aegypti* Linnaeus, followed by simulated studies using transgenic *Anabaena* PCC7120 expressing the most toxic combination of genes. Comparative analyses of responses of these key species revealed a hierarchy of toxin activities with synergistic interactions, and resolved some inconsistencies recorded in the literature. The following clones were the most toxic to these mosquito species (*C. quinquefasciatus*, *An. Arabiensis* and *Ae. Aegypti*, respectively): pVE4-ADRC expressing all four genes (LC_{50}s of 0.59, 3.2, and 0.68 μg ml^{-1}); pVE4-ARC expressing *cry4Aa, cyt1Aa, p20* (LC_{50}s of 0.93, 6.2 and 0.87 μg ml^{-1}), and pVE4-AD expressing *cry4Aa* and *cry11Aa* (LC_{50}s of 1.51, 7.5 and 1.3 μg ml^{-1}), concluding that clone pVE4-ADRC is undoubtedly the most effective. It is anticipated that introduction of another gene, *cry4Ba*, would further enhance toxicity of this system.

The role of appropriate promoter(s) in enhancing toxicity was demonstrated by comparing expression of the same gene combination under a strong *E. coli* promoter (P_{A1}) either singly, in pVRE4-DRC or two (the second preceding *cyt1Aa* as well in pVE4-DRC); the latter produced more Cyt1Aa, which is less toxic, at the expense of the more toxic Cry11Aa, thus quenching toxicity. On the other hand, the combination under pVRE4-DRC had an apparent correct stoichiometry to enhance toxicity. This observation implies that further toxicity fine-tuning could be reached by manipulating promoters to enhance toxicity in the recombinant systems.

In an effort to reduce premature processing of toxin proteins, a series of protease-deficient *E. coli* mutants were transformed with expression plasmids containing *cry11Aa* (the product of which is known to be degraded in this host bacterium), alone or in combination with the other *Bti* genes. The recombinant mutants displayed reduced growth rates and yields compared to the non-recombinant and the recombinant protease-proficient counterparts. On induced production of the toxins, overproduction of a battery of chaperonins (protective proteins) was observed in all the recombinant mutants but not visible at all in the non-recombinant control. The most prominent of the overproduced protective *E. coli* proteins (quantified using EZQuant-gel analyzer) were the 57.2 kDa GroEL (37% identity with GroEL of *E. coli* 0157:H7), with an average of 136 ± 20 units, and the 18.7 kDa Dps (72% identity with that of *E. coli* 0157:H7), with an average of 105 ± 98 units. These proteins seemed to regulate the accommodation of the toxins in their new niche while maintaining their role of housekeeping in the host cells. Contrary to the expectations, there was an evident massive degradation, especially when Cry11Aa was expressed alone in the protease-deficient strains. This could be attributed to the overproduction of the protective proteins. However, simultaneous expression of *cry11Aa*, *p20* and *cyt1Aa* under the same promoter significantly reduced the level of degradation, which in some mutants was un-noticeable. The amount of protective proteins produced was not necessarily related to the quantity of recombinant protein. It is noteworthy that compromising the constitutive endogenous protease network triggered a visibly increased presence and activity of molecular chaperones, resulting in increased recombinant protein degradation. An observation of transmission electron microscope images of the recombinant mutants and their non-recombinant counterpart expressing *cry11Aa* showed no evidence of inclusion bodies. Toxicity was however not dramatically affected in the tested recombinant mutants.

In simulated semi-field experiments, the transgenic *Anabaena* PCC 7120 protected the toxins from premature degradation and retained toxicity for up to 20 days, compared to commercial *Bti* preparation, which lost toxicity within 3 days due to environmental factors. The ability of the transgenic *Anabaena* to float and thus avoid adsorption to bottom mud, coupled with phototaxicity, which enables it to move freely to get solar energy for its physiological needs, should improve the interaction of this model system with the mosquito larvae tested. This system is anticipated to be even better for the surface feeding species of the genus *Anopheles* (which was not tested in the simulation studies).

The roles of gene interactions in the transgenic microorganisms and improved delivery systems for the insecticidal proteins, suggesting possibilities for environmental healing process, are discussed.

Keywords are underlined above

Publications

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