Bacteria from roots of six drought-tolerant plant species inhibit growth of sensitive plant species: A physiological characterization

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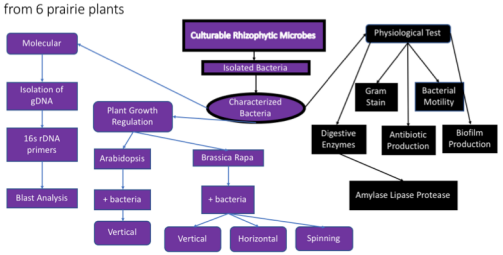
Schematic Representation

A schematic representation of the isolation and characterization of Rhizobacteria from 6 prairie plants



Abstract

The role of Plant-Growth-Promoting Rhizobacteria (PGPR) is well documented, but little has been reported about root bacteria that play an antagonist role in plant development, outside of plant pathogenic bacteria. We have characterized six bacterial isolates from drought-tolerant local plant roots. When co-cultivated with seedlings of *Brassica rapa* and *Arabidopsis thaliana* under sterile conditions, the six isolates inhibited root hair production of the seedlings after 7 days for *Brassica*, and 3 weeks for *Arabidopsis*. We characterized the six bacterial isolates using Gram staining, motility assays, digestive enzyme production (amylase, lipase, caseinase), antibiotic production, and biofilm production. All six isolates were Gram-positive rods of varying size and arrangement. None had the enzyme ACC deaminase - which is common to PGPRs. As might be expected, our isolates did not stimulate plant growth. All six isolates had caseinase activity; five isolates produced the extracellular enzyme amylase, and one isolate - from *Monarda fistulosa:* (wild bergamot root) - had lipase activity. Isolates from *Monarda fistulosa*, *Agastache foeniculum* (anise hyssop root), *Verbesina alternifolia* (ironweed root), and *Platycodon grandiflorus* (balloon flower) root produced biofilms. Was the inhibition of *Brassica rapa* root hair growth by the six isolates due to a lack of ACC deaminase activity or due to an antagonism between native *Brassica rapa* bacteria and the 6 isolated bacteria from the drought-tolerant plants? To answer this question, lawns of the six root bacterial isolates were tested against 12 native *Brassica rapa* bacterial isolates for antibiotic activity. Of the 12 native *Brassica rapa* isolates, several isolates exhibited antibiotic production against five of the six root bacteria. The production of biofilms and antibiotic activity might explain the inhibitory effects of the *Brassica rapa* native bacteria on the growth of the six isolates from the drought-tolerant plants. In a second poster we have done a molecular characterization of the six isolates.

Introduction

Plant growth-promoting rhizobacteria (PGPR) characterized in previous studies (Brennan et. al.) typically increased biomass and root hair numbers and reduced the plant's susceptibility to abiotic stresses. Eighty percent of rhizobacteria and fungi produce hormone auxin and after its delivery to the plant root, the hormone ethylene increases root hair number, elongation, and cell proliferation. Why would a drought-tolerant bacterial species instead, inhibit root hair formation in sensitive plants such as *Brassica rapa*? Is there a connection to biofilms or antibiotic production by these bacteria?

Biofilms are colonies of bacteria that attach to almost any surface (including plant roots) and secrete a sticky matrix of polysaccharides, proteins, and DNA. The first cells to attach themselves to the surface form weak bonds, then use adhesion molecules to make a stronger attachment. In order to facilitate the arrival of other cells to form the biofilm, the original bacterial cells provide more diverse adhesion sites and begin to build the sticky matrix that holds the biofilm together. Once the biofilm is made, communication is done through quorum sensing, using chemical messaging to sense how many bacteria are around them and the type of bacteria (http://bacteriality.com/2008/05/biofilm/).

Some bacteria also have antagonistic properties that give them an environmental advantage by inhibiting the growth of surrounding bacteria. These antagonistic bacteria, in turn, have greater access to the environment's nutrients and resources (Benson). Beltagy outlined the antimicrobial and antioxidant characteristics of *Brassica rapa* in his 2014 study.

The research showed that *Brassica rapa* had inhibitory effects against *Candida albicans*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*.

In this study, we have characterized six rhizobacterial strains from roots of six drought-tolerant local prairie plants, that inhibited root hair formation of *Brassica rapa* in sterile culture.

Results

Table 1. Six root-associated aerobic bacterial isolates were characterized according to digestive enzyme production [Figure 7], motility, gram staining [Figures 1-6], and morphology. For enzyme activity, (-) means no production, a single (+) means weak production, two (++) means medium production, and three (+++) means high production.

Bacterial plant source	Protease (casein) (mm)	Lipase (Spirit blue)	Amylase activity (starch agar)	Motility	Gram Stain	Arrangement and shape of bacteria
Wild bergamot	+++	-	+++	non-motile	+	Clusters of small rods
Anise hyssop	+++	+	+++	non-motile	+	Single, x-large bacilli
Black-eyed susan	++	+	+++	non-motile	+	Long chains of bacilli
Ironweed	++	+	+++	non-motile	+	Medium length chains of bacilli
Prairie sage	++	+	+++	non-motile	+	Isolated large bacilli
Balloon flower	+++	+	+++	non-motile	+	Small, clustered rods

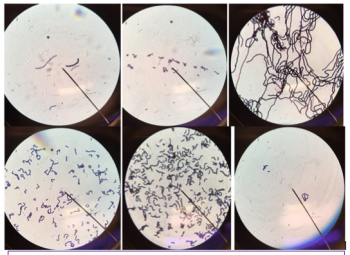


Fig. 1 Representative gram stains (1000x) of a gram positive bacilli from a root bacterial isolate. Left to right, top to bottom: Anise Hyssop, Balloon Flower, Black-Eyed Susan, Ironweed, Prairie Sage, Wild Bergamot.

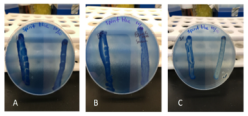


Fig. 2 Lipase Activity on Spirit Blue Plates. A-C (left to right) Anise Hyssop, Ironweed, Balloon Flower, Black – Eyed Susan, Prairie Sage, Wild Bergamot

Table 2. Six prairie bacterial tester strains were streaked onto TS agar medium. To generate a bacterial lawn, a single prairie bacterial strain was swabbed onto a TS agar plate, then patched from single colonies of the thirteen Brassica colony isolates. Strains were co-cultured overnight at 32°C. Antagonistic (antibiotic) activity was assessed by the presence of a zone of clearing around each patch. Colony "B3" was removed after the colony was destroyed. Negative- no antagonistic response. Positive zone of clearing indicates antagonistic response.

	Bacterial Plant Source:					
Brassica Colony Bacteria	**Wild Bergamot root**	**Anise hyssop root**	**Black Eyed Susan root**	**Balloon Flower root**	**Ironweed root**	**Prairie Sage root**
B1	-	-	-	-	-	-
B2	+	-	-	-	-	-
B4	+	-	-	+	+	-
B5	-	-	-	-	-	-
B6	-	+	-	-	-	-
B7	-	-	-	-	-	-
B8	-	-	-	-	-	-
B9	+	+	-	+	+	+
B10	-	-	-	-	-	-
B11	+	+	-	+	+	-
B12	-	-	-	-	-	-
B13	-	-	-	-	-	-
Total positive responses	4	3	0	3	3	1

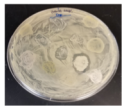


Fig. 3 Effect of antagonistic Brassica bacteria colony B9 on Prairie Sage bacteria.

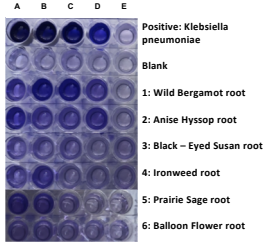


Fig. 4 Biofilm Production in Six Root Bacteria. Lanes A) Undiluted bacteria, B) 1/10 dilution, C) 1/100 dilution, D) 1/1000 dilution, E) negative control- no bacteria (TSB only).

Methods

Bacterial Characterization: The six root-associated aerobic bacterial isolates were tested for motility on SIM media and Gram-stained according to Benson. The presence of extracellular enzymes was evaluated on differential growth media: skim milk agar, starch agar, and Spirit Blue agar (HiMedia). The presence of protease activity on skim milk agar and amylase activity on starch agar was indicated by a clear halo surrounding the bacterial colonies. On Spirit Blue agar, the indicator of lipid digestion to glycerol and fatty acids was the presence blue colonies.

Biofilm Production: Six plant root bacterial isolates and a positive control for biofilm production (*Klebsiella pneumoniae*) were grown in TSB overnight. Serial dilutions were performed, and 100 µL of each dilution (or TSB as a negative control) was pipetted in wells of 96-well ELISA plates, then incubated at 32 °C for 24hr. The plates were then immersed in distilled water, shaken over a tub and inverted on paper towels. Crystal violet (125 µL) was then added to each well and incubated 10-12 minutes at room temperature. The stain was discarded, and the plates were immersed in distilled water 3 times and allowed to dry overnight. Ethanol (150 µL of 95%) was added to each well and allowed to dissolve the stain (~10 minutes) and the presence of biofilms was determined by OD measurements at 550 nm in a spectrophotometer with a plate reader.

Antibiotic Activity: Twelve native *Brassica rapa* root bacterial isolates were patched on to lawns of six prairie root bacterial strains to determine if the *Brassica* bacterial root strains produced antibiotic compounds. To generate a bacterial lawn, each of the six root bacterial strains were swabbed onto TSA plates, dried for 10 min., then single colonies of twelve *Brassica* root isolates were patched onto each of the six plates. The bacteria were co-cultured overnight at 32°C. Antagonistic (antibiotic) activity was assessed by the presence of a zone of clearing around and within each patch (Hernandez).

Results

Bacterial Characterization: The six isolates were negative for motility via SIM media as indicated by no growth from the stab lines. The six isolates were found to be Gram positive rods varying in size and length (Fig. 1). All six isolates tested positive for extracellular amylase and protease activities. With respect to protease activity, the bacterial root isolates of Black-Eyed Susan, Ironweed, and Prairie Sage plants showed moderate enzyme production, while the isolates for Wild Bergamot, Anise Hyssop, and Balloon Flower plants showed high enzyme production. The isolate from Wild Bergamot root was negative for lipases, while the other five root isolates were positive for the lipid-digesting enzyme but had weak enzyme production.

Biofilm Production: Biofilm production was observed in bacteria from Balloon Flower roots, Anise Hyssop roots, and Wild Bergamot roots. Weak biofilm production was detected in bacteria from Prairie Sage root (Fig. 4).

Antibiotic Activity: Four of the twelve *Brassica* bacterial isolates produced antimicrobial compounds (Table 2 and Fig. 3) as indicated by the (+). These strains exhibited a halo around and within the patched bacteria, preventing the native prairie root bacterial isolates from growing. The results indicate that isolates B11, B9, and B4 had the most significant antimicrobial activity.

Discussion

Bacterial Characterization: Typical characteristics of PGPR are variable and ambiguous. While most are gram negative bacteria, some are classified as gram positive. All PGPR have rod morphology that ranges in size. They have slow to fast motility, and there are no known non-motile species. The starch hydrolysis results in the literature are can be negative or positive. All six of our prairie root species were tested for starch hydrolysis and tested positive. ACC deaminase production is common among many species of PGPR, but is not a required characteristic. It coincides with IAA(auxin) production and phosphate solubilization. It is also commonly engineered into PGPR to encourage growth and speed of production. The literature results differ from our own isolated root strains, as ours did not exhibit motility, were gram positive, but did exhibit ACC deaminase production.

Biofilm Production: Biofilm production has been found to be beneficial to, or promoting of, plant growth (Backer). Three benefits of biofilm production are enhancing bacterial survival, enhances plant growth through various PGPR mechanisms, and having higher resistance to antibiotics. However, we observed contradictory results: the root bacteria with the greatest biofilm production - Balloon Flower, Anise Hyssop, and Wild Bergamot were killed by antibiotics produced by some *Brassica rapa* root bacteria. Prairie Sage root bacteria, which had weak biofilm production was killed by only one isolated Brassica Bacteria that produced antibiotics. Black Eyed Susan root bacteria produced no biofilms and lawns of this bacteria were not killed at all, when *Brassica rapa* root bacteria were patched on top. In contrast, Ironweed root bacteria, which also had no biofilm production, were killed by three *Brassica rapa* bacterial strains. In a future study, this will be explored further.

Antibiotic Activity: Antibiotic production has both harmful and beneficial effects on plant growth. The antibiotic activity can protect the plant from invading bacteria that would harm, or possibly kill the plant. In this case, it would be very beneficial for the plant to produce antibiotics. Unfortunately, this can also deter bacteria that are helpful in defending the plant against pathogens. It is difficult to separate at this time – the contribution of antibiotic activity and biofilm production - on plant root hair growth. Considering the *Brassica* plants could not produce seed in the controlled environment, it is more likely that the antibiotic activity prevented helpful bacteria from promoting plant growth and reproduction. The *Brassica* plants did not develop seeds when treated with any of the 6 prairie root bacteria in the controlled environment

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Acknowledgments

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