Mode of action of a multi-microbial inoculant on pathogen suppression and impact on soil microbial communities

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Introduction

Recently, a new product was introduced with potential to control plant diseases. The multi-microbial inoculant (MI) product consists of numerous species of benign and probiotic microorganisms that are co-cultured in an aqueous medium. The MI product mediates diverse activities including: (1) suppressing plant pathogen activity and (2) enhancing microbial activity (i.e., decomposition of crop residues). Modes of action driving these activities are unknown. We hypothesize that the modes of action contributing to microbial efficacy include: (1) the enhancement of soil microbial community diversity and (2) interference with pathogen quorum sensing (QS) pathways.

We present an approach for testing these hypotheses and share preliminary results. The first hypothesis is approached by analyzing microbial DNA samples from soils treated with MI. To test the second hypothesis we examine possible breakdown of QS autoinducers (AI) upon exposure to MI using Gas Chromatography-Mass Spectrometry (GC-MS).

Methods

Objective 1

Analyze the diversity of soil microbial communities after MI application to soil.

Approaches

MI was applied to soils in the field and intact soil microcosms incubated in a growth chamber.

1. Field Setting

Land Management (Fig. 1):
- Cultivated Soil or
- Reintroduced Grassland

Treatments:
- Three spray applications of MI (100 L ha^{-1}) or
- Three spray applications of H_2O (100 L ha^{-1}) (control)

Replication:
- Each land management x treatment is replicated in triplicate (12 plots total)

Plot dimensions: 3m x 5m

Study initiated in March 2012 and plots were sampled eight times between initiation and October 2012.

2. Growth Chamber Study of Intact Soil Microcosms

Soil microcosms:
- 15 intact soil cores (ISC) were obtained from cultivated soil control plots and planted to tomato (*Solanum lycopersicum*)

Treatments (MI inoculation rates and dates)
- 40L ha^{-1} (April) and 3x20L ha^{-1} (May-October)
- 40L ha^{-1} (April) and 60L ha^{-1} (May)
- 40L ha^{-1} (April) and 60L ha^{-1} (July)
- 60L ha^{-1} (October)
- Control received corresponding amounts of H_2O whenever MI was applied

Replication:
- Each treatment and control is replicated in triplicate (15 ISC total)

Methods (continued)

Objective 2

Monitor changes in QS autoinducer (AI) concentrations during exposure to MI or H_2O.

Approach **(Fig. 2.)**

Two AIs examined
1. N-(3-Oxo**hexa**noyl)-L-homoserine lactone (AHLEc)
2. N-(3-Oxo**deca**noyl)-L-homoserine lactone (AHL)

Standard curves for AI were developed using GC-MS and used for sample analyses

AIs were exposed to MI or H_2O for 2, 4, and 8 hours

After exposure, AIs were extracted by liquid-liquid extraction with chloroform

Chloroform extracts were analyzed using a GC-MS (Fig. 2) to determine AI concentrations

Figure 1. Experimental Approach (Objective 1)

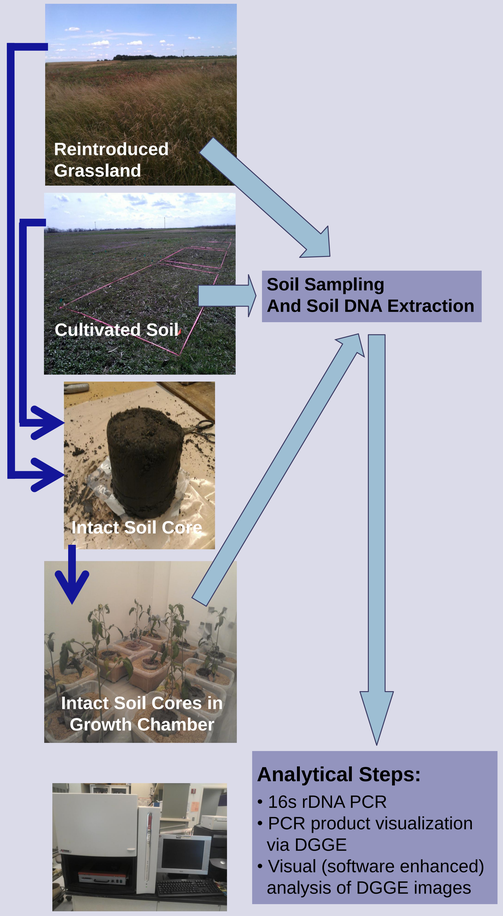


Figure 2. Experimental Approach (Objective 2)

