

Molecular detection of six high importance endosymbiotic bacteria in Belgian wild-caught mosquitoes



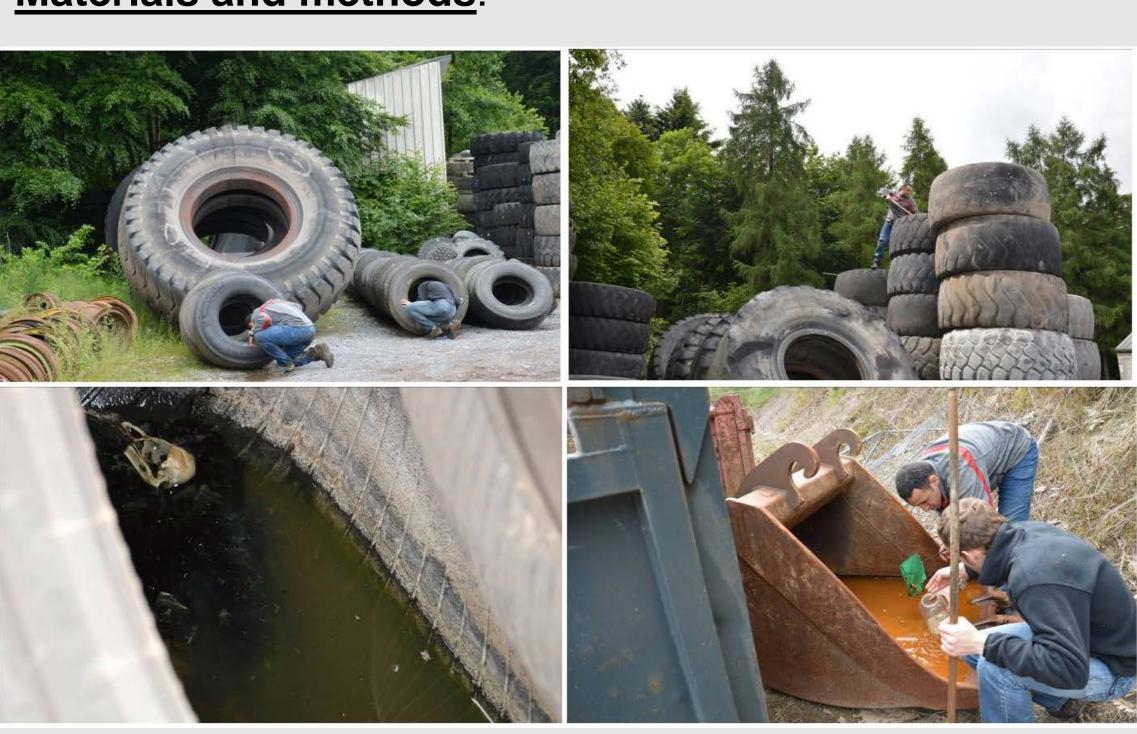
Raharimalala F.N.^{1, 2,} Boukraa S.¹, Bawin T.¹, Zimmer J.-Y.¹, Francis F.¹ ¹Functional and Evolutionary Entomology – University of Liege (GxABT) – Belgium ²Institut Pasteur – Madagascar; Email: <u>entomologie.gembloux@ulg.ac.be</u>



Introduction:

Due to increasing resistance to various pesticides currently used, research of alternative solution is promoting the use of endosymbiotic bacteria (1). In this study, eleven species of Culicidae (Culex pipiens s.l., Cx. torrentium, Cx. hortensis, Anopheles claviger, An. maculipennis s.l., An. plumbeus, Culiseta annulata, Ochlerotatus geniculatus, Oc. dorsalis, Aedes albopictus and Coquillettidia richiardii) from eight sites of Belgium were used for the screening of the presence of six genera of important endosymbiotic bacteria (2, 3): Wolbachia sp, Commamonas sp, Delftia sp, Pseudomonas sp, Acinetobacter sp and Asaia sp. PCR of 176 individual mosquitoes (144 larvae and 32 adults) were used for the screening.

