

## Abstract

# A Mycovirus Mediates the Virulence of an Insect-Killing Fungus against the Malaria Mosquito Vector <sup>†</sup>

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**Abstract:** The cosmopolitan insect-pathogenic fungus and popular biocontrol agent *Beauveria bassiana* can be used to control *Anopheles* mosquito populations and restrict the spread of malaria, the deadliest vector-borne infectious disease in the world caused by the protozoan parasite *Plasmodium*. Here, we establish that infection with a double-stranded (ds)RNA mycovirus, *Beauveria bassiana* polymycovirus (BbPmV)-1, significantly reduces *B. bassiana* virulence against *A. coluzzii*, the main vector of malaria. The BbPmV-1-mediated hypovirulence can be at least partially attributed to slow fungal growth on the mosquitos. Analysis of the dual next-generation sequencing of the *B. bassiana* and *A. coluzzii* transcriptomes provided insight into the molecular mechanisms of the BbPmV-1-mediated effects. BbPmV-1-free *B. bassiana* has a wide impact on the *A. coluzzii* transcriptome, affecting immunity and metabolism, and led to the identification of novel immune response proteins. BbPmV-1 regulates the gene expression profile of its fungal host, directing the use of available resources towards sporulation and suppressing the mosquito immune system. Additionally, BbPmV-1-infected and -free *B. bassiana* strains differentially modulate mosquito gut microbiota; the former reduces the bacterial genus *Elizabethkingia* and the latter *Serratia*. Co-transfection of mosquitos with *B. bassiana* and *P. berghei* revealed a reduction of ookinetes in the presence of BbPmV-1, potentially due to the upregulation of a mycotoxin. Finally, BbPmV-1-mediated hypovirulence is at least partially dependent on the *A. coluzzii* RNAi pathway, and silencing of the *dicer-2* gene restores virulence. Taken together, our data clearly demonstrate the crucial role of mycovirus infection in mediating *B. bassiana* virulence against *A. coluzzii* and suggest that BbPmV-1 protects *A. coluzzii* from *B. bassiana*, the mosquito's own immune system, potentially harmful gut microbiota, and *Plasmodium* parasites.

**Keywords:** mycovirus; *Beauveria bassiana*; *Anopheles coluzzii*; malaria; *Plasmodium*; microbiome; transcriptome; immune response



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