Potential of *Burkholderia seminalis* as biocontrol agent against *Fusarium oxysporum* evaluated by mass spectrometry imaging

Francisca Diana da Silva Araújo[1] (PQ), Welington Luiz Araújo[2] (PQ), Marcos Nogueira Eberlin[1] (PQ)

*[1]ThoMSon Mass Spectrometry Laboratory, Chemistry Institute, University of Campinas, POB 6154, 13084-970, Campinas, SP, Brazil. [2]Biomedical Sciences Institute, University of São Paulo, São Paulo, SP, Brazil. *eberlin@iqm.unicamp.br*

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Abstract

We have demonstrated that *B. seminalis* uses pyochelin for inhibition of *F. oxysporum*, probably in addition to unidentified antibiotics.

Introduction

Burkholderia seminalis strain TC3.4.2R3 was isolated from surface disinfected sugarcane roots and presented antagonism against *Fusarium verticiloides* and *Xanthomonas albilineans*.[1] Thus, here we apply the matrix-assisted laser desorption/ionization-time of flight mass spectrometry imaging (MALDI-TOF MSI)[2] to characterize the spatial and temporal distribution of *B. seminalis* metabolites in monoculture and co-culture with *F. oxysporum*, a fungus that causes diseases in several crops of agricultural interest.[3]

Results and Discussion

Wild-type *B. seminalis* colonies inoculated on MALDI glass slides containing a thin film of PDA medium were evaluated at 24, 48 and 72 h of incubation. Many compounds were detected at 24 h, and colony-localized ions were differentiated from diffusible metabolites. The ions of *m/z* 186, 198, 329, 373, 399, 428, 450, 714 and 799 showed to be associated with the bacterial colony. The compounds of *m/z* 247, 275, 277 and 279 were diffuse within the agar but more abundant into bacterial colony, and the diffuse metabolite of *m/z* 325 was more intense outside the colony, pointing to higher level of involvement in the inhibitory activity (Figure 1). An increase in the spatial distributions of diffuse metabolites was observed in 48 and 72 h of incubation. The ion of *m/z* 714 analyzed by MALDI-TOF/TOF was identified as polyglutamate, a marker for extracellular matrix previously found in *Bacillus subtilis*,[4] *P. aeruginosa*, and *B. pumilus*.[5] The ion of *m/z* 325 was identified by ESI-FT-ICR as pyochelin (*m/z* 325.0672), a known siderophore also found in *P. aeruginosa*.[3] Diffuse metabolites were almost totally absent at 24 h in mutant strains (M3, M4 and M7) carrying disruptions in antimicrobial compound gene clusters. However, it was possible to detect a gradual increase in the production of these metabolites with the incubation time (Figure 1), demonstrating that their production have not been fully silenced but only delayed.

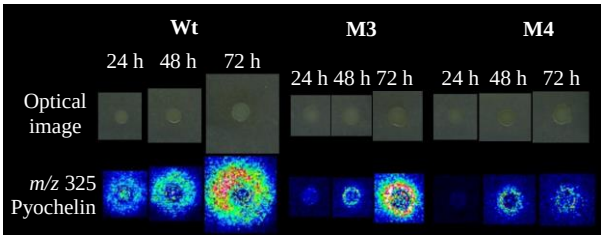

Figure 1. MALDI MSI images (spatial resolution of 200 μm) of pyochelin in wild type *B. seminalis* and mutant M3 and M4 on PDA medium.

The micotoxins bikaverin (protonated, sodiated and potassiated) and fusarin C (sodiated) were detected in *F. oxysporum* by MALDI-TOF MSI, besides polyglutamate. In co-culture of the bacteria with the fungus were not detected by MSI additional metabolites of *B. seminalis*, and the pyochelin production were similar to the monocultures. *B. seminalis* antagonism probably is resulted of the antifungal activity of pyochelin, and perhaps together other molecules unidentified in this work. The interaction zone revealed additional molecules produced by fungus.

Conclusions

Our study confirms that MALDI-TOF MSI is an excellent technique to evaluate metabolites involved in polymicrobial interactions, and points for potential use of *B. seminalis* as biocontrol agent of diseases caused by *F. oxysporum*.

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