Materials and methods:



Different types of breeding sites (larvae collections)





Different methods of adults captures



DNA extracted + PCR with specifics primers

All positives PCR were sequenced and confirmed with comparison of data deposited in GenBank. Sequences of positives endosymbiotic bactria are also deposited in GenBank.

Key words: Mosquito, Endosymbiotic bacteria, Belgium, Wolbachia

Results:

Table 1: Mosquito larvae species, number of individuals tested and endosymbiotic bacteria screened

Total number:	48	36	6	6	18	24
Asaia	0 (0%)	7 (19,4%)	1 (16,7%)	1 (16,7%)	6 (33,3%)	7 (29,2%)
Acinetobacter	25 (52,1%)	13 (36,1%)	3 (50%)	2 (33,3%)	9 (50%)	13 (54,2%)
Pseudomonas	45 (93,8%)	30 (83,3%)	2 (33,3%)	3 (50%)	13 (72,2%)	18 (75%)
Delftia	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Commamonas	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Wol 16S	17 (35,4%)	0 (0%)	1 (16,7%)	0 (0%)	0 (0%)	0 (0%)
Species	Cx. pipiens s.l.	Cx. torrentium	Cx. hortensis	An. claviger	An. maculipennis s.l.	Cs. annulata

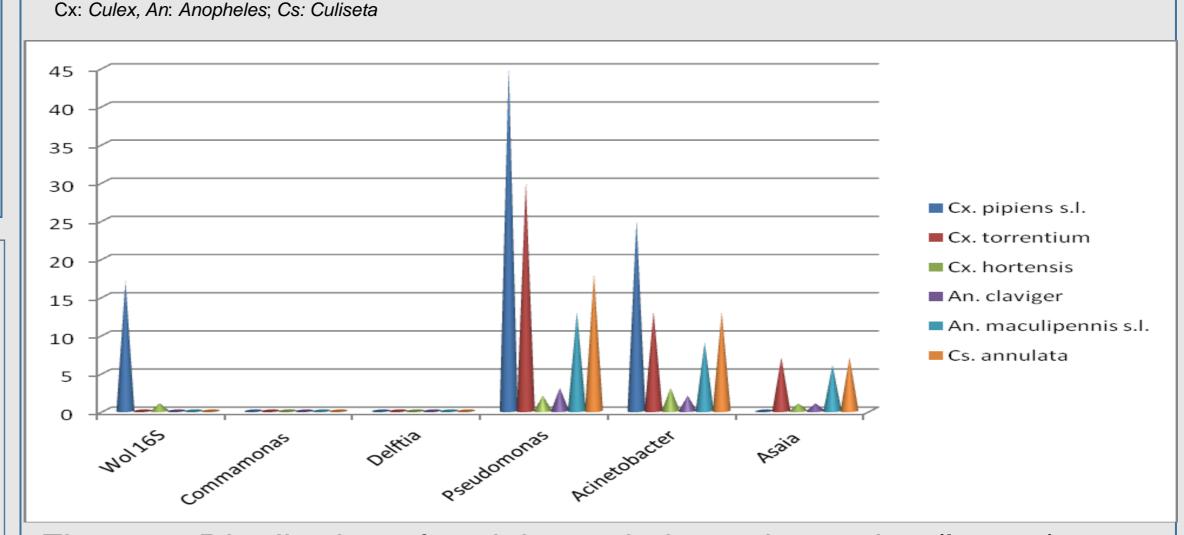


Figure 1. Distribution of endobacteria in each species (larvae)

Table 2: Mosquito adult species, number of individuals tested and endosymbiotic bacteria screened

