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Selective soil bacteria to manage downy brome, jointed goatgrass, and medusahead and do no harm to other biota

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ABSTRACT

Downy brome (cheatgrass, *Bromus tectorum* L., DB), jointed goatgrass (*Aegilops cylindrica* L., JG), and medusahead (*Taeniatherum caput-medusae* L. Nevski, MH) are annual invasive grass weeds that reduce cereal yields in cropland, negatively impact plant diversity, wildlife habitat, and forage potential in rangeland, and fuel wildfires. Methodical and comprehensive screening and selection of soil microbial populations identified cold-loving, weed-suppressive bacteria that had exclusive selectivity and did not harm other plants, even in stressed conditions. These weed-suppressive bacteria were further selected to contain benign, non-antagonist traits that minimized any negative effect on the general microbial populations, aquatic invertebrates, and insects. Three strains were selected for field studies across Washington State from east with 508 mm annual precipitation to west with 152 mm annual precipitation. Weed inhibition only occurred when bacteria were applied in the late fall or early winter to cool, moist soil (< 10 °C) during overcast skies and rain or snow at application or one or two days later. Field studies consistently showed a 50% reduction in DB, JG, and MH within three years of bacterial application. Almost complete suppression of these fall annual grass weeds was seen 5–7 years after one application, when desirable plants (pasture grass, winter wheat (*Triticum aestivum* L.), perennial bunchgrasses, or natives) were present. In weed monocultures, weed reductions only reached 50% in 7 years. Because of their selectivity, these bacteria can be used in management of the invasive weeds DB, JG, and MH in rangeland, cropland, forest, pasture, turf, road sides, construction sites, and rights-of-way.

1. Introduction

Downy brome (cheatgrass, *Bromus tectorum* L., DB), jointed goatgrass (*Aegilops cylindrica* L., JG), and medusahead (*Taeniatherum caput-medusae* L. Nevski, MH), are annual invasive grass weeds (Forest Service, 2014; Skinner et al., 2008; Stannard et al., 2010) infesting large portions of the west (Duncan et al., 2004). Today, the three grass weeds are found throughout the US, as well as many provinces of Canada and several states in Mexico (EDDMapS, 2017; NRCS, 2017a,b,c). In cropland, these three grass weeds devastate cereal yields, especially at the edge of fields and in cropland bordering rangeland, by utilizing the limited available water before the crop matures (Dahl and Tisdale, 1975; Donald and Ogg, 1991; Stahlman and Miller, 1990). The three weeds negatively impact rangeland plant diversity, wildlife habitat, and forage potential (Epanchin-Niell et al., 2009; Forest Service, 2014; Knapp, 1996; Thill et al., 1984). Invasive annual grass weeds also reduce tribal plants used for food and medicine (Borins, 1995). Unfortunately, the dead, above-ground biomass left by annual grass weeds results in a dense, contiguous mat of highly flammable fuel susceptible to ignition (Abatzoglou and Kolden, 2011; Balch et al., 2013; Brooks et al., 2004; USGS, 2002). Rangeland fire size, intensity, and frequency have increased dramatically with the expansion of annual grass weed infestations (Balch et al., 2013; Haubensak et al., 2009; Jackson and Sullivan, 2009; USGS, 2002).

In cropland, these three weeds can be managed with crop rotation, tillage, and annual applications of various herbicides (Lyon et al., 2015). However, herbicides are not always effective and they do not affect the annual germination of the copious amount of seed generated by annual grass weeds that become part of the weed-seed bank. Current methods of rangeland restoration, including drill-seeding native grasses or near-native grasses (Epanchin-Niell et al., 2009) are being used. Near-native grasses are non-native plants that are similar in growth habit to native plants that they can take the place of natives in reseeding efforts. In addition, the use of herbicides (Mangold et al., 2013), the lowering nutrient availability (Mazzola et al., 2008; Vasquez et al., 2008) and/or moderate grazing (Davies et al., 2009) have also been

Abbreviations: d, day; DB, downy brome; h, hour; JG, jointed goatgrass; KB, King's B; MH, medusahead; *P.f.*, *Pseudomonas fluorescens* (Flügge) Migula; SR, Sands and Rovira selective medium

