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Identification of a *Streptomyces* specialized metabolite involved in antifungal activity, plant defense stimulation and bacteria fitness in the rhizosphere

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Introduction

Identifying new strains from rhizosphere and understanding their biological activities offers new approaches to plant protection. *Streptomyces* sp. AgN23 interacts with *Arabidopsis thaliana* and activates hypersensitive response (HR) in Arabidopsis root.

Streptomyces sp. AgN23 was isolated from grapevine rhizosphere and screened for its abilities to produce

Gaining deeper understanding of the interaction between AgN23 and *Arabidopsis thaliana* is necessary to assess its potential as a biocontrol agent.

Findings

- 1 Identification of a candidate specialized metabolite in AgN23 culture extracts
- 2 Implementation of a reverse genetic approach
- 3 genes, metabolomic rerouting
- 4 Importance of the compounds biosynthesis for the strain fitness

1 *Streptomyces* sp. AgN23 interacts with *Arabidopsis* root and reroutes plant specialized metabolome

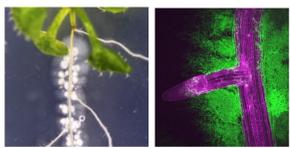


Figure 1: Left, Photograph of *Arabidopsis thaliana* rhizosphere colonized by AgN23 10 days after inoculation. Right, Confocal fluorescence image of AgN23-GFP around *Arabidopsis* root.

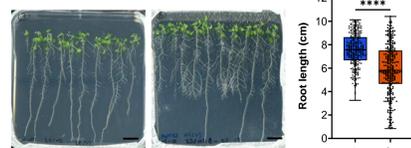


Figure 2: Observation of *Arabidopsis thaliana* dwarf root phenotype 10 days after inoculation with AgN23.

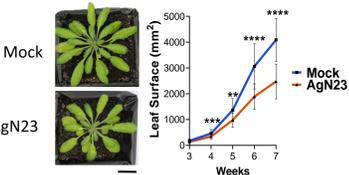


Figure 3: Arabidopsis leaf area measurement following growth within non- or inoculated potting soil.

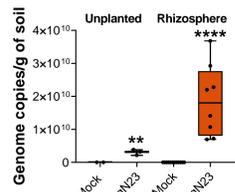


Figure 4: AgN23 genome copy number in *Arabidopsis* rhizosphere 8 weeks after soil inoculation.

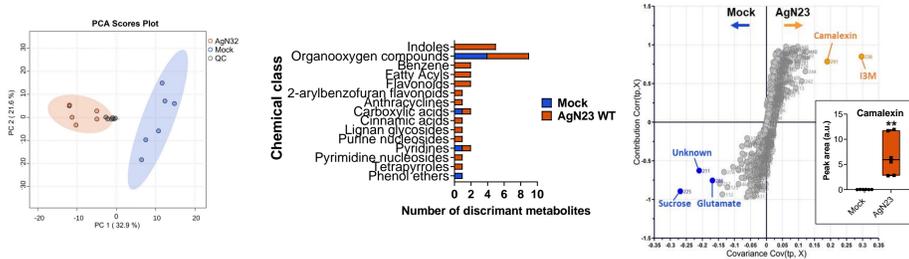


Figure 5: AgN23 modulates *Arabidopsis* root metabolome by inducing biosynthesis of indolic compounds such as camalexin.

2 AgN23 produces galbonolides, antifungals inhibiting ceramide synthesis in *Arabidopsis thaliana*

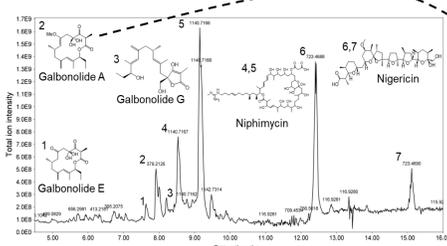


Figure 6: Typical UHPLC-HRMS chromatogram of supernatant of AgN23 culture

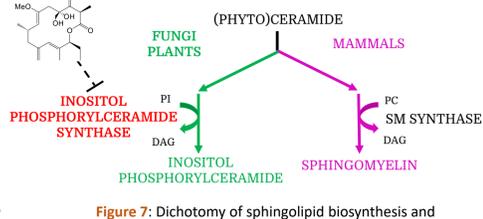


Figure 7: Dichotomy of sphingolipid biosynthesis and inhibition of IPCs by galbonolides

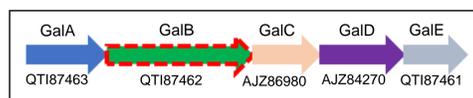


Figure 8: Galbonolides biosynthetic gene cluster with putative AgN23 genomic loci (NCBI assembly GCA_001598115.2). Knock-out gene is shown in red broken line.

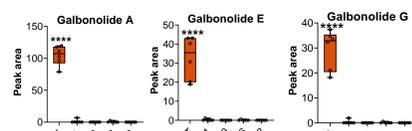


Figure 9: Peak area of the different galbonolides structures based on HRMS and MS/MS spectra from WT and KO strains.



