

## INVESTIGATING THE LARVAL MYCOBIOTA IN ITALIAN MOSQUITOES

Zubair M.S.\*<sup>[1]</sup>, Cappelli A.<sup>[2]</sup>, Damiani C.<sup>[2]</sup>, Favia G.<sup>[2]</sup>, Ricci I.<sup>[2]</sup>

<sup>[1]</sup>School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy; <sup>[2]</sup> School of Biosciences and Veterinary Medicine, University of Camerino, CIRM Italian Malaria Network, Camerino, Italy

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**INTRODUCTION:** Every year Mosquito-borne diseases (MBDs) cause almost 1.2 million fatalities worldwide (Weaver et al., 2010. *Antivir Res*, 85:328-45). The major MBDs include Zika, Yellow fever, Dengue, West Nile, Chikungunya, Rift Valley fever, and Malaria (Sanfelice et al., 2022. *Health Econ*, 31:73-93). Due to increasing pesticide resistance and safety concerns related to the use of synthetic chemicals, it is essential to develop alternative eco-friendly mosquito control methods. Mosquito larvae interact with many microorganisms, such as bacteria and fungi, in both water and land environments. These microbial communities can be recovered from larvae. In particular, research on fungus-mosquito interactions might lead to the identification of attractive or entomopathogenic agents to be used as bioinsecticides for treatment of breeding sites (Tawidian et al., 2021. *Msphere*, 6:101-28). In the present study the larval mycobiota of Italian mosquitoes has been characterized to investigate potential entomopathogenic or symbiotic fungi.

**MATERIALS AND METHODS:** Mosquito larvae of different species were collected from different regions of Italy. Larvae were identified by morphological characters. After identification, the larval samples from each collection sites and species were pooled for the fungal isolation. Larval homogenates were plated on YM agar medium and incubated overnight at 30°C. Fungal colonies were categorized and selected based on colony morphology and grown in YM medium overnight at 30°C. For the DNA extraction fungal samples were inoculated in YPD medium and incubated overnight at 30°C. To identify the yeast species, the DNAs were amplified with universal primers for ribosomal target ITS1/4 (Wasinpiyamongkol et al., 2019. *bioRxiv*, 518241). Amplicons were sequenced using the Sanger method by Microsynth company and the sequences were analyzed using ClustalW and BLAST software.

**RESULTS AND CONCLUSIONS:** About a thousand *Aedes koreicus*, *Aedes albopictus* and *Culex pipiens* larvae from the Veneto and the Marche regions have been analysed for the associated mycobiota. Both Ascomycota and Basidiomycota have been identified. Those belonging to Ascomycota included: *Schwanniomyces vanrijiae*, *Candida parapsilosis*, *Barnettozyma californica*, *Wickerhamomyces anomalus*, and *Metschnikowia pulcherrima*. Fungi belonging to Basidiomycota included: *Sporobolomyces* sp. and *Rhodothorula paludigena*.

Here we provided a list of fungi associated with mosquito larvae that are proposed for next functional studies to investigate their attractive and entomopathogenic properties. Knowledge coming from this research is essential for the development of fungal product to lure and kill mosquitoes. Findings will disclose innovative bioproducts as larvicidal tools for the MBDs control of MBDs.