Species	Cx. pipiens s.l.	An. plumbeus	Oc. geniculatus	Oc. dorsalis	Ae. albopictus	Cq. richiardii
Wol 16S	9 (100%)	0 (0%)	0 (0%)	1 (100%)	1 (100%)	1 (100%)
Commamonas	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Delftia	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Pseudomonas	6 (66,7%)	6 (75%)	8 (88,9%)	1 (100%)	1 (100%)	1 (100%)
Acinetobacter	1 (11,1%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)
Asaia	1 (11,1%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)
Total	9	8	9	1	1	1

Cx: Culex, An: Anopheles; Oc: Ochlerotatus; Cq: Coquillettidia; Ae: Aedes

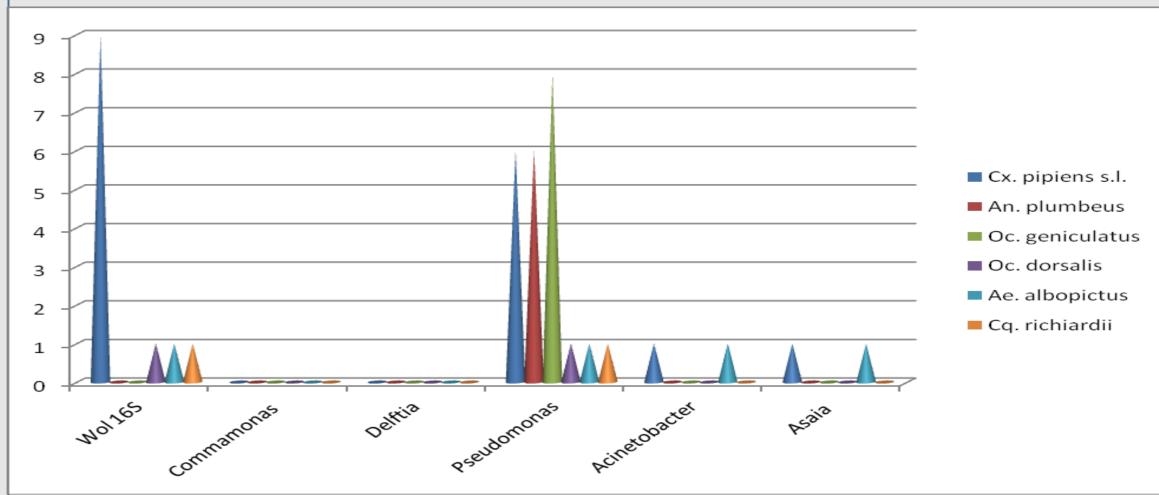


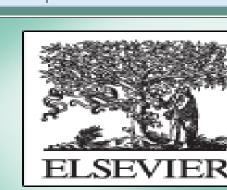
Figure 2. Distribution of endobacteria in each species (adults)

Discussion and conclusion:

- Wolbachia, Pseudomonas, Acinetobacter and Asaia were detected in all species with different proportions
- Commamonas and Delftia were totally absent in all mosquito species tested
- Considering its predominance in all species studied in this research, Pseudomonas would be a perfect candidate for a perspective study focusing on its role on mosquito biology and the possible impact of its removal via the use of antibiotics.

(1) Christodoulou M., 2011. Biological vector control of mosquito-borne diseases. Lancet Infect Dis 11: 84–85. (2) Minard, G., Tran, F.H., Raharimalala, F.N., Hellard, E., Ravelonandro, P., Mavingui, P. & Valiente Moro, C., 2013. Prevalence, genomic and metabolic profiles of Acinetobacter and Asaia associated with field-caught Aedes albopictus from Madagascar. FEMS Microbiol Ecol 83: 63-73. (3) Zouache, K., Raharimalala, F.N., Raquin, V., Tran-Van, V., Raveloson, L.H.R., Ravelos

(4) Lindh, J., 2007. Identification of bacteria associated with malaria mosquitoes –Their characterisation and potential use. Doctoral thesis, comprehensive summary (Other academic). Stockholm University, Faculty of Science, Department of Genetics, Microbiology and Toxicology.









Seventh International Symposium on Molecular Insect Science