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Bioaugmentation of microbes to restore coastal wetland plants to protect land from coastal erosion

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ABSTRACT

Microbes with beneficial effects to plant growth and health have been dubbed plant growth-promoting rhizobacteria (PGPR). PGPR has been extensively studied in crop plants; however, our study investigates the effects of PGPR on the wetland grass *Spartina alterniflora*. *S. alterniflora* is the dominant vegetation in coastal marshes and is often used in wetland restoration projects. Greenhouse raised *S. alterniflora* were subjected to three treatments: One of a consortia of microbes with freshwater, one of a consortia of microbes with 10 parts per thousand (ppt) saline water, and one with a pure culture and freshwater. Plant growth and soil nitrogen and phosphorus content were measured over 60 days and all plants were sacrificed at the end of the experiment to quantify biomass. Of the three treatments, the treatment receiving the consortia plus salt water had the most growth (41.1 ± 4.4 cm) and greatest biomass (108.03 g) followed by the pure culture treatment with freshwater (34.9 ± 3.2 cm, 96.25 g), the consortia treatment with freshwater (39.7 ± 5.0 cm, 89.04 g), and lastly the control treatment (7.7 ± 1.5 cm, 51.85 g). All treatments were significantly different from the control but not significantly different between each other. In consortia plus 10% saline water treatment, mean stem growth was almost six times greater, total biomass was doubled, and the number of additional stems was three times greater compared to the control. This study shows a positive relationship between microbial activity, soil nutrient cycling of nitrogen and phosphorus, and plant growth in greenhouse grown *S. alterniflora* inoculated with PGPR.

1. Introduction

Coastal wetlands are some of the most vulnerable ecosystems on earth. Marshes and mangroves protect coastal regions from storms. Tidal wetland conversion to open water through sea-level rise is expected to accelerate. Large areas of marsh are being converted to open water in the Gulf of Mexico and especially the state of Louisiana in the US is losing land at an alarming rate due to coastal erosion. Although coastal wetlands have long been considered vulnerable to sea-level rise, many recent studies indicated feedback between plant growth and geomorphology allows wetlands to actively resist coastal erosion and land loss (Kirwan and Megonigal, 2013).

Coastal wetlands act as a transition zone from open-ocean to estuarine areas (Mitsch and Gosselink, 2007). Estuaries provide a host of ecosystem services including erosion protection, flood protection, breeding grounds for coastal birds, and habitat for many harvested species including fish, shrimp, and crabs (Gomes et al., 2010; Polidoro et al., 2010). Wetland plants are also important in nutrient cycling and biogeochemical processes of wetlands including carbon sequestration and mineralizing soil organics (Gomes et al., 2010; Polidoro et al., 2010; Miransari, 2011). Coastal wetlands are among the most productive ecosystems on earth and vegetation tends to stabilize their relative elevation. The growth of the grass *Spartina alterniflora* is positively correlated with interannual variations in sea level (Kirwan and Guntenspergen, 2012). Enhanced growth of *S. alterniflora* in coastal wetlands will protect the coastal land from erosion and land loss. Thus, it is imperative to find a natural means to enhance the growth of this most important coastal plant.

Kloepper and Schroth (1981) first coined the term “plant growth-promoting rhizobacteria” in a study investigating the effects of soil bacteria on the growth of radishes, sugar beets, and

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potatoes. In this study plant growth-promoting rhizobacteria (PGPR) are defined as free-living soil bacteria that can form close relationships within the rhizosphere of plants and can enhance plant growth and health. Although some microbial populations act as plant pathogens, PGPR benefits plants directly by increasing availability of essential plant nutrients like nitrogen and phosphorus (Glick et al., 1999) and by producing plant hormones such as indole acetic acid (IAA), which encourages root growth (Patten and Glick, 2002). Various crop plants such as radishes and potatoes (Kloepper and Schroth, 1981), cotton, sweet corn (McInroy and Kloepper, 1995), cucumber (Wei et al., 1996; Raupach and Kloepper, 1998) canola (Patten and Glick, 2002), soybean (Bai et al., 2002), and barely (Canbolat et al., 2006) have shown positive responses in growth and pathogen resistance when treated with microbial consortia or biofertilizers. Biofertilizers are defined as a microbial enrichment applied to the soil to enhance plant growth and health (Vessey, 2003). Fertilizers supply essential nutrients to plants such as nitrogen and phosphorus. Microbes can mobilize nitrogen and phosphorus trapped in soil making it available to plants (Glick et al., 1999). Bacteria transform organic nitrogen into ammonia (NH_3), nitrite (NO_3), and nitrate (NO_2), which can be utilized by plants and microorganisms (Glick et al., 1999; Mitsch and Gosselink, 2007). Phosphorus is typically abundant in nature but the mineral readily binds to clay, ferric iron, calcium, and aluminum making it insoluble and unavailable to plants (Mitsch and Gosselink, 2007). Some bacteria such as *Pseudomonas putida* and *Bacillus* spp. are known to solubilize phosphorus making it available to plants (Glick et al., 1999; Ahmad et al., 2008).