Figure 10: Left, Conversion of NBD-C6-ceramide to NBD-C6-IPC by *Arabidopsis thaliana* inositol phosphoceramide synthase (AtIPCS2). Right, AtIPCS2 activity following treatments with extracts from culture supernatant of AgN23 WT or KO mutants

3 Validation of the role of the candidate compound in plant defense pathways activation (HR, defense)



Figure 11: Necrosis symptoms in *Nicotiana benthamiana* leaves infiltrated with extracts from culture supernatant of AgN23 WT or KO mutants. Concentrations in $\mu\text{g/mL}$.

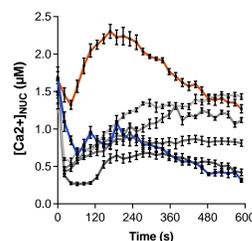


Figure 12: Left, Kinetics of AgN23 or KO mutants culture extracts-induced enhancement of the nuclear calcium concentration in *Arabidopsis* seedlings. Right, Ion leakage from *Arabidopsis* leaf discs 48 hours after infiltration.

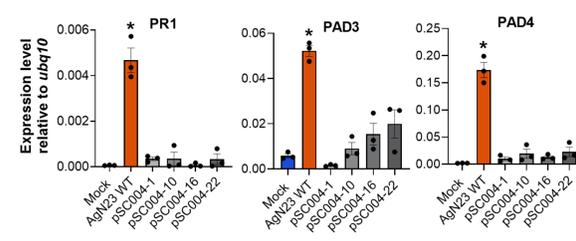
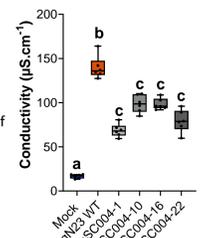


Figure 13: In planta expression level of *pr1*, *pad3*, and *pad4* genes responsible for salicylic acid-dependent systemic acquired resistance, synthesis of camalexin and positive regulation of SA accumulation, respectively

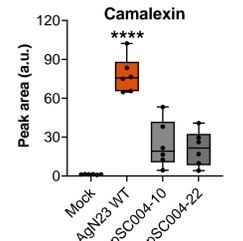
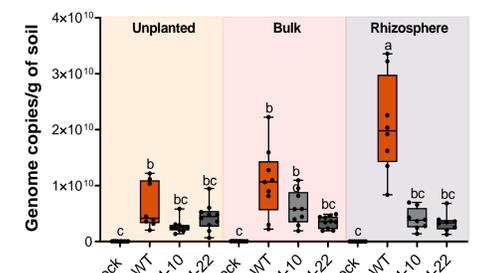


Figure 14: Peak area of camalexin in *Arabidopsis* 10 days after inoculation with WT or KO strains

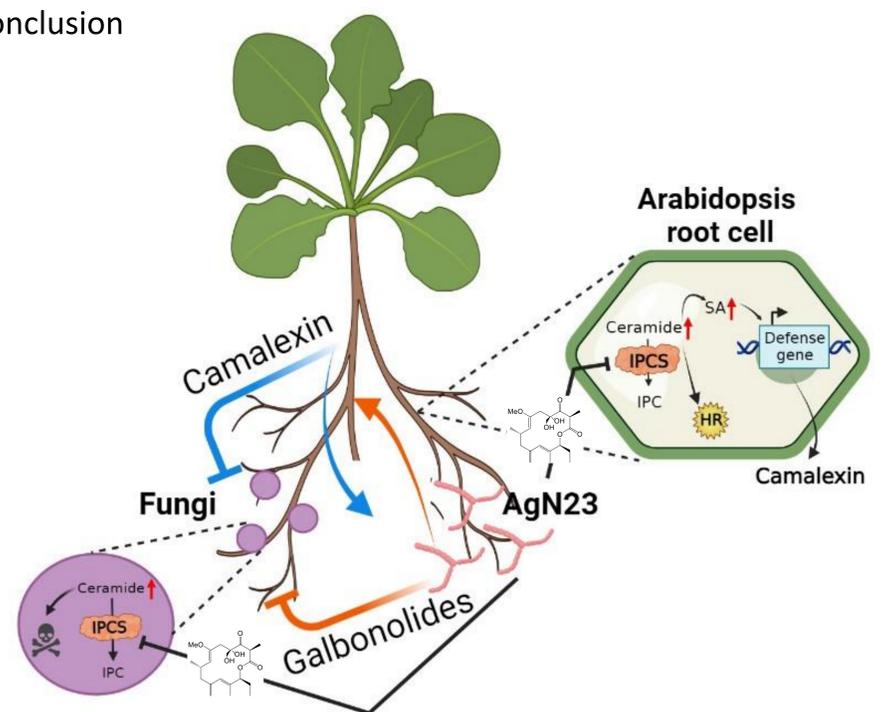
4 Galbonolides biosynthesis modulates AgN23 fitness in the rhizosphere of *Arabidopsis thaliana*



Figure 15: Left, Photographs of *Arabidopsis* 6 weeks within WT or KO inoculated potting soil. Right, AgN23 genome copy number in *Arabidopsis* rhizosphere 6 weeks after inoculation.



Conclusion



References

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