Low-input production of switchgrass using plant growth promoting rhizobacteria

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Introduction

Production of second generation biofuel crops, such as perennial rhizomatous grasses (PRGs), are considered advantageous over first generation annual crops because it can be grown on less than prime and marginal lands with minimal agricultural inputs and thus do not displace food production [1,2]. Moreover, tropical PRGs such as sugarcane and elephant grass can be cultivated with low input of nitrogen (N) fertilizer, in large part due to biological nitrogen fixation (BNF) from associative free-living diazotrophs and other plant growth promoting rhizobacteria [3]. Therefore, research into temperate PRGs that can be grown in a northern climate, and the identification of potential N_2-fixing bacteria that associate with temperate PRGs are key to improving biofuel production systems.

Site History

Switchgrass (SW, *Panicum virgatum),* a temperate PRG, is a prime candidate for biofuel feedstock production in North America. Variety trials were conducted in Ste-Anne-de-Bellevue, Quebec, Canada (45°28'N 73° 45'W) on 11 different upland SW varieties including Cave-In-Rock, Carthage, and several cultivars from the U. of Nebraska and Oklahoma. The trial was initiated in 1996, and managed until 2000 after which no fertilizer was applied. The SW has continued to produce high biomass yields without N fertilization, which suggests an alternative N source perhaps through BNF.

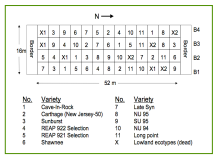


Figure 1a (above left): Photo of field plot taken on July 11, 2008.
Figure 1b (above right): Diagram of REAP-field.

Hypotheses

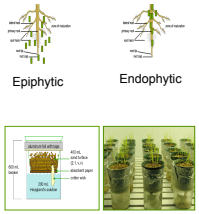
We hypothesized that 1) indigenous populations of rhizosphere N_2-fixing bacteria have provided SW with the N necessary for substantial biomass growth, and 2) productivity differences amongst varieties are due to variation and abundance of N_2-fixing bacterial populations.

Experimental Design

❑ Root, rhizome and rhizosphere soil samples from each plot (4 m x 4m) were collected in July, 2008 over a 4 week period.

❑ Serial dilutions were performed to estimate most probable number (MPN). Putative epiphytic (> 500) and endophytic (> 300) N_2-fixing bacteria were isolated on N-free solid media.

❑ Bacterial screening bioassays were performed in the growth chamber on singly and batch (mixture containing 8-10 isolates) inoculated and uninoculated (control) SW plants (*P.virgatum* cv. Cave-In-Rock) grown in modified Leonard assemblies and fertilized with N-free Hoagland's solution. A batch inoculation containing N_2-fixing isolates purchased from the American Type Culture Collection (ATCC) was also included in the bioassay.



❑ Statistical analyses were performed at $P < 0.05$ using SAS CONTRAST.

❑ Isolates that show plant growth promotion will be selected for identification using phylogenetic analysis of the 16S rRNA. Amplification of *nifH* gene will be used as a marker for N_2-fixation capability.

❑ Those isolates that show production of the greatest amount of plant biomass will be chosen for further field experimentation.

❑ Field harvest of SW varieties was performed on November 11, 2008. Subsamples (0.5 kg) were oven-dried at 55ºC for 72 h to determine dry weight (DW) yields.

Results

❑ **Field harvest:** SW varieties, Cave-In-Rock (V1), REAP 921 (V5), Late Syn (V7) and NU 94 (V10) had the greatest dry weight yields in 2008.

❑ **Growth chamber (Fig. 2a):** Endophytic bacterial isolates corresponding to the batch inoculations from the ATCC and V1 treatments ($P < 0.05$) enhanced SW DWs. Batch inoculation of endophytic isolates corresponding to treatments V5, V7 and V10 also improved SW DWs.

❑ **Fig. 2c & 2d:** Among the ATCC, V1, V5 ,V7 and V10 treatments, several individual isolates enhanced ($P < 0.05$) SW biomass.

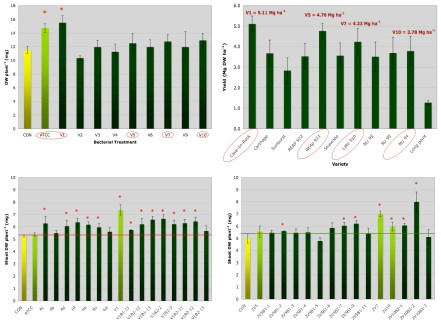


Figure 2a (above left): DW yields (mg plant^{-1}) of batch-inoculated SW grown in the growth chamber.
Figure 2b (above right): DW yields (Mg ha^{-1}) of SW field varieties harvested in November 11, 2008.
Figure 2c (below left): Shoot DW yields (mg plant^{-1}) of singly-inoculated SW grown in the growth chamber.
Figure 2d (below right): Shoot DW yields (mg plant^{-1}) of singly-inoculated SW grown in the growth chamber.

Discussion & Future Outlook

❑ **Findings:** The bacterial treatments that improved SW biomass in the growth chamber experiments also correspond to SW varieties that produced the greatest biomass production in the field **(Fig. 2b).**

❑ **Bacterial classification:** Identification of the bacterial isolates; confirmation of its N_2-fixing capabilities through *nifH* gene amplification.

❑ **Determination of fixed N transfer from bacteria to plant:** %C and %N in plant tissue and $^{15}N/^{14}N$ isotope dilution analyses.

❑ **Field experimentation:** Seed and spray-inoculated field trials are currently being conducted.

❑ **Future challenge:** To develop low N input SW agroecosystems and bioinculant technology that will enhance the use of biofuels as a sustainable energy source.

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References:
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[2] Hill et al. (2006). *PNAS* 103: 11206–11210.
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