Plant Growth-Promoting Bacteria for Phytostabilization of Mine Tailings

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Eolian dispersion of mine tailings in arid and semiarid environments is an emerging global issue for which economical remediation alternatives are needed. Phytostabilization, the revegetation of these sites with native plants, is one such alternative. Revegetation often requires the addition of bulky amendments such as compost which greatly increases cost. We report the use of plant growth-promoting bacteria (PGPB) to enhance the revegetation of mine tailings and minimize the need for compost amendment. Twenty promising PGPB isolates were used as seed inoculants in a series of greenhouse studies to examine revegetation of an extremely acidic, high metal content tailings sample previously shown to require 15% compost amendment for normal plant growth. Several isolates significantly enhanced growth of two native species, quailbush and buffalo grass, in tailings. In this study, PGPB/compost outcomes were plant specific; for quailbush, PGPB were most effective in combination with 10% compost addition while for buffalo grass, PGPB enhanced growth in the complete absence of compost. Results indicate that selected PGPB can improve plant establishment and reduce the need for compost amendment. Further, PGPB activities necessary for aiding plant growth in mine tailings likely include tolerance to acidic pH and metals.

Introduction

Mining activities have created a global problem in the form of mine tailings, with heavily impacted regions located in arid and semiarid environments in northern Mexico and the western United States, the Pacific coast of South America (Chile and Peru), southwestern Spain, western India, South Africa, and Australia (e.g., ref *8*). Mine tailings are generated during mineral ore processing and are the primary component of mining waste (*19*). Acidic pH, high metal content, low nutrients, impacted microbial community, and lack of soil structure leave these sites barren of vegetative cover and highly susceptible to both eolian dispersion and water erosion. The Klondyke mine tailings (Klondyke, AZ), constitute a state Superfund site that contains approximately 70 000 m^3 of acidic tailings with elevated levels of As, Cd, Pb, and Zn (*31*). The latter two metals are considered most problematic for plant and microbial growth because they are present at solid phase concentrations up to several thousand mg kg^{-1}. Erosion of these tailings is of concern because they are adjacent to Aravaipa Creek, an important riparian system in southern Arizona, and elevated Pb and Cd have been detected in fish downstream of the tailings site (*16*).

Efforts are currently underway to investigate the potential for remediation of Klondyke using phytostabilization. The goal of this strategy is to stabilize tailings piles by establishing a vegetative cover that does not hyperaccumulate metals. The desired outcomes include a significant decrease in eolian and waterborne tailings dispersion and a visually attractive plant cover with above ground tissues meeting acceptable metal levels to protect surrounding wildlife. Prior greenhouse research has indicated that optimal growth of a model desert native plant, quailbush, in Klondyke tailings requires the addition of 15% compost (w/w) (*18*). Such a compost requirement is feasible but adds significantly to the cost and logistics of remediation. Thus, it is desirable to investigate possibilities for reducing the amount of compost necessary for plant establishment.

Previous studies have demonstrated the benefits of utilizing plant growth-promoting bacteria (PGPB) for establishment of vegetation on desertified or contaminated sites (*2, 4, 9*), and a limited number of studies have explored the use of PGPB in mine tailings (*22, 34*). PGPB have been shown to enhance plant growth through a variety of mechanisms including the production of growth hormones such as indoleacetic acid (IAA), siderophore production to aid in nutrient acquisition and suppression of plant pathogens, phosphate solubilization activity, and the production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase which is thought to be an important mechanism for reducing plant ethylene concentrations under stressed conditions (*7, 10, 13, 20, 21, 23, 24, 28, 33*).

Here we report the use of PGPB to reduce the amount of compost necessary to establish two native plants, quailbush and buffalo grass, in the Klondyke tailings. A collection of 131 bacterial isolates was obtained from the rhizospheres of quailbush plants grown in composted Klondyke tailings, bulk mine tailings, and our laboratory collection. The objectives of our research were (*1*) to evaluate this collection of bacterial isolates for their potential plant growth-promoting (PGP) activities, the ability to grow in acidic conditions, and their tolerance to metal stress and (*2*) to evaluate a subset of 20 isolates from this collection that displayed desirable PGP mechanisms for the ability to enhance the growth of quailbush and buffalo grass in Klondyke tailings.

