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Endophytic Microbes for Growth Promotion of Crop Plants and Suppression of Aggressive Invasive Plant Species

By
James F. White
Kurt P. Kowalski
Kathryn L. Kingsley
Matthew T. Elmore

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Field of the Invention

This invention relates to compositions comprising endophytic bacteria and methods of use thereof to promote plant growth and suppress aggressiveness in invasive plant species.

Background of the Invention

Several publications and patent documents are cited throughout the specification in order to describe the state of the art to which this invention pertains. Each of these citations is incorporated by reference herein as though set forth in full.

Plant seeds carry embryonic plants and nutrients for early stages of seedling growth; seeds also carry small communities of symbiotic microbes (primarily bacteria and fungi) that are needed for defense from pathogens, modulation of plant development, and nutrient acquisition in seedlings. Seed-vectored symbiotic microbes are adapted to their host plant and may enable seedlings to survive and thrive (Compant, Clement and Sessitsch, 2010; Kandel et al., 2017). Without symbiotic microbes, seedlings do not develop properly—often lacking normal root gravitropic response where roots do not grow downward into the soil or other substrate—sometimes growing upward—where roots may not produce root hairs—or hairs may be sparse or short (Holland, 1997; Verma et al., 2017a, 2017b; White et al., 2012). Seedlings without their microbes are more susceptible to diseases and oxidative stresses (abiotic and biotic in nature), drought, heat, heavy metals, herbivory, etc. (Rodriguez et al., 2009; Torres et al., 2012; Waller et al., 2005; White and Torres, 2010).

Some seed-associated tissues appear to harbor adaptations to vector microbes on seeds. Dried paleas and lemmas that adhere closely to grass seed coats (or caryopsis testa) vector bacteria and sometimes fungi that colonize roots and shoots of the germinating seedlings as they emerge from the seeds (White et al., 2012). The characteristically winged seeds of species in the plant family Polygonaceae vector

bacteria that colonize germinating seedlings. In cotton (*Gossypium* spp.; Malvaceae) elongated trichomes (cotton fibers) carry bacteria that may stimulate seedling growth and protect cotton plants from diseases. Removal of the cotton fibers by acid delinting as is commonly done makes seeds easier to process in mechanical planters—but also removes symbiotic bacteria from cotton seeds, leaving the seedlings defenseless from pathogens, insect pests, and compromised developmentally (Irizarry and White, 2017, 2018). As a consequence cotton is often considered to be "the world's dirtiest crop" due to the amount of agrochemicals frequently used in its cultivation (Environmental Justice Foundation, 2007). In many grasses of subfamily Pooidae fungal *Epichloë* endophytes colonize the ovules of the maternal plant and grow into the embryo inside caryopsis—thus germinating seedlings already contain the fungal endophyte (White and Cole, 1986).

Summary of Invention

This invention pertains to the use of endophytic microbes as bioherbicides to suppress growth and development of weed plants. The endophytes were obtained from plant species *Abrus precatorius*, *Digitaria ischaemum*, *Froelichia gracilis*, *Lycopersicum esculentum* and *Poa annua*. Several of these endophytes were shown to suppress development of seedlings of dandelion (*Taraxacum officionale*), curly dock (*Rumex crispus*), clover (*Trifolium repens*), Japanese knotweed (*Fallopia japonica*) and annual bluegrass (*Poa annua*), *Amaranthus hypochondriacus* and *Amaranthus viridis*. Inhibitory microbes were found to enter into root cells at the root tip meristem, becoming located within the periplasmic spaces, between the cell wall and plasma membrane. Normally microbes in root symbiosis play roles in modulation of plant development, including stimulation of root gravitropic response (trigger roots to grow downward) and increasing root and shoot growth. Maladaptive endophytic microbes from other hosts displaced native endophytes and disrupted functions of the symbiosis, and led to increased seedling mortality. We apply the term 'endobiome interference' to denominate this maladaptive symbiosis. None of these microbes appeared to be pathogenic or inhibitory of growth in their original hosts based on growth of seedlings containing microbes on agarose media.

Applications of endobiome interference to control invasive or weedy plant species

Invasive and weedy plant control generally employs use of chemical herbicides. Endobiome interference offers a non-chemical means whereby particular weeds could be controlled without herbicides (Kowalski et al., 2014). It may be possible to enhance growth of crop species and simultaneously repress growth of weedy competitor species through applications of microbes that are growth promotional in crops—but produce endobiome interference in competitor plants. Such an approach could reduce applications of agrochemicals in crops with economic and environmental benefits.

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