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used in rangeland restoration. Unfortunately, these methods have had limited success in reestablishing native or near-native grass species and reducing annual grass-weed abundance (Davies, 2010). Weed-suppressive bacteria that reduce the weed-seed bank and inhibit seedling growth may help curb the increase of the three weeds in cropland and rangeland (Kennedy, 2016; Kennedy et al., 1991). These bacteria inhibit seed germination as well as root growth (Johnson et al., 1993; Tranel et al., 1993) and are especially attractive as biocontrol agents for annual grass weeds because these bacteria are at their highest, most active levels in the soil at the same time the weed is increasing root growth. Previously, DB, JG, and MH growth was found to be inhibited by *Pseudomonas fluorescens* (Flügge) Migula strain D7 (*P.f.* D7) and related species that were isolated from soil of the Palouse region of Eastern Washington state (Kennedy et al., 1991; Kennedy and Stubbs, 2007). The bacteria colonize roots and produce metabolites that inhibit DB, JG, and MH root growth (Gurusiddaiah et al., 1994), allowing other plant species to be more competitive. Unfortunately, *P.f.* D7 is not as selective as hoped and has been found to injure several native grasses under stressed conditions. In light of this, comprehensive screening and selection of additional soil microbial populations were initiated to identify weed-suppressive bacteria that have greater exclusive selectivity and do not harm other plants, even in stressed conditions. In addition, the bacteria selected must not harm or negatively impact aquatic invertebrates, beneficial insects (e.g., ladybugs), and other biota.

The objectives of this study were to:

1. Isolate naturally-occurring soil bacteria that specifically inhibit downy brome, jointed goatgrass, and medusahead;
2. From the weed-suppressive bacteria identified in (1), select for those that do not harm crops, natives, near-natives, and other plants during soil conditions near field capacity and also during drought;
3. Select from the weed-suppressive bacteria those that contain benign, non-antagonist traits to minimize any negative effect of the introduced bacteria on the general microbial populations, aquatic invertebrates, plants, and insects.
4. Evaluate the efficacy and survival of these new benign, weed-suppressive bacteria in the field.

This stringent selection process will ensure that the introduced bacteria, while reducing the DB, JG, and MH seed bank, will not be harmful to other plants, will not be competitive and perturb the native soil microbial community, and will have relatively short-term survival. These benign traits will safeguard the existing natural biota while still inhibiting DB, JG, and MH.

2. Materials and methods

2.1. Isolation and propagation of soil bacteria

Soil was sampled in early spring during a thaw following a hard freeze from areas of stunted grass species growing in the midst of healthy grass species. The soil was stored at 4 °C and processed within 5 d. Serial dilutions were plated onto Sands and Rovira (SR) agar, which selects for fluorescent pseudomonads (Sands and Rovira, 1970). The plates were incubated 48 h at 24 °C as described in Kennedy (2016). Individual colonies were used to inoculate King's B broth (KB, King et al., 1954). Cultures were incubated for 32 h to mid-log stage (5×10^8 colony forming units (cfu) mL^{-1}) at 22 °C with shaking at 180 rpm and stored in 50% sterile glycerol at -80 °C until needed. Isolates used in this study were taken from cryostorage, plated onto SR agar, and incubated for 48 h at 24 °C. Unless stated otherwise, mid-log phase KB cultures (5×10^8 cfu mL^{-1}) were used in the assays described below.

Table 1
Plant species tested and not suppressed by *Pseudomonas fluorescens* (Flügge) Migula strains ACK55, NKK78, and SMK69 in agar root length bioassay and greenhouse plant/soil studies under two moisture regimes (either 25% soil water content (−0.03 MPa) or 10% soil water content (−0.10 MPa)). *Pseudomonas fluorescens* strains ACK55, NKK78, and SMK69 inhibit downy brome (cheatgrass, *Bromus tectorum* L.), jointed goatgrass (*Aegilops cylindrica* L.), and medusahead (*Taeniatherum caput-medusae* [L.] Nevski).

