Exploration of secondary metabolites as biocontrol agents against sugar beet disease

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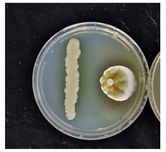
Introduction

Sugar beet pathogens are a major issue that greatly impact the yield of sugar beets harvested each year. Developing new strategies to combat sugar beet disease is vital for the growth and survival of the sugar beet industry. In this study, we have detected and identified secondary metabolites from *Burkholderia contaminans,* a sugar beet leaf bacterial symbiont, possessing antifungal activity against the sugar beet foliar pathogen *Cercospora beticola.*

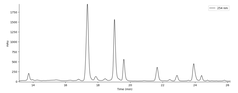


Antagonism plate of *Burkholderia contaminans* (left) against *Cercopsora beticola* (right)

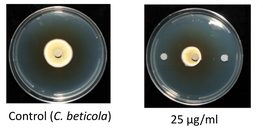
Another aim of the study is to better understand the chemical mechanisms utilized by *C. beticola* to infect sugar beet leaves. Therefore, we have set out to identify secondary metabolites produced by *C. beticola* and the underlying genetic structures. Utilizing various analytical approaches such as HPLC, LCMS, bioinformatics, and genetic manipulations, we have identified secondary metabolites produced by *C. beticola* and have gained insight into the biosynthesis of the yellow pigmented metabolite, fulvic acid.

Metabolites identified and purified from *Cercospora beticola*

Secondary metabolites identified from *B. contaminans* possess antifungal activity against *C. beticola*



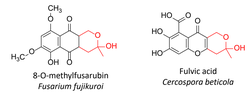
HPLC chromatogram showing the presence of metabolites in the EtOAc extract of *Burkholderia contaminans* growth medium

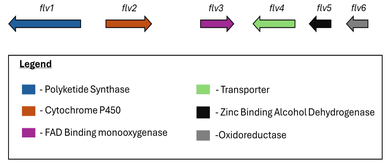


Disk diffusion assay of metabolite identified from metabolomic studies showing inhibitory activity against *C. beticola*

Genome mining for the fulvic acid biosynthetic gene cluster (BGC)

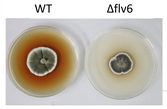
Fulvic acid shares structural similarities with the metabolite, 8-O-methylfusarubin, whose biosynthesis has been previously studied. The backbone of 8-O-methylfusarubin is formed by a polyketide synthase (PKS). The thioesterase domain of the PKS is responsible, in part, for the formation of the dihydro methylhydroxy pyran moiety[1] (highlighted in red). A BLAST analysis of the 8-O-methylfusarubin producing PKS and its thioesterase domain revealed a homologous PKS with a thioesterase domain showing 61.4% and 71% sequence homology respectively.



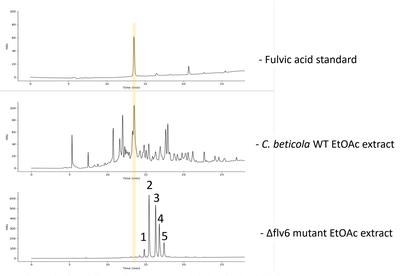


Putative Fulvic acid BGC in *C. beticola*

Genetic knockout of *flv6* shows absence of fulvic acid and accumulation of additional metabolites



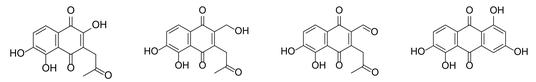
C. beticola WT and Δflv6 mutant show obvious phenotypic differences



HPLC analysis of *C. beticola* WT and *C. beticola Δflv6*

Fulvic acid is present in the EtOAc extract of the WT *C. beticola* but absent in the EtOAc extract of the *C. beticola* Δflv6 mutant. Additional metabolites were shown to accumulate in the *C. beticola* Δflv6 mutant EtOAc extract.

Putative structures of metabolites accumulating in the extract of *C. beticola* Δflv6 based on LCMS and UV spectra of purified metabolites

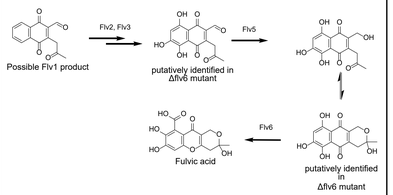


1 $C_{13}H_{10}O_6$ **2** $C_{14}H_{12}O_6$ **3** $C_{14}H_{10}O_6$ **5** $C_{14}H_8O_6$

Putative structures of metabolites shown in the chromatogram of the *C. beticola* Δflv6 mutant

Metabolites accumulating in the EtOAc extract of the *C. beticola* Δflv6 mutant were purified and analyzed by high-resolution mass spectrometry. The UV spectra and molecular formulae of the purified metabolites are consistent with that of napthoquinones[2]. This shows that the PKS likely releases a napthoquinone intermediate and the *flv6* gene is likely responsible for the conversion of the napthoquinone product into the subsequent benzopyrone.

Proposed biosynthesis of fulvic acid



Summary

1. A metabolite was identified from the sugar beet leaf bacterial symbiont *Burkholderia contaminans* that possesses antifungal activity against the sugar beet foliar pathogen *Cercospora beticola.*
2. A putative biosynthetic gene cluster was identified in *C. beticola* for the production of the metabolite fulvic acid which has been shown to be abundant in aggressive strains of *C. beticola*[3].
3. Through chemical analyses and genetic manipulations, a biosynthetic pathway for fulvic acid has been proposed.

Acknowledgements

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