Sebacina for Switchgrass: Application and Benefits

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Project Goals: The BioEnergy Science Center (BESC) is focused on the fundamental understanding and elimination of biomass recalcitrance. BESC's approach to improve accessibility to the sugars within biomass involves (1) designing plant cell walls for rapid deconstruction and (2) developing multi-talented microbes or converting plant biomass into biofuels in a single step (consolidated bioprocessing). BESC biomass formation and modification research involves working directly with two potential bioenergy crops (switchgrass and *Populus*) to develop varieties that are easier to break down into fermentable sugars. We are testing large numbers of natural variants and generating specific and modified plant samples as well as developing genomics tools for detailed studies into poorly understood cell wall biosynthesis pathways.

The Sebacinales belong to a taxonomically, ecologically, and physiologically diverse group of fungi in the Basidiomycota. *Sebacina vermifera* is a mycorrhizal fungus that was first isolated from the Australian orchid *Cyrtostylis reniformis*. Research from our laboratory on this fungus clearly indicates its plant growth—promoting abilities in non-orchid host plants. *S. vermifera* colonization enhanced seed germination, biomass production and drought tolerance of the native warm-season grass, switchgrass, an important bioenergy crop for cellulosic ethanol production in the United States. Towards this end, we have developed a method for large-scale dissemination of inoculum containing this growth-promoting fungus for switchgrass field trials. Further, we demonstrate the effectiveness of this carrier-based method for colonization of switchgrass in greenhouse trials for biomass enhancement of wild type and transgenic, low lignin (COMT downregulated) switchgrass lines compared to an efficient *in vitro* colonization method. *S. vermifera* colonization enhanced plant biomass regardless of delivery method, although the percentage of fungal biomass *in planta* increased with the clay-based delivery system. Further, we found that release of some clay minerals in solution was enhanced in the presence of *S. vermifera*, while others were seemingly reduced. Intriguingly, the presence of *S. vermifera* has little or no impact on cell wall composition, including lignification. This clay-based inoculum is not prone to contamination and can be stored at room temperature. These features together with the mineral composition of the clay illustrate the potential for packaging the fungus with a nutrient source en masse for large-scale delivery to the field. This research is the first report documenting the development of a bentonite clay particle-based delivery system for mass production of any symbiotic microbe and suggests that *S. vermifera* can be packaged with a mineral composite and effectively delivered to a target host plant. We are currently taking this next step, initiating field trials of transgenic and natural varieties of switchgrass for *S. vermifera*-mediated crop improvement studies.

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