Experimental Section

Bacterial Isolates. Ninety three isolates were obtained from the rhizosphere of quailbush plants grown in Klondyke tailings (*18*), 21 from bulk tailings samples collected from the Klondyke site (*18*), 5 from the Boston Mill mine tailings site on the San Pedro River (*25*), and 12 isolates from our laboratory culture collection. Samples were plated in triplicate on R2A (EM Science, Gibbstown, NJ) amended with 200 mg L^{-1} cyclohexamide to inhibit fungal growth and incubated for five days at 23 °C. Colonies were selected and isolated based on morphological differences. All 131 bacterial isolates were maintained on R2A at 22 °C, gram-stained, and screened as described below.

Native Plants. Two plants native to the desert southwest ecosystem surrounding Klondyke were used in this study, the shrub *Atriplex lentiformis* (Torr.) S. Wats., commonly

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know as quailbush and the grass *Buchloe dactyloides* (Nutt.) Engelm., commonly known as buffalo grass. Seeds were obtained from Carter Seeds, Vista, CA (quailbush) and Western Native Seed, Coaldale, CO (buffalo grass).

Mine Tailing and Soil Control. A single composite Klondyke tailings sample was oven-dried at 105 °C, sieved through a 2 mm mesh screen, and analyzed for texture (*5*). Tailings mineralogy was derived from powder X-ray diffraction and X-ray absorption spectroscopy analysis. Total organic carbon (TOC) and total nitrogen (TN) were measured in the solid phase using a Shimadzu high temperature combustion TOC/TN analyzer (nitrogen includes both organic and inorganic forms). The pH, electrical conductivity (EC), dissolved organic carbon (DOC), and dissolved nitrogen (DN, organic plus inorganic) were measured in a saturated paste extract (1:1) in deionized water. Samples were prepared by microwave acid digestion using EPA method 3051 (*32*) for total element (As, Cd, Cu, Fe, K, Mn, Na, Pb, Zn) analysis by ICP-MS. Vinton Loamy Sand, previously characterized (*15*), was employed as a uncontaminated control soil.

PGPB Assays. Isolates were tested for the ability to solubilize phosphate on dicalcium phosphate medium (DCP) (*11*). Isolates producing a zone of clearing on DCP plates after 14 days at 23 °C were considered positive for phosphate solubilization activity.

Qualitative Siderophore Assay. Siderophore production was assayed for on iron-deficient chromazurol S (CAS) plates (*26*). Colonies were transferred from R2A onto the CAS medium and allowed to incubate at 23 °C for 14 days. Colonies that produced orange halos on CAS plates were considered positive for siderophore production.

Quantitative Siderophore Assay. Siderophore production was quantified from isolated colonies from an R2A plate as described previously (*26*). Siderophore quantification was conducted in triplicate, and mean siderophore production values are reported in μg 10^8 CFU.

Indoleacetic Acid Assay. Isolates were assayed for the ability to produce IAA (*20*), except R2B was used as the growth medium. Isolates were transferred from R2A plates to 5 mL of R2B and incubated on a rotary shaker (200 rpm) at 23 °C for 48 h. Turbid R2B (50 μL) culture were then transferred to 5 mL of fresh R2B supplemented with 0, 100, or 500 mg L^{-1} of tryptophan and incubated for 48 h. A 1.5 mL aliquot was removed and centrifuged at (22 000*g*) for 10 min. Cell supernatant (1 mL) was removed and mixed with 4.0 mL of Salkowski's Reagent (150 mL concentrated H_2SO_4, 250 mL distilled–deionized H2O, and 7.5 mL 0.5 M $FeCl_3$-$6H_2O$), incubated for 20 min at room temperature, and then IAA was quantified spectrophotometrically at 535 nm. A standard curve was created using R2B containing known concentrations of IAA (Fisher Scientific). IAA production was measured in triplicate at 500 mg L^{-1} tryptophan and mean values were reported as μg per 10^7 CFU.

