

Symposium. Thursday, 15:30. **234**

Vibrio and the intraphagosomal environment: how an oyster pathogen evades intracellular killing in oyster hemocytes

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Vibrio tasmaniensis LGP32 is a *V. splendidus*-related strain pathogenic for *Crassostrea gigas* oysters. We recently showed that LGP32 invades the oyster immune cells, the hemocytes, through phagocytosis. Oyster hemocytes are professional phagocytes harboring microbicidal activities including a potent oxidative response. Interestingly, the phagocytosed LGP32 survives inside the oyster hemocytes, evading the host defense by preventing acidic vacuole formation and limiting reactive oxygen species production. When hemocytes were invaded by numerous LGP32, we observed cytotoxic effects such as membrane disruptions and cytoplasmic disorders. Cytotoxicity was shown to entirely depend on LGP32 entry into hemocytes, as cytochalasin D was sufficient to inhibit hemocyte death. By developing a transcriptomic approach based on RNA sequencing, we identified a series of *Vibrio* antioxidant genes whose expression is strongly induced within oyster hemocytes. We also observed an overexpression of genes involved in cation efflux. Overexpression of these molecular functions in the intraphagosomal stage was confirmed by RT-PCR. To determine how far those LGP32 genes are involved in resistance to intracellular killing and subsequent virulence, we constructed isogenic deletion mutants for two overexpressed antioxidants and two overexpressed cation transporters. Those mutants were phenotyped for intracellular multiplication, cytotoxicity and virulence in oyster experimental infections. Our data show that resistance to reactive oxygen species and efflux of cations are two important functions required for LGP32 intracellular survival, cytotoxic effects and virulence.

CONTRIBUTED PAPERS Thursday, 14:00-16:00

MICROBIAL CONTROL 4

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Establishing the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales) as an endophyte in cucurbits for managing Zucchini Yellow Mosaic Virus (ZYMV)

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The fungal entomopathogen *Beauveria bassiana* (Balsamo)

Vuillemin (Ascomycota: Hypocreales) is known to survive as an endophyte in a wide range of plants and offer protection against an increasing number of insect pests. Although recent discoveries suggest that the fungus can also protect plants against plant pathogens, no studies are currently available on the efficacy of endophytic *B. bassiana* against plant viruses. We conducted experiments to determine whether endophytic *B. bassiana* could provide protection against Zucchini Yellow Mosaic Virus (ZYMV), one of the most economically important diseases of cucurbits worldwide. Four selected *B. bassiana* strains were able to successfully colonize squash plants following the foliar inoculation of plants with the conidial suspension of each respective strain. Disease incidence and severity, sampled weekly following the challenge inoculation of plants with ZYMV, were significantly lower in *B. bassiana*-inoculated plants as compared to control plants; irrespective of the *B. bassiana* strain being inoculated. Our study demonstrates, for the first time, that endophytic *B. bassiana* has the biocontrol potential for managing plant viruses. Further studies should be conducted to determine whether such endophytic *B. bassiana*-mediated protection against ZYMV in squash extends to other cucurbits.

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Bean plant *Phaseolus vulgaris* endophytically colonized by *Beauveria bassiana* and *Hypocrea lixii* acquires protection against *Liriomyza huidobrensis* (Diptera: Agromyzidae) in the field

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Field trials were carried out for two cropping seasons in two sites (Sagana and Narumoro, Central province of Kenya) to evaluate the prospects of endophyte isolates of *Beauveria bassiana* and *Hypocrea lixii* for the control of leafminer *Liriomyza huidobrensis* in *Phaseolus vulgaris*. Autodissemination device treated with conidia of *Metarhizium anisopliae* was also added as a treatment. The effects of endophytes on leafminer infestation (punctures and mines), number of pupae and parasitoids, and yield were evaluated. Both isolates successfully colonized different parts of *P. vulgaris* plants; however, colonization was greater with *H. lixii* than *B. bassiana* in both sites. Leafminer infestation was not significantly different during the first season while it was higher in the controls than in endophyte treatments at both sites during the second season. The number of pupae varied between 150-250 and 320-400 in endophyte and control treatments, respectively, during the first season; and from 100-200 and 350-500, respectively, in endophyte and control treatments during the second season. The number of parasitoids that emerged from pupae did not differ significantly among the treatments. Higher yield was obtained in endophyte than in control treatments. With exception to yield during season two, the inclusion of autodissemination device treatment did not have significant effect on all the parameters evaluated. There were no significant differences between the fungal isolates. Results of the present study suggest that both endophyte fungal isolates hold potential and could be considered for the control of leafminer. There is the need however to confirm these results on large-scale trials.