Biochar quieting of microbial chatter varies with production conditions

http://biochar.rice.edu

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Microbes use cell-cell communication to control behaviors

Low density

A diffusible **signal** (S) is synthesized continuously by each bacterium

Quorum density

When QUORUM is achieved, S accumulates and **flips molecular switch** that changes biosynthesis

Higher density

Newly synthesized molecules accumulate and **affect cell behaviors**
Diverse behaviors are coupled to population density (growth & health)

Symbiosis

- Legumes
  - (Rhizobium leguminosarum)
  - (Bradyrhizobium japonicum)
  - (Sinorhizobium meliloti)

- N₂
- NH₃

Virulence

- Carrot/Potato (Erwinia carotavora)
- Maize (Pantoea stewartii)
- Fruit/nut (Agrobacterium tumefaciens)
- Onion (Burkholderia cepacia)
- Lettuce (Pseudomonas aeruginosa)

Quorum sensing

Biofilm formation

Spore formation

Chemical languages vary among species

**Bacteria** (MW = 300 to 2300 amu)

- Acylhomoserine lactones
- Oligopeptides

**Fungi**

- Farnesol
- Tyrosol
- dimethoxycinnamate
- A factor

Other: furanosyl borate diesters, quinolones, indole


*Eukar. Cell.* 2006. 5: 613-619
A single microbial species can have parallel conversations

Low population density

Multiple signals (S1 & S2) synthesized by each bacterium

Quorum density

When QUORUM is achieved, S flips different molecular switch that controls biosynthesis reactions (can be different densities)

Higher density

Newly synthesized molecules accumulate and affect different cell behaviors

Some N₂ fixing symbionts for plants (e.g., *Rhizobium leguminosarum*) uses five distinct signals to make decisions!
Other organisms can silence conversations by making signal-degrading enzymes.

Virulence by *Erwinia carotovora* requires quorum signal (S).

No virulence when organisms present that degrade signal used by *Erwinia*.

Like quorum quenching organisms, biochar can alter cell growth

Sorhgem root (+Fungi)

Symbiosis

Parasitism

Biochar, 400°C (+N nutrient)

Hypothesis

1. Biochars sorb some of the signals used for microbial cell-cell communication (focus on 12C acyl homoserine lactones, AHL).

![AHL molecule](image)

2. AHL sorption occurs on the time course of microbial signaling and gene expression.

3. Biochar production conditions influence the extent to which biochar quiets microbial signaling.

4. Biosensors can rapidly assess biochar effects on signaling and provide insight into biological observations.
Programming *Escherichia coli* to report on one acyl homoserine lactone (AHL)

1. Insert DNA that encodes an AHL-dependent transcriptional regulator (LasR) from *Pseudomonas aeruginosa*

2. Insert DNA encoding green fluorescent protein (GFP) under LasR regulation

3. Use AHL addition to switch LasR “ON” so that it binds DNA, drives GFP synthesis, and makes cells green (which are easy to image)
Add AHL to *E. coli* cultures

*Escherichia coli* display AHL-dependent fluorescence

![Graph showing relative fluorescence with AHL concentration](image)
Strategy for comparing effects of different biochars on AHL availability

Incubate AHL with varying [biochar]

Add soluble fraction to *E. coli*

**[AHL] that makes cells green**

1 µM in 1 mL

Biologically relevant concentration of AHL within the environment

Time is near the doubling rate of microbes within rich nutrient conditions
Slow pyrolyzed mesquite was used to study effects of production conditions

- Incubate AHL with varying [biochar]
- Add soluble fraction to *E. coli*

[AHL] that makes cells green

1 µM in 1 mL

Hardwood slow-pyrolyzed at a range of temperatures

Surface area (m²/g)

Temperature, °C
700°C biochar inhibits AHL-induced fluorescence

[AHL] that makes cells green

Add soluble fraction to E. coli

Incubate AHL w. varying [biochar]

1 hr incubation

1 µM in 1 mL

Relative fluorescence

0 0.25 0.5 0.75 1

mg charcoal/mL

1% by mass is comparable to level that elicits biological effects within soils

700°C biochar inhibits AHL-induced fluorescence
Production conditions alter biochar inhibition of cellular fluorescence

- **Weakest inhibition**: half-maximal inhibition at 50 mg/mL char
- **Strongest inhibition**: half-maximal inhibition at ≤1 mg/mL char
Relationship between surface area, production temperature, and GFP expression

![Graph showing the relationship between biochar surface area, production temperature, and GFP expression. The graph includes data points for 300°C, 350°C, 400°C, 450°C, 550°C, 600°C, and 700°C, with an exponential fit line and an R² value of 0.99. Biochar (mg/mL) needed for GFP inhibition (50%) is plotted on the y-axis, and biochar surface area (m²/g) on the x-axis.]
Microbial growth is not altered by charcoal treated water

Suggests that these charcoal aren’t releasing compounds that mimic AHL.
(would be interesting to screen biochars for such compounds)
Inhibition occurs on the time scale of microbial signaling and gene expression

[AHL] that makes cells green

1 μM in 1 mL

biochar

Incubate AHL w. varying [biochar]

6 min incubation

Add soluble fraction to *E. coli*

Relative fluorescence

Note: Under optimal growth conditions (37°C, rich medium), *Escherichia coli* doubles every 20-30 minutes
An addressable cell-cell signaling system for evaluating hard-to-image biochars
Agar plate assay for quantifying biochar inhibition of microbial chatter
Cells are added to plates, grown for 18 hours, and then imaged.

Receiver cell colony #1

Sender cell colony

Receiver cell colony #2

Chatter inhibition = \frac{\text{ratio green on right}}{\text{ratio green on left}}
Biochars inhibit chatter to varying extents!

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<th>Agar plate #1</th>
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Relative green emission of receiver cells adjacent to biochar (+BC) and empty agar (-BC)

300°C

0.24

700°C

0.02
Conclusions

Wood biochars disrupt cell-cell communication mediated by AHL signals \( \rightarrow \) other signals from bacteria/fungi?

Inhibition of coordinated cell behaviors (GFP expression) varied 10 fold \( \rightarrow \) other biochars? aged biochr?

Biochar effects on gene expression needs to be better characterized \( \rightarrow \) tradeoffs? symbionts vs pathogens?

Synthetic biology is a simple way to build biosensors to compare biochars \( \rightarrow \) better reporters?

Unclear how biochars impact cell-cell communication when mixed with soils \( \rightarrow \) additive effects? non-additive? relationship to short term vs long term priming?
Questions?