ACC Deaminase Activity. Isolates were screened for their ability to obtain nitrogen from ACC a potential mechanism for decreasing plant ethylene levels (*10*). Isolates were grown on a rotary shaker (200 rpm) at 22 °C in 5.0 mL R2B until turbid and then 50 μL were transferred to 5.0 mL of DF medium, supplemented with 30 mM NH_4^+ as $(NH_4)_2SO_4$, pH 7.2. Cultures reaching visual turbidity in this medium were transferred to 5.0 mL of fresh DF medium containing 3.0 mM ACC as a sole source of N. Isolates producing visible turbidity in DF-ACC medium were assumed to have the ability to use ACC as a sole source of N. Each isolate was transferred four additional times to fresh DF-ACC medium for confirmation.

Tolerance of Acidic pH. To determine tolerance to acidic pH, isolates were initially grown in 5.0 mL R2B (pH 7.2) and then transferred to 5.0 mL R2B (pH 5.0). Cultures were monitored and isolates displaying visible turbidity within 8 days were considered positive for growth. All cultures positive for growth at pH 5.0 were transferred (50 μL) to 5.0 mL R2B (pH 4.0) and again monitored for 8 days.

Metal Tolerance Screening. Isolates were screened for the ability to grow on modified R2A (g L^{-1}; 1.0, glucose; 0.3, proteose peptone no. 3; 0.3, yeast extract; 0.3, starch; 0.3, sodium pyruvate; 0.1, K_2HPO_4; 0.1, $Na_5O_{10}P_3$; 0.1, $MgSO_4 \cdot 7H_2O$; 15 noble agar, adjusted to pH 6.5 using $C_8H_5O_4K$) amended with either 0, 0.25, 0.5, 1.0, or 1.5 mM $Pb(NO_3)_2$ or $ZnSO_4$. Nitrate and sulfate concentrations were normalized to 10 mM in all Pb and Zn plates using KNO_3 and $MgSO_4 \cdot 7H_2O$, respectively. Isolates that grew on these plates after 5 days were considered to show tolerance to the respective metal concentration.

Isolate Identification. Selected isolate colonies were inoculated into 5 mL of R2B and incubated on a shaker (180 rpm) at 23 °C until turbid. A 1 mL aliquot of bacterial culture was centrifuged at 14 000*g*, decanted, resuspended in 1 mL of sterile distilled water, and cells were lysed using two freeze–thaw cycles followed by 15 min in a boiling water bath. The 16S rRNA gene was amplified from 3 μL cell lysate as previously described (*14*). Products were purified using the QIAquick PCR purification kit (QIAGEN Inc., Valencia, CA) and then submitted to the University of Arizona Research Laboratories Genomic Analysis and Technology Core for sequencing with an ABI3730xl DNA analyzer (Applied Biosystems, Foster City, CA). Nucleotide sequences were used to identify bacterial isolates by BLAST analysis and then were submitted to the GenBank database under accession numbers DQ507202 to DQ507211 and DQ898296 to DQ898301.

PGPB Inoculation and Growth of Quailbush in Vinton Soil and in Klondyke Tailings. A series of three greenhouse experiments were performed to evaluate the selected PGPB. The first experiment examined growth of quailbush in Vinton soil to assess whether any of the PGPB were pathogenic or otherwise inhibitory for growth. Vinton soil was wetted to field capacity 48 h prior to planting and then placed into 50 cm^3 pots. Quailbush seeds were surface sterilized by placing approximately 200 seeds into a sterile 50 mL centrifuge tube containing 30 mL of a 2% bleach solution for 10 min followed by a 3 min rinse in a 0.01% sodium thiosulfate solution to neutralize the bleach. The seeds were then rinsed three times in 30 mL of sterile water. PGPB cultures were prepared 48 h prior to inoculation by transferring single colonies from R2A plates to 5.0 mL of R2B and incubating on a rotary shaker (200 rpm) at 23 °C. Immediately prior to inoculation, the cultures were centrifuged at 12 100*g* for 10 min, culture supernatant was removed and cells were resuspended in sterile PBS (g L^{-1}): 8.0 NaCl, 0.2 KCl, 1.44 Na_2HPO_4, 0.24 KH_2PO_4, adjusted to pH 7.4. Sterilized seeds were aseptically transferred to each individual isolate suspension and allowed to incubate for 10 min with a 5 s vortexing period every minute. Inoculated seeds were planted 0.5 cm deep with a sterile tweezers at depth of approximately 0.5 cm and the pots were placed into a controlled greenhouse environment at 30 °C receiving irrigation for three min three times daily. Noninoculated, sterile seed control treatments were created by subjecting seeds to the same sterilization and planting procedures minus the inoculation (referred to as the sterile control). Nonsterilized seeds, soaked in sterile water, were used as a second control (referred to as the nonsterile control). Each treatment consisted of five seeds per pot and 10 replicate pots. After 30 d, seedlings were counted to determine germination and then were thinned to one plant per pot. Dry plant biomass was determined 75 days after planting. At this time each plant was carefully harvested (roots and shoots were separated) and rinsed gently under running water to remove all tailings and compost material. Plant roots and shoots were then placed in individual aluminum foil packets, dried for 48 h in a 60 °C oven, and weighed.

