

## A METAGENOMIC APPROACH TO INVESTIGATE THE MOSQUITO MYCOBIOTA FOR THE DEVELOPMENT OF FUNGAL BASED INNOVATIVE BIOPRODUCTS FOR ECO-FRIENDLY CONTROL OF MOSQUITOES

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**INTRODUCTION:** Mosquito control is a crucial aspect of public health and environmental management, especially in regions where mosquitoes pose a threat due to disease transmission. Traditional vector control methods, such as insecticides, have proven effective but are often associated with environmental concerns and the development of insecticide resistance. In recent years, there has been growing interest in alternative and environmentally sustainable approaches to be used, in conjunction with traditional methods, to control the risks associated with mosquitoes. The characterization of the microorganisms inhabiting the mosquitoes has emerged as a significant area of research due to its implications in various aspects of mosquito biology, ecology, and vector competence. Understanding the composition and function of the microbial communities could have profound implications for vector control strategies. While the bacterial community of mosquitoes has been deeply studied, the fungal component is still little appreciated (Malassigné et al., 2020. *Pathogens*, 9:564). This study represents an in-depth investigation of the fungal community associated with Italian mosquito larvae aimed to identify suitable candidates for 'lure and kill' products leveraging attractant and entomopathogenic microbial properties.

**MATERIALS AND METHODS:** In 2022 and 2023, around a thousand mosquito larvae were collected in the Marche (*Aedes albopictus* and *Culex pipiens*) and in the Veneto regions (*Aedes koreicus*). Metagenomic analysis was performed on pools of ten L4 larvae utilizing an Illumina sequencing strategy, targeting the fungal ITS1-4 region. Furthermore, fungal strains were isolated from the same larval samples using culture-dependent methods.

**RESULTS AND CONCLUSIONS:** The analysis unveiled a diverse fungal community including Ascomycota (budding yeasts) and Basidiomycota. Some fungi are specifically associated with mosquito species, such as *Hyaloraphidium* and *Microidium* in *Cx. pipiens* and *Ae. koreicus*, respectively. Whereas other fungi, such as *Wickerhamomyces anomalus*, *Metschnikowia pulcherrima*, and *Candida parapsilosis* were detected across all analysed species. Metagenomic outcomes were confirmed using culture-dependent methods. Isolated fungi are under characterisation for both entomopathogenic activity against larvae and attractant properties mediated by volatile organic compounds towards gravid mosquitoes. To our knowledge, this represents the first in depth comparative description of the larval mycobiota in *Ae. albopictus*, *Ae. koreicus* and *Cx. pipiens*. Selected attractant and entomopathogenic fungi would provide a microbial repository available for functional tests. Fungal blends might be used for the implementation of 'lure and kill' formulations to be released in artificial or natural breeding sites or to be added in gravid mosquito traps. Such innovative fungal-based bio-products might contribute to the mosquito control following a sustainable 'ready to use' technology.