

Bacterial Oxalotrophy as a Biocontrol Mechanism against Fungal Pathogens

Aislinn Estoppey¹, Fabio Palmieri* (fabio.palmieri@unine.ch), Geoffrey L. House², Debora F. Rodrigues³, Jamey D. Young⁴, Saskia Bindschedler¹, Pilar Junier¹, **Patrick S. G. Chain**¹.

¹Laboratory of Microbiology, Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland;

²Bioscience Division, Los Alamos National Laboratory, Los Alamos, New Mexico; ³Civil and Environmental Engineering, University of Houston, Houston, Texas; ⁴School of Engineering Vanderbilt University, Nashville, Tennessee

<https://genomicscience.energy.gov/research/sfas/lanlbfi.shtml>

Project Goals: The worrisome rise of antifungal drugs resistance leads to the need of finding alternative therapeutic strategies to fight against fungal pathogens. Here we propose to apply bacterial nutritional interference as an alternative biocontrol strategy in order to control the growth of these pathogenic fungi.

The worldwide emergence of drug-resistant pathogenic fungi in the last decades is a major problem not only in human health, but also in agriculture and food security. Nowadays, only a very limited number of therapeutic alternatives are available for the treatment of fungal infections. Generally, the same compounds are used in both agriculture and medicine, creating an interconnected system favoring the spread of resistance. This is particularly worrisome for pathogens with a broad range of hosts. Therefore, there is an urgent need to find more sustainable therapeutic approaches to mitigate the rise of antifungal drug resistances.

Some phytopathogenic fungi such as *Sclerotinia sclerotiorum* or *Botrytis cinerea* are known to use oxalic acid as a pathogenicity factor. Indeed, oxalic acid chelates calcium ions in the middle lamella of the plant cell wall in order to access and degrade pectin. Moreover, oxalic acid often precipitates as calcium oxalate (CaOx) crystals. These crystals mechanically break the plant cell wall, facilitating penetration and infection of the plant tissues. Oxalic acid is one of the most common low molecular weight organic acids (LMWOA) produced by fungi and it plays multiple roles aside pathogenicity in processes such as interspecies competition, mineral weathering, and lignocellulose degradation. In soil, it is primarily present in the form of CaOx crystals. This pool of CaOx can be consumed by oxalotrophic bacteria, leading to a local pH increase. The effect of bacteria on CaOx and pH are the stepping stones of this biocontrol project. In the case of normal infection, the secretion of oxalic acid by the fungal pathogen leads to the acidification of the host tissues and makes the environment favorable for infection. However, when oxalotrophic bacteria are added to the system, the bacterial consumption of oxalic acid leads to a pH increase and makes the environment less favorable for infection.

Confrontation experiments in Petri dishes were conducted with different phytopathogenic fungi (*Botrytis cinerea* and *Sclerotinia sclerotiorum*) and oxalotrophic bacteria (*Cupriavidus necator*, *Cupriavidus oxalaticus*, and *Burkholderia phytofirmans*) in different nutritional conditions. In addition, a non-oxalotrophic bacterium (*Pseudomonas putida*) and a Δ oxc-mutant of *B. phytofirmans* were considered as controls. *C. necator* and *C. oxalaticus* inhibited the growth of both *B. cinerea* and *S. sclerotiorum*. This was also the case for *B. phytofirmans*, with a pronounced growth inhibition in confrontation with *S.*

sclerotiorum. The Δoxc -mutant of *B. phytofirmans* still inhibited the growth of *S. sclerotiorum* but to a lesser extent. Moreover, oxalic acid concentration was quantified in liquid mono- and co-cultures. Plant-bacterium-fungus interactions are proposed to be investigated in the future with the plant model *Lactuca sativa* (lettuce).

This study was supported by a U.S. Department of Energy Biological and Environmental Research Science Focus Area grant (grant no. DE-AC52-06NA25396).