

Natural infection by *Beauveria bassiana*

The genes encoding the antifungal peptides drosomycin and metchnikowin can be selectively induced after massively covering the adults with spores of the entomopathogenic fungus *Beauveria bassiana*. The level of antifungal peptide gene expression increases over several days and is similar to the level obtained after microbial injection challenges (Lemaitre et al., 1997). *Beauveria bassiana* is pathogenic for many insect species and has the ability to cross the cuticle of insects through the secretion of proteases and lipase (Clarkson and Charnley, 1996). Natural infection or injection of these fungi causes a significant mortality in wild-type adult flies.

In this study we infected *Drosophila OregonR* adult males, aged 3-4 days at 25°C. Natural infection was initiated by shaking anesthetized flies in a petri dish containing a sporulating culture of *Beauveria bassiana* and incubated at 25°C (5) for specific times (12, 24, 48, and 96 hours). Compared to septic injury, natural infection by *B. bassiana* induces a slow but more specific antifungal response that starts 12-24 hours after infection (5).

Detailed protocol: The most efficient and natural way to infect flies with fungi is to place the CO₂-anesthetized flies on a 5.5-cm or 9-cm dish with a well-sporulated carpet of fungi. Hand-shake the Petri dish until the flies are totally covered with spores (see figure). Infected flies are transferred into clean vials of normal medium and are incubated at 29°C. Vials should be changed every 2 days.

References

Clarkson, J. M., and Charnley, A. K. (1996). New insights into the mechanisms of fungal pathogenesis in insects, *Trends Microbiol* 4, 197-203.

Lemaitre, B., Reichhart, J., and Hoffmann, J. (1997). *Drosophila* host defense: differential induction of antimicrobial peptide genes after infection by various classes of microorganisms., *Proc Natl Acad Sci USA* 94, 14614-14619.

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