Development of entomopathogenic fungi in mosquito control: which kind of production for which efficiency?

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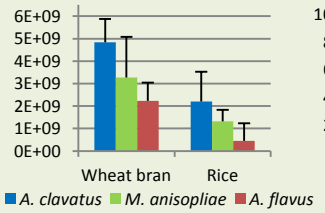
Context

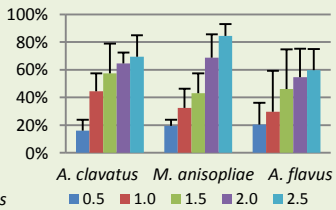
- **Mosquitoes (Diptera: Culicidae)** are zoonotic vectors responsible for numerous infectious diseases of medical and veterinary importance as filariasis, malaria and encephalitis.
- **As part of an integrated vector control**, entomopathogenic fungi (EF) could be developed as biopesticides in two ways: spores and metabolites recognized as effective virulence factors.
- **This project** aims to develop new research areas for the production of insecticidal compounds from these fungi focusing on solid-state and biofilm fermentation.

Classical fermentation systems

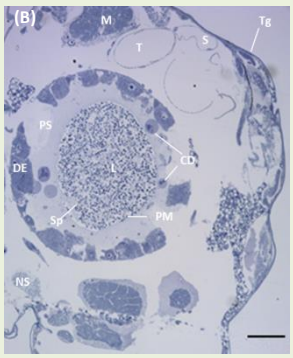
- **Solid-state fermentation** enhances **spores and metabolites** production.
- Submerged fermentation impairs metabolic efficiency.
- We showed high spore productivity of solid-state media based on **agro-industrial substrates** as wheat bran for *Metarhizium anisopliae, Aspergillus clavatus* and *A. flavus*.
- Spores remained pathogenic on *Culex quinquefasciatus* larvae as revealed by classical toxicity tests and microscopy observations.
- However, the absence of free water makes culture parameter variations difficult to control in large-scale.



Spore productivity (+SD) of EF (expressed as spores/g of substrate) grown on solid-state media (wheat bran and rice).

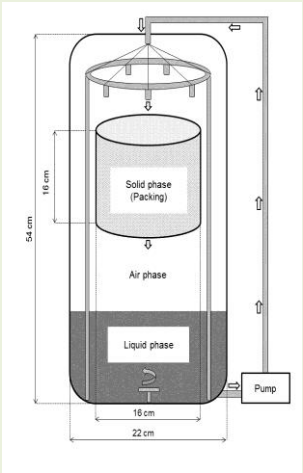


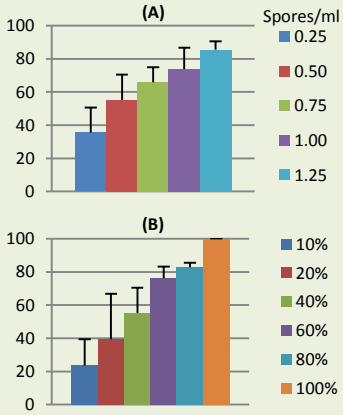
Corrected mortalities (+SD) induced on *Cx quinquefasciatus* larvae after 72h with concentrations ranging from 0.5 to 2.5x10[8] spores per ml grown on wheat bran.



Transverse sections of (A) untreated and (B) treated *Cx quinquefasciatus* larvae showing *A. clavatus* spore activity on the midgut epithelium after 24h. L = lumen, Sp = spores, PM = peritrophic membrane, PS = peritrophic space, CD = cellular debris, DE = digestive epithelium, M = muscles, NS = nervous system, T = tracheae, S = blood sinus, Tg = tegument. Scale bar = 50 µm.

- Recently, we performed a **bioreactor design** intended for simultaneous **spores and metabolites production** combining the technological advantages of submerged and solid-state fermentations.





Corrected mortalities (+SD) induced by *A. clavatus* on *Cx quinquefasciatus* larvae after 72h with (A) spores concentrations ranging from 0.25 to 1.25x10[8] per ml and (B) metabolites concentrations ranging from 10 to 100% of culture filtrates.

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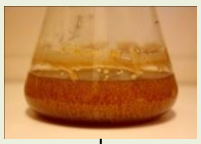
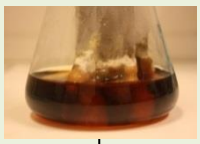
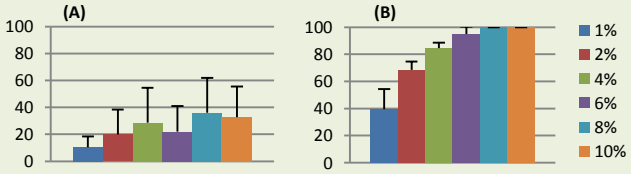
A new trend: Biofilm fermentation

- **Biofilm fermentation** (i.e. growth of fungal biomass on an inert support immerged in a nutrient medium) is a tremendous production system favouring the secretion of insecticidal metabolites in the liquid medium as we showed recently.
- This is also an interesting tool to provide an overview of the complexity of the metabolic pathways involved in the regulation of **extracellular metabolites secretion** because corresponding genes are reported to be differentially expressed from classical fermentation systems.







Corrected mortality (+SD) induced by *A. flavus* metabolites on *Cx quinquefasciatus* larvae after 72h with concentrations ranging from 10 to 100% of culture filtrates from (A) submerged and (B) biofilm fermentation.

Perspectives. Researches in vector control are currently intensified. In this context, the identification of genes and metabolites specifically expressed during biofilm fermentation will help to develop new technologies related both to the design of bioreactor and the production of insecticidal proteins.

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