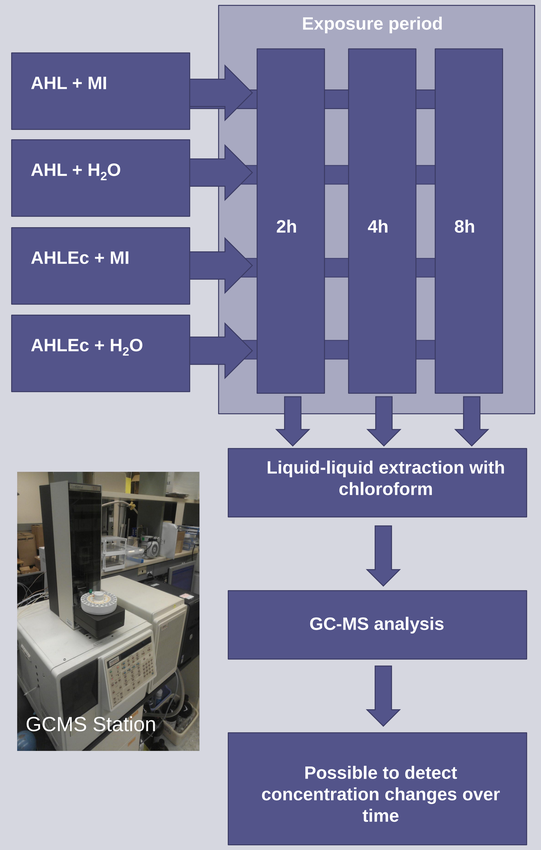


Fig. 3. Chromatograms of AHL extracts; 2h exposure to water (red) and MI (blue)

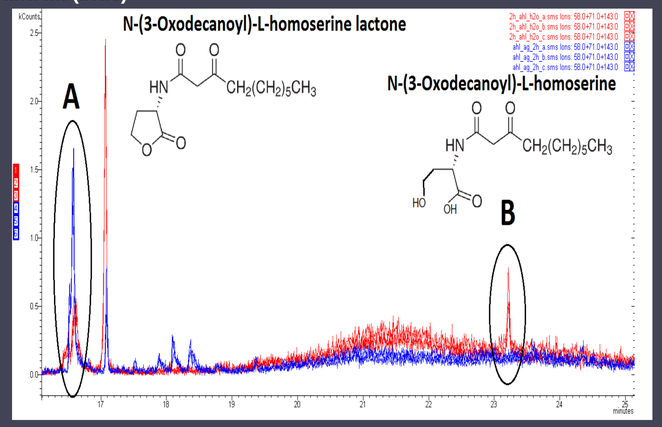
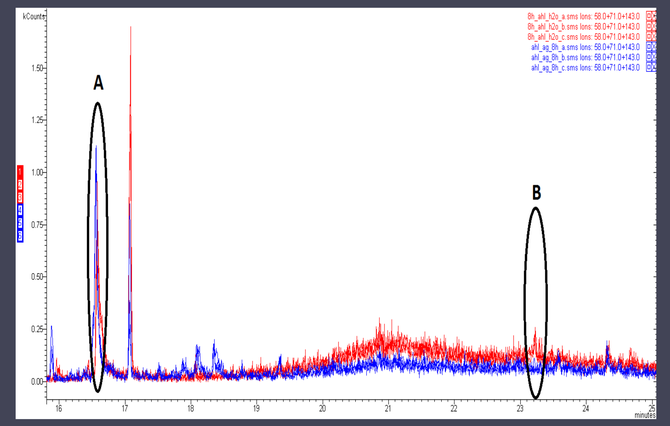


Fig. 4 Chromatogram of AHL extracts; 8h exposure to water (red) and MI (blue)



Results

Objective 1

•No results; research in progress

Objective 2

•GC-MS analysis of AHL standards revealed two major compounds (Fig.3). Most likely they are:
- •N-(3-Oxodecanoyl)-L-homoserine lactone (Peak A) and
- •N-(3-Oxodecanoyl)-L-homoserine (Peak B).

The former is known as the active AI while the latter is inactive.

•In MI treatment, the decrease in the concentration of N-(3-Oxodecanoyl)-L-homoserine lactone (Peak A) was not followed by an increase in the concentration of N-(3-Oxodecanoyl)-L-homoserine (Peak B) (Fig. 4).

•AHLEc analysis is in progress

Preliminary Conclusions

Results support the hypothesis that MI affect QS Autoinducers. However, AI degradation appears to be not the only indicator of MI impact; MI may rapidly convert AH to AHLA for subsequent degradation.

Acknowledgments

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