Increasing environmental concerns surrounding chemical fertilizer overuse such as the 13,080 km^2 "dead zone" in the Gulf of Mexico (Rabalais et al., 2010) make PGPRs an attractive alternative to traditional fertilizers and herbicides (Adesemoye and Kloepper, 2009; Miransari, 2011). Other concerns of traditional fertilizers use are changes in soil structure, pH, biogeochemical processes (Miransari, 2011), and the accumulation of herbicides and insecticides in the environment (Patten and Glick, 2002). Biofertilizers can help reduce the use of chemical fertilizer thereby alleviating the negative ecosystem issues they cause (Patten and Glick, 2002; Vessey, 2003; Adesemoye and Kloepper, 2009; Miransari, 2011).

Interest in PGPR has largely been focused on crop plants. However, halo-tolerant coastal plant species that endure unique environmental stresses like flooding and salinity may host unique and potentially beneficial PGPR (Bashan and Holguin, 2002; Kathiresan and Selvam, 2006). There may also be benefit in microbial treatments of wetland restoration plants (Bashan and Holguin, 2002). Research conducted in the area of mycorrhizae interactions with *Spartina alterniflora* and *Spartina cynosuroides* resulted in a minimal plant growth response (McHugh and Dighton, 2004). However research by Gomes et al. (2010) using mangrove species demonstrated that nursery raised plants transplanted into the wild influence the resulting microbial community of the rhizosphere. Understanding the microbial ecology of coastal plants can help improve restoration efforts by improving stabilization of soil after restoration (Bashan and Holguin, 2002; Kathiresan and Selvam, 2006; Miransari, 2011).

The purpose of this study is to enhance the growth of *S. alterniflora*, through bioaugmentation process by adding natural bacterial amendments to the soil. *S. alterniflora* is a facultative halophyte that dominates intertidal coastal marshes along the Atlantic coast to Louisiana and Texas (Tiner, 1993; Bush, 2002). It is a perennial grass that can reach up to 2.5 m with the shorter form in higher areas less affected by tidal events (Tiner, 1993). Bacterial consortia similar to those found in wild plants may harbor unique microbial communities, which can potentially be used as a biofertilizer for the production of restoration plants. Biofertilizers could potentially improve restoration efforts by producing healthier plants that would also increase soil health by introducing beneficial microbial communities to the restoration area thereby stabilizing the area. The major goal of this study is to test the effects of PGPR treatments on greenhouse raised *S. alterniflora* by measuring nitrogen and phosphorus in the soil and plant growth over the duration of the experiment.

2. Materials and methods

2.1. Collection of sample

Samples were collected from the rhizospheres of *S. alterniflora* in Elmer's Island, LA (Fig. 1). Elmer's Island is located on the Louisiana coast, which is indicative of *Avicennia germinans* (black mangroves) and *S. alterniflora* habitats. The rhizospheric soil sample plot was located at N29° 11′ 15.5″, W90° 03′ 58.9″ and at N29° 11′ 19.9″, W90° 04′ 00.2″ (Fig. 1). Each site was chosen based on a visual assessment that 90% of the area was dominated by the target plant species to ensure collection of microbes that are specific to plant species. Samples were collected by digging up approximately 15 cm of the root system with the above ground biomass. Above ground biomass was collected to ensure preservation of rhizosphere conditions as found at the collection sites. Samples were stored in plastic bags with the above ground biomass exposed on ice while in transit and upon returning to the lab samples were stored at 4 °C in a walk-in cooler. At each site, soil temperature, pH, and salinity were recorded using an Aquaterr EC-300 salinity multimeter.

2.2. Isolation of soil bacteria

Bacteria were enriched and isolated on King's B medium and a halophilic rhizosphere medium. King's B medium was chosen because it is selective for *P. putida* and *P. flourescens*, which are commonly found in the rhizosphere of plants and have known plant growth-promoting properties. *Pseudomonas* and *Bacillus* spp. have been reported as nitrogen transformers, phosphorus solubilizers, and siderophore producers, making it a good genera to investigate for plant growth-promoting properties (Glick et al., 1999; Kumar et al., 2012). The halophilic rhizosphere medium was selected to increase chances of isolating halophilic or salt tolerant microbial species since collected plants are found in brackish environments. Various rhizospheric soils were inoculated into the above-mentioned media for isolation of pure culture. Bacterial isolates were identified using the Biolog GEN III analyzer (BIOLOG Model GEN III, Hayward, CA, USA).

2.3. Propagation of S. alterniflora

The collected *S. alterniflora* were cut into small sections of 0.25 m × 0.25 m × 0.25 m. Individual stems were separated and roots were rinsed of soil before transplanting. Eighty stems were transplanted, entire stem with roots, were transplanted into ¾ Trade gallon pots (Classic 300S, Nursery Supplies, Inc.). Pots were filled one inch from the top of the pot with a 50/50 mix of potting soil (Hapigro®; Hope, AR) and top soil (Hapigro®; Hope, AR) that was first autoclaved for 1 h at 15 psi and 121 °C to kill pre-existing microbial communities. After initial transplantation, specimens were allowed to grow for two weeks before initial measurements were recorded and various treatments were administered. Each treatment consisted of 15 plants. For the duration of the experiment plants were watered every three days. Treatments one, two, and four received freshwater and treatment three received a 10 ppt saline solution (Instant Ocean®). The sampling scheme was to