The second greenhouse experiment was performed to evaluate the impact of the PGPB on growth of quailbush in Klondyke tailings amended with either 0 or 10% compost (w/w) (obtained from the University of Arizona Controlled Environment Agricultural Center, Tucson, AZ). Preparation and inoculation was as described for the first experiment. After 30 days of growth, germination was evaluated, and seedlings were thinned to one plant per pot. Dry plant biomass was determined for each plant after 75 days of growth.

The third greenhouse experiment was performed to further evaluate promising PGPB from the first two studies in larger pots and a second plant, *B. dactyloides*, was tested in addition to *A. lentiformis*. Each plant was grown in tailings amended with either 0 or 10% compost (w/w). This study was performed using larger (1625 cm^3) pots and a 90 day growth period after which dry plant biomass was determined for each plant. Each treatment had five replicate pots with 10 seeds per pot. For *A. lentiformis*, pots were thinned to one plant per pot after 4 weeks. The *B. dactyloides* treatments were not thinned.

In each study, plants received 0.6 cm irrigation pot^{-1} three times daily. Daily temperature fluctuations were held constant with high humidity at 20 °C during the evening and approximately 35 °C during the day, simulating typical growth conditions during a Sonoran Desert monsoon season (mid July to late August). No supplemental lighting was used.

Statistical Analysis. Statistical analysis was conducted using SAS version 9.1 (SAS Institute Inc. Cary, NC). Significant treatment effects for plant root, shoot, and total biomass and root-to-shoot data were evaluated using a one-way ANOVA ($p < 0.05$). For each treatment, significant differences between means were determined by the Duncan's Multiple Range Test ($p < 0.05$).

Results and Discussion

Bacterial Isolation and Screening. This study reports the screening, identification, and testing of PGPB for use in phytostabilization of mine tailings found in arid and semiarid environments with the goal of minimizing compost amendment. PGPB have been widely studied for agricultural applications (*6, 12, 17*), and some studies have demonstrated that PGPB can aid in revegetation of desertified regions (*2*). However, little is known about phytostabilization of mine tailings; previous studies performed have investigated the use of soil-isolated PGPB for the phytostabilization of mildly toxic mine tailings at high compost amendment rates (*22, 34*).

PGPB useful for establishment of plants in mine tailings may require specific traits beyond those normally associated with PGPB effective for agriculture. For example, Burd et al. (*4*) reported that a heavy-metal resistant, siderophore producing, ACC-deaminase positive strain of *Kluyvera ascorbata* increased plant growth in a Ni-contaminated soil. The authors proposed that this inoculant acted by reducing Ni toxicity and alleviating Ni-induced stress via ACC-deaminase activity. Based on this consideration, we hypothesized that microbial populations residing in bulk mine tailings or those associated with plants grown in tailings are subject to selective pressures that may make them more suitable for use in revegetation of tailings than isolates not exposed to these conditions. For example, desirable traits, in addition to traditional PGP mechanisms, might include the ability to grow at acidic pH and the ability to tolerate elevated metal concentrations. Thus, our initial collection of 131 isolates was a diverse assortment of bacteria obtained primarily from bulk mine tailings and from the rhizosphere of quailbush plants grown in composted tailings. Of these isolates, 86 were Gram positive and 45 were Gram negative (Supporting Information, Table S1). A majority (62%) produced siderophores while smaller proportions of the collection had the ability to solubilize calcium phosphate (40%), both abilities important for providing plants with essential nutrients (*24*). Nearly 21% of the isolates possessed ACC-deaminase activity, whereas 57% of the isolates produced IAA. A large proportion of the isolates (80%) were able to grow at pH 5, and 16% of isolates grew at pH 4, demonstrating their potential to survive in the acidic tailings environment. In general, PGP mechanisms were distributed evenly among the Gram-positive and Gram-negative isolates in the collection.