Class	Common Name	Latin Name
Dicot	Alfalfa	*Medicago sativa* L.
Dicot	Annual agoseris	*Agoseris heterophylla* (Nutt.) Greene
Dicot	Apple	*Malus* spp. Mill.
Dicot	Austrian winter pea	*Pisum sativum* subsp. *arvense (L.) Poir.*
Dicot	Ballhead sandwort	*Arenaria congesta* Nutt.
Dicot	Beans	*Phaseolus vulgaris* L.
Dicot	Big sagebrush	*Artemisia tridentata* Nutt.
Dicot	Bladderpod	*Lesquerella kingii* (S. Watson)
Dicot	Broccoli	*Brassica oleracea* L. var. *botrytis* L.
Dicot	Button snakeroot	*Eryngium yuccifolium* Michx.
Dicot	Camelina	*Camelina sativa* L.
Dicot	Canola	*Brassica napus* L.
Dicot	Celery	*Apium graveolens* L.
Dicot	Chick peas	*Cicer arietinum* L.
Dicot	Clover	*Trifolium* L.
Dicot	Common lambsquarters	*Chenopodium album* L.
Dicot	Common vetch	*Vicia sativa* L.
Dicot	Cotton	*Gossypium hirsutum* L.
Dicot	Cucumber	*Cucumis sativus* L.
Dicot	Daisy	*Bellis perennis* L.
Dicot	Desert yellow fleabane	*Erigeron linearis* (Hook.) Piper
Dicot	Fava bean	*Vicia faba* L.
Dicot	Flax	*Linum usitatissimum* L.
Dicot	Foothill daisy	*Erigeron corymbosus* L.
Dicot	Joint vetch	*Aeschynomene* spp. L.
Dicot	Lentil	*Lens culinaris* Medik.
Dicot	Lettuce	*Lactuca sativa* L.
Dicot	Magnolia	*Magnolia* spp. L.
Dicot	Mayweed chamomile	*Anthemis cotula* L.
Dicot	Milk-vetch	*Astragalus leibergii* L.
Dicot	Milk-vetch	*Astragalus purshii* L.
Dicot	Mint	*Mentha* spp. L.
Dicot	Northern bedstraw	*Galium boreale* L.
Dicot	Pea	*Pisum sativum* L.
Dicot	Peanuts	*Arachis hypogaea* L.
Dicot	Pepper	*Capsicum* spp. L.
Dicot	Phlox	*Phlox* spp. L.
Dicot	Potato	*Solanum tuberosum* L.
Dicot	Prairie clover	*Dalea* spp. L.
Dicot	Purple sage	*Salvia dorrii* (Kellogg) Abrams
Dicot	Rannucula	*Ranunculus* spp. L.
Dicot	Rapeseed	*Brassica rapa* L.
Dicot	Redroot pigweed	*Amaranthus retroflexus* L.
Dicot	Rose	*Rosa* spp. L.
Dicot	Rush skeletonweed	*Chondrilla juncea* L.
Dicot	Safflower	*Carthamus tinctorius* L.
Dicot	SageBrush	*Artemisia* spp. L.
Dicot	Sagebrush false dandelion	*Nothocalais troximoides* (A. Gray) Greene
Dicot	Shaggy fleabane	*Erigeron pumilus* Nutt.
Dicot	Silky lupine	*Lupinus sericeus* Pursh
Dicot	Slender hawksbeard	*Crepis atribarba* A. Heller
Dicot	Soybeans	*Glycine* max L. Merr.
Dicot	Squash	*Cucurbita* spp. L.
Dicot	Sugar beets	*Beta vulgaris* L.
Dicot	Sulfur lupine	*Lupinus sulphureus* Douglas ex Hook.
Dicot	Sunflower	*Helianthus* spp. L.
Dicot	Threadleaf fleabane	*Erigeron filifolius* (Hook) Nutt.
Dicot	Tomato	*Solanum lycopersicum* L.
Dicot	Tumble mustard	*Sisymbrium altissimum* L.
Dicot	Vetch	*Vicia* spp. L.
Dicot	Wild parsnip	*Pastinaca sativa* L.
Monocot	Annual bluegrass	*Poa annua* L.
Monocot	Barley	*Hordeum vulgare* L.
Monocot	Basin wildrye	*Leymus cinereus* (Scribn. & Kunth) Lag. ex Griffiths
Monocot	Bluebunch wheatgrass	*Pseudoroegneria spicata* (Pursh) Á. Löve
Monocot	Bottlebrush squirreltail	*Elymus elymoides* (Raf.) Swezey

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