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Plant signaling compounds alter secondary metabolite production among antagonistic *Streptomyces*

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Abstract

Streptomycetes have been implicated in the control of soil-borne plant pathogens, and are known to produce an extensive array of antimicrobial secondary metabolites. We investigated the hypothesis that plants manipulate the production of secondary metabolites by streptomycetes. We tested a collection of diverse *Streptomyces* isolates for responses to potential signaling molecules produced by plants, including plant hormones, flavonoids, sesquiterpene lactones, and crude root exudates. Secondary metabolite production was investigated with the use of high performance liquid chromatography (HPLC) and bioassays for inhibitory activity. We found evidence that streptomycetes respond to plant-produced compounds with altered patterns of secondary metabolite production. *Streptomyces* isolates in our study had the ability to chemically modify and produce close analogs of plant-derived compounds. The production of similar chemical compounds may facilitate cross-kingdom communication. Our work suggests the potential for plants to manipulate the activities of soil microbial communities, which may confer a selective advantage in suppression of plant pathogens. These results concur with studies from many different systems showing that microbial activity is tightly linked with the health and functioning of higher organisms.

Objective

Our aim was to investigate the possibility that plants use chemical compounds to manipulate the behavior of soil microorganisms. Plants could improve their health by enhancing beneficial microbial activities, such as antibiotic production leading to pathogen suppression.

Materials & Methods

Streptomyces isolates were tested in vitro for response to exogenous chemicals known to be involved in other plant-microbe symbioses (Table 1), using paper disc assays or amendments to liquid cultures. *Streptomyces* colony development, pigment production, spore formation, and antibiotic production were evaluated in the presence of these compounds at various concentrations. Antibiotic production was quantified by inhibition of an overlay Bacillus strain. Changes in secondary metabolite production due to the presence of the plant compounds were observed observed via HPLC and tandem HPLC-mass spectrometry (LC-MS).

Table 1 – A subset of the plant compounds tested in this study.

Chemical Class	Known Activities	Compounds Tested
Flavonoids	Nodulation Mycorrhizae *stimulation*	Chrysin Rutin Luteolin
Sesquiterpene Lactones	Parasitic plant germination Mycorrhizae formation	Artemisinin Parthenolide
Plant Hormones	Plant growth & development	Trans-zeatin riboside Indole-3-acetic acid

Results

• Plant signaling compounds alter phenotypes related to secondary metabolism (including pigment production, colony morphology, and sporulation; Figure 1), and can both enhance and repress antibiotic production (Figure 2).

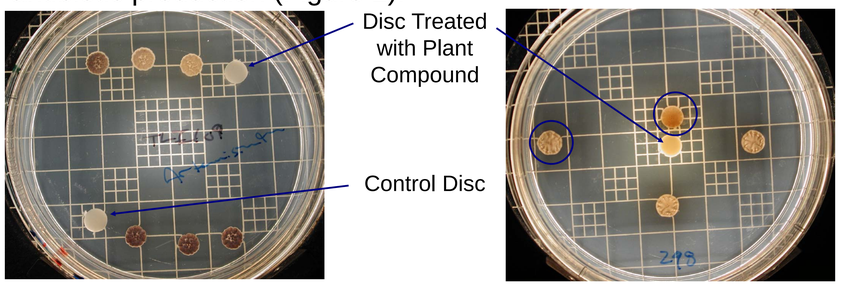


Figure 1 - Plant signaling compounds can reduce (artemisinin; left panel) or enhance (morin; right panel) pigment production, and can alter colony development (smooth colony morphology at the highest dose of morin; right panel). The same *Streptomyces* isolate was spotted multiple times on a single plate.

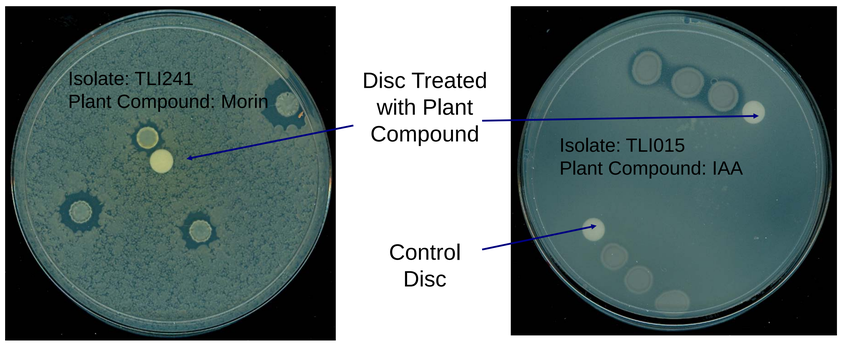


Figure 2 - Plant signaling compounds can both repress (morin; left panel) and stimulate (indole-3-acetic acid; right panel) the production of toxic secondary metabolites by *Streptomyces spp.*, as revealed by *in vitro* inhibition of an overlay *Bacillus* strain.

• *Streptomyces* sp. *can* chemically modifying plant signaling compounds (Figures 3 & 4) and may respond to plant signaling compounds with the production of novel secondary metabolites (Figure 3).

Figure 3 - HPLC chromatograms of culture extracts. Top: solvent control. Bottom: chrysin treatment. Three new peaks are evident in the treated sample. Visible are chrysin (18.566 min), a modified derivative of chrysin (16.379 min; see Fig 4), and a new peak (13.598 min) which may be a derivative of chrysin, or a new *Streptomyces* metabolite that is induced by chrysin.

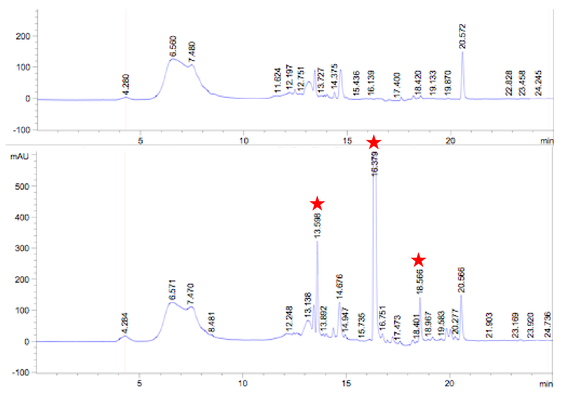
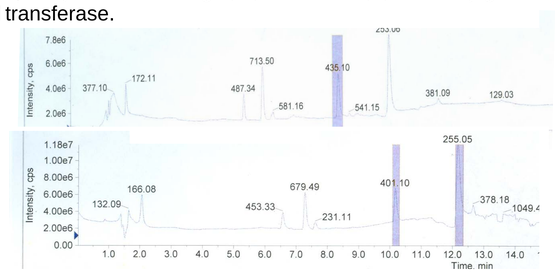
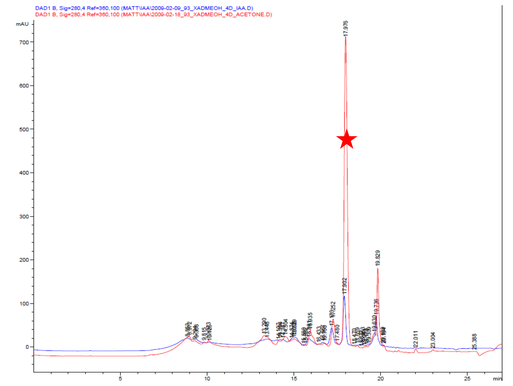


Figure 4 - LC/MS data showing the induction of a new compound in the presence of chrysin (at 13.598 min; Figure 3, bottom panel). The mass of the compound eluting in this peak is 400 amu (M+H = 401 in positive mode, $M+Cl^-$ = 435 in negative mode). Since the UV spectrum of this compound is almost identical to that of chrysin, the compound is most likely a modification of chrysin. The mass difference of 146 suggests the addition of a known streptomycete amino sugar, 2,4 diamino 2,4,6 trideoxy glucose by the action of a glycosyl transferase.



• Plant signaling compounds can dramatically alter the quantity of some secondary metabolites produced by *Streptomyces* isolates (Figure 5).

Figure 5 – HPLC chromatogram showing dramatic increase in production of an unknown secondary metabolite (peak marked with red star) in a culture treated with indole-3-acetic acid (red trace) compared to the solvent control (blue trace).



• *Streptomyces* are capable of producing close analogs of plant hormones and signaling compounds.

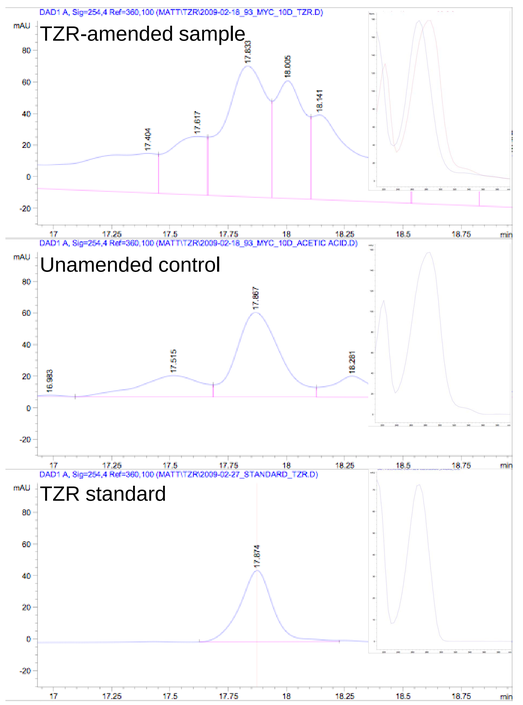


Figure 6 - HPLC chromatograms showing the production of a close analog of trans-zeatin riboside (TZR; a plant hormone) by a *Streptomyces* isolate. The top panel corresponds to extracts from a culture amended with TZR. Both TZR and an analog are evident (peaks at 17.833 min and 18.005 min). The middle panel is the unamended control culture. Only the TZR analog is observed (peak at 17.867 min). The bottom panel shows the TZR standard. Exogenous TZR and the compound produced by *Streptomyces* sp. have nearly identical retention times and UV absorbance characteristics.

Conclusions

This work illustrates the potential for plants to have direct and significant effects on soil microbial activities related to pathogen suppression. Antimicrobial secondary metabolites have been implicated repeatedly in successful biocontrol, and our work demonstrates the potential for plant compounds to impact antibiotic production both positively and negatively. Multiple features of streptomycete growth and development, including colony morphology, pigment production and sporulation are also altered by the presence of plant signaling molecules. Streptomycetes in turn exhibit the potential to alter plant development and symbioses with other organisms by modifying or creating mimics of plant hormones and signaling molecules produced by plants. Cross-taxa signaling interactions may be vital in the regulation of costly metabolic processes such as antibiotic production, and may impact microbial community evolution or important ecosystem functions such as plant disease biocontrol.

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