A further quantitative analysis was performed for two of the above PGP activities; siderophore and IAA production (Supporting Information, Table S2). Results show that 27% of isolates produced siderophores at a concentration of 10 mg L^{-1} or greater, while 15% produced at 100 μg mL^{-1} or greater. These levels of siderophore production are comparable to production by soil rhizobacteria (56−140 mg L^{-1}) (*1*). In terms of IAA production, whereas no isolates produced IAA without the supplementation of tryptophan, 20% of isolates produced IAA in concentrations from 10 to 25 μg mL^{-1} medium when supplemented with 100 μg mL^{-1} tryptophan, and 76% of isolates produced IAA when supplemented with 500 μg mL^{-1} tryptophan (Table S2). Thakuria et al. (*30*) report comparable levels of IAA production, ranging from 2.0 to 21.6 mg L^{-1} in culture supernatants from soil rhizosphere bacteria; however, these isolates were supplied with substantially higher amounts of L-tryptophan. In summary, the collection of 131 isolates from bulk and planted tailings exhibited a variety of PGP activities and a large proportion of the collection grew at a moderately acidic pH of 5.

Following screening of the collection, a subset of 20 isolates was subjectively selected based on the PGPB assay results (Supporting Information Table S3). Selection criteria included isolates exhibiting only one PGPB activity (e.g., K6–19, K6–11B, and MTR-18) and isolates with different mixtures of PGPB activities (e.g., *B. vietnamensis*, MTR-21A, and MTR-61). One isolate, SP-1, did not have any of the measured PGPB activities and was chosen as a control. Many of the genera represented by this subset have been previously reported to enhance plant growth. For example, MTR-1, MTR-61, M22, and K6–19 represent bacterial genera previously identified as exhibiting biological control against plant pathogens (*7*).

Mine Tailings Analysis. Selected physical−chemical analyses showed that the tailings have a pH of 4.54 ± 0.02, TOC and TN of 360 ± 68 and 67 ± 12 g kg^{-1}, respectively, DOC and DN of 38 ± 6.9 and 20 ± 1.2 g kg^{-1}, respectively, and EC of 3.0 ± 0.12 dS m^{-1}. The tailings texture is 51.9% sand, 26.4% silt, and 21.7% clay with major mineral constituents of quartz, orthoclase feldspar, and jarosite, and minor contributions from plumbojarosite and goslarite, which are the principal Pb and Zn bearing constituents, respectively. The major metals in the tailings are (mg kg^{-1} ± relative standard deviation) As, 91 ± 10%; Cd, 2.4 ± 0.7%; Cu, 653 ± 1%; Fe, 26 560 ± 1%; Mn, 2811 ± 1%; Pb, 4620 ± 1%; Zn, 1400 ± 1%.

Greenhouse Studies. An initial greenhouse study, conducted in the Vinton soil, showed that none of the 20 isolates inhibited growth of quailbush, e.g., there was no difference between the inoculated treatments and control plants ($p < 0.05$) (data not shown).

A second greenhouse study was performed to screen each of the 20 selected isolates for the ability to enhance growth of quailbush in either 0 or 10% compost-amended tailings (w/w). These amendment levels were chosen because a previous study showed that while 15% compost addition restored growth to levels comparable to that observed in an off-site soil control, the 0 and 10% treatments did not (*18*). We therefore wanted to test whether PGPB could enhance plant growth at these suboptimal compost treatment levels.

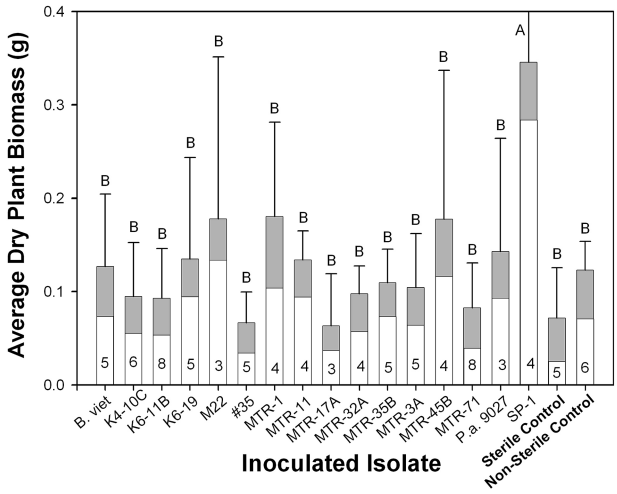

FIGURE 1. The effect of PGPB inoculation on the total average dry plant mass of quailbush grown in Klondyke tailings in the absence of compost for 75 days (mean + 1 SD for total plant biomass). Lower and upper bars represent the average dry root biomass and average dry shoot biomass for each treatment, respectively. PGPB isolate IDs correspond to those given in Table S3. The number at the bottom of each bar represents the surviving plants out of 10 replicates. Surface-sterilized (sterile control) and nonsterilized (non-sterile control) *A. lentiformis* seeds served as controls. Four isolates, MTR-18, MTR-21A, MTR-61, and MTR-70, are not included because there were less than three surviving plants. Means with different letters are significantly different in total dry plant biomass at $p < 0.05$.

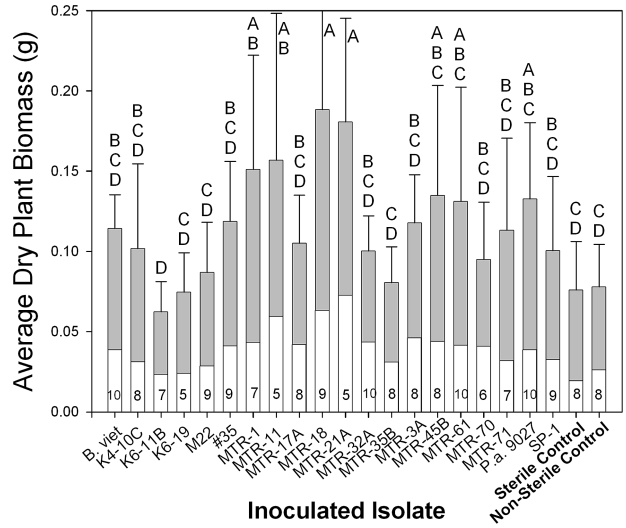

FIGURE 2. The effect of PGPB inoculation on the total average dry plant mass of quailbush grown in Klondyke tailings in the presence of 10% compost for 75 days (w/w) (mean + 1 SD for total plant biomass). Lower and upper bars represent the average dry root biomass and average dry shoot biomass for each treatment, respectively. PGPB isolate IDs correspond to those given in Table S3. Surface-sterilized (sterile control) and nonsterilized (non-sterile control) *A. lentiformis* seeds served as controls. The number at the bottom of each bar represents the surviving plants out of 10 replicates. Means with different letters are significantly different at $p < 0.05$.

This 75 day experiment revealed that the average total biomass production in the 0% compost control treatments (0.071 ± 0.066 g) was similar to the biomass produced in the 10% compost control treatments (0.077 ± 0.027 g), although there was less variability in growth among plants in the 10% treatment. One noticeable difference between two compost levels in this experiment was that the root:shoot biomass ratio was greater for the 0% than for the 10% compost treatment. This suggests that in the absence of compost (and the nutrients provided therein) the plants must put most of their energy into establishing a root system with large surface area to aid in nutrient uptake.

For the inoculated 0% compost treatments, only one isolate, SP-1 (most closely related to *Microbacterium)*, significantly ($p < 0.05$) increased total plant biomass in the noncomposted tailings by approximately 4-fold to 0.35 ± 0.28 g although, again, there was great variability in individual plant growth (Figure 1). Although *Microbacterium* isolates have previously been shown to enhance growth in field-grown potato plants (*27*), SP-1 did not display any PGPB activities nor did it show tolerance to either metals or to low pH (Table S3) so its PGPB activity is not apparent. Further, SP-1 did not stimulate growth in the third longer-term study suggesting it may not have good survival potential for tailings.

The average quailbush survival rate in the 0% compost treatment was 4.8 ± 1.5 out of 10. Two isolates, K6–11B and MTR-71, enhanced plant survival to eight (Figure 1). This suggests that some PGPB may play a role in facilitating plant survival while not necessarily enhancing plant biomass and could be used as part of a PGPB mixture to enhance overall survival and growth. It should also be noted that four isolates (MTR-18, MTR-21A, MTR-61, and MTR-70) in the 0% compost treatment resulted in less than three surviving plants and were not included in Figure 1.

In the 10% compost treatment, plant survival was improved to 7.9 ± 1.6 which was significantly higher than survival in the 0% treatment ($p < 0.0001$). In terms of plant growth, plants inoculated with 18 out of the 21 isolates had an average total dry biomass (0.12 ± 0.031 g) that was 1.5-fold greater than the control plants (0.077 ± 0.027 g). Four of these isolates (MTR-21A, MTR-1, MTR-18, and MTR-11) had significantly ($p < 0.5$) higher average dry plant biomass (0.016 ± 0.018 g) than the control plant treatments with the increase ranging from 2 to 2.5-fold (Figure 2). Three of these isolates, representing the genera *Clavibacter* (MTR-21A), *Microbacterium* (MTR-18), and *Streptomyces* (MTR-1), have been reported to improve plant growth in previous studies of PGPB for agricultural applications (*3, 29*).

One intriguing observation is that MTR-18 and MTR-21A, the isolates that resulted in the greatest plant biomass increase in the 10% compost treatment, were not effective in the 0% compost treatment. These results suggest that some isolates may display PGP activities under different environmental conditions, e.g., plant type, compost rate. For example, neither MTR-18 or MTR-21A showed tolerance to Pb and Zn (Table S3), which may have resulted in their ineffectiveness at the 0% compost treatment in which there was no compost material present to buffer metal toxicity. Thus, a minimum amount of compost amendment may be necessary to allow growth of some PGP isolates such as MTR-18 and MTR-21A.

A third 90 day greenhouse study was performed with selected isolates that enhanced quailbush growth in the previous experiment. The goals of this study were to confirm the previous results, scale up the size of the pots used for the study to allow for a longer growth period, and to extend the study to a second plant, buffalo grass. Results with quailbush showed that the addition of compost alone doubled the biomass in control plants from 1.2 ± 0.5 g (0% compost, data not shown) to 2.1 ± 0.4 g (10% compost). This is in contrast to the previous study where there was no difference between the control plants in the 0 and 10% treatments. Also, there was up to a 30-fold increase in total biomass produced in the controls from the 75 and 90 day experiments. These data suggest that the length of the study is an important consideration.

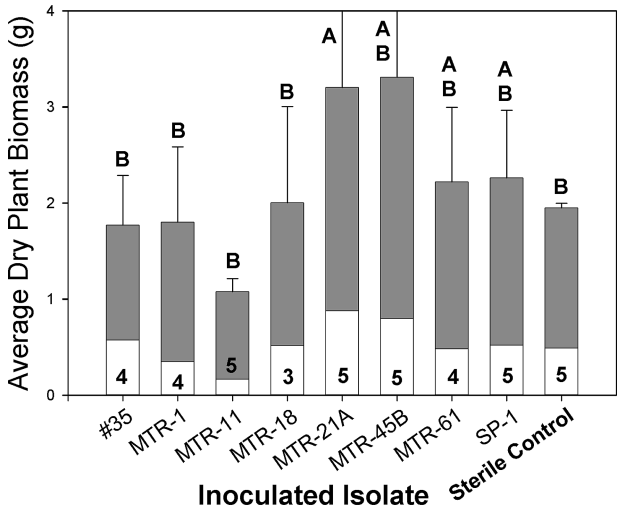

FIGURE 3. The effect of PGPB inoculation on the total average dry plant mass of quailbush grown in Klondyke tailings in the presence of 10% compost for 90 days (w/w) (mean + 1 SD for total plant biomass). Lower and upper bars represent the average dry root biomass and average dry shoot biomass, respectively, for each treatment. PGPB isolate IDs correspond to those given in Table S3. Surface-sterilized (sterