Soil augmentation with a *Bacillus* biocatalyst improves crop growth metrics and drives ecological succession in the rhizosphere

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Introduction

Many bacteria of the genus *Bacillus* are known to function as Plant Growth Promoting Bacteria (PGPB, Esitken *at al* 2002, Esitken *at al* 2010, de-Bashan *at al* 2010) and as endophytes within the roots of crop species such as wheat (Liu *at al* 2009) and corn (Bacon and Hinton, 2002). BiOWiSH® Crop is a commercially available biocatalyst consisting of active microbial cultures (*Bacillus licheniformis*, *B. subtilis*, *B. pumilus* and *B. amyloliquefaciens*) and metabolites, which has been shown to drive increases in yield and plant growth metrics for a variety of crops when applied according to standard farming practices (Table 1).

	Yield Increase vs. Control
Corn	3,379 kg/hectare
Rice	689.46 kg/hectare
Tomatoes	15.6% increase
Wheat	484.21 kg/hectare

Table 1 – Benefits of BiOWiSH® application for a variety of commercially relevant crops. Crops were grown at 3rd party research facilities under standard growing practices for the species in question (full study data available upon request).

In addition to direct benefits provided to plants as PGPB and as endophytes, a microbial assemblage may benefit plants indirectly by stimulating or restructuring soil microbial communities through nutrient mobilization or the production of antimicrobial peptides. Laboratory microcosm studies of BiOWiSH® Crop products suggest that they drive changes (Figure 1) in the abundance of PGPB bacterial colonies on agar plates. Based on these observations, we endeavored to determine whether the addition of the BiOWiSH ® *Bacillus* assemblage drove changes in the species composition of the rhizosphere.

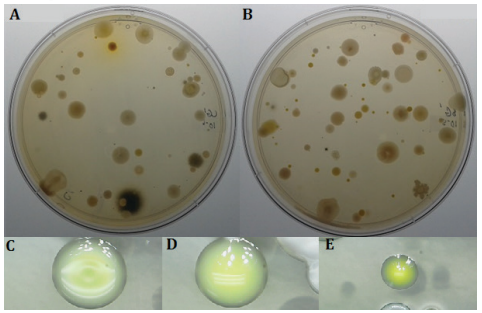

Figure 1 – Qualitative assessments of shifts in microbial species composition subsequent to *Bacillus* biocatalyst addition showed a significant (G-Test for Goodness of Fit, G = 18.38, p < 0.001) increase in yellow bacterial colonies (C, D, and E) in biocatalyst soil (B) relative to control soil (A). Identification via 16s sequencing determined that these colonies were members of the genera *Sphingomonas* and *Novosphingobium*.

Methodology

To determine whether shifts in endophytic diversity occurred, we examined the roots of laboratory-grown corn (*Zea mays*) for endophytic *Bacillus*. To determine whether shifts in edaphic microbial diversity occurred, samples of soil collected from the rhizosphere were examined for changes in species composition using next-generation genetic sequencing.

Microcosm setup – Soil was collected from a working farm and dispensed in 100kg portions into ethanol-disinfected plastic bins with holes drilled for drainage. Corn seed planting and nitrogenous fertilizer application were conducted according to the guidelines of the Purdue University Agricultural Extension. Microcosms were maintained under T5 4-lamp grow lights suspended 18" above the soil surface. Treatments included an abiotic control and a duplicate treatment which received the *Bacillus* biocatalyst. Each treatment was conducted in duplicate. Assuming homogeneous distribution, *Bacillus* were dosed at 3.0 x 10^5 CFU/g of soil. Plants were allowed to grow until reaching the V8 growth stage.

Gentamicin Protection Assay – The presence of endophytes in root masses was detected using a gentamicin protection assay for plant tissues (Xicohtencatl-Cortes *at al* 2009) modified with a recovery medium and a second antibiotic shock to eliminate "false positives" due to the presence of recalcitrant, extracellular endospores.

Microbiome Characterization – The microbial species composition of the rhizosphere in each soil microcosm was characterized using an NGS-based 16s rRNA amplicon diversity assessment (Illumina) at Molecular Resource DNA Laboratories (Shallowater, Texas, USA). An amplicon diversity assessment was conducted before the addition of nitrogen and biocatalyst (if applicable) and again at the termination of the study. Percent change relative to the baseline assessment was calculated for bacterial and fungal taxa for each treatment.

Results

Gentamicin Protection Assay – Corn plant root masses were extracted at the termination of the study, and qualitative differences (Figure 2) in root mass size were observed. Results of gentamicin protection assays are displayed in Figure 3. Hundredfold dilutions of BiOWiSH® treated soils plated on Tryptic Soy Agar (Carolina Biological Supply) with a gentamicin disc in the center showed near complete inhibition of edaphic bacterial taxa. Root masses harvested from both treatments showed putatively endophytic bacteria; however, only BiOWiSH® root masses showed colonies exhibiting distinct biocatalyst colony morphology.

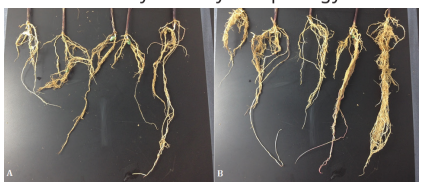

Figure 2 – Qualitative differences in the robustness of root masses collected at random from control (A) and *Bacillus* biocatalyst (B) soil treatments. Root masses were cleaned and subjected to a Gentamicin Protection Assay.

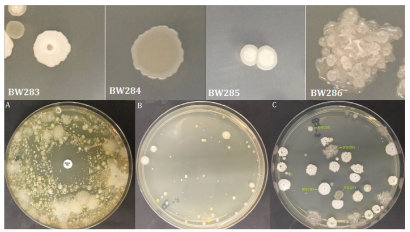

Figure 3 – Results of gentamicin protection assays of laboratory grown corn plant root masses. The four distinct colony morphologies present in BiOWiSH® Crop products (BW283, BW284, BW285 and BW286) are displayed above for reference. Plate screenings (A) showed near complete inhibition of edaphic soil taxa by a gentamicin disc, demonstrating technical right to succeed for the assay. Serial dilutions of homogenized root masses previously subjected to gentamicin shocks showed that control root masses (B) did not present unique BiOWiSH® colony morphologies whereas the BiOWiSH® root masses (C) showed all four.

Microbiome Characterization – Results of NGS-based 16s rRNA amplicon diversity assessments (Illumina) are displayed in Figure 4 (bacterial diversity) and Figure 5 (fungal diversity). Changes in the species composition of edaphic microbial communities over time were compared for the control and biocatalyst treatments.

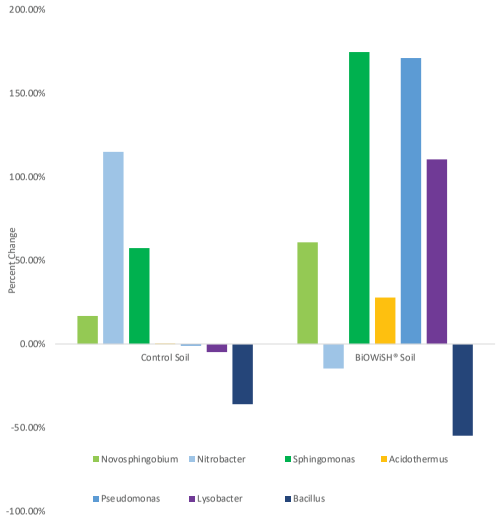

Figure 4 – Shifts in rhizosphere bacterial diversity detected via 16s amplicon sequencing. Greater percent increases were detected for known PGPB genera such as *Sphingomonas*, *Novosphingobium* and *Pseudomonas* in the biocatalyst treated soil than in the control soil. No net increase was detected among bacteria of the genus *Bacillus*, suggesting that the biocatalyst assemblage did not proliferate in the soil.

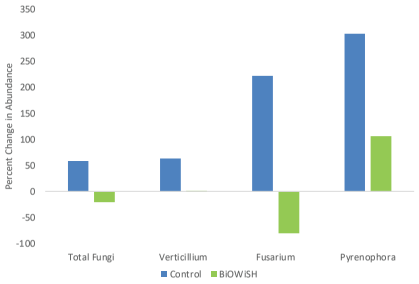

Figure 5 – Shifts in rhizosphere fungal diversity detected via 16s amplicon sequencing. Decreased fungal proliferation was observed in *Bacillus* biocatalyst treated soils relative to controls.

Discussion

In the present study, we examined the impact of a commercially available biocatalyst (BiOWiSH® Crop Liquid), containing a mixture of *Bacillus* species and metabolites, on the microbial species composition of the rhizosphere. In field trials, the effects of this assemblage include increased plant yield, increased plant growth metrics, and increased Brix score. These results compare favorably with the effects of both extracellular and endophytic PGPB; however, whether these effects were attributable to *Bacillus* directly or to their impact upon native soil microbial communities was not known.

Results of gentamicin protection assays carried out on corn root masses suggest that the *Bacillus* organisms are indeed capable of crossing the cell membrane and taking up residence within root tissues as endophytes. The observed (qualitative) increase in root mass size and the increase in plant growth metrics reported in field trials all compare favorably with the documented effects of endophytic PGPB.

Results of 16s amplicon diversity assessments showed that biocatalyst soil treatments experienced a shift in bacterial diversity that favored known PGPB taxa such as *Sphingomonas* and *Novosphingobium*. Importantly, the addition of 3.0 x 10^5 CFUs of *Bacillus* per gram of soil did not lead to a sustainable increase in edaphic *Bacillus* titer. This suggests that the direct action of these taxa as PGPB may not be as important to the benefits shown during field trials as is their effect as drivers of ecological succession in the rhizosphere, leading to the proliferation of other PGPBs. Finally, NGS sequencing data show that fungal taxa were inhibited as a whole in biocatalyst soil treatments.

A more detailed understanding of the mode of action of agricultural biocatalysts, such as BiOWiSH® Crop Liquid, can assist with the engineering of more effective products for the agronomy marketplace as the industry strives to meet an increased demand for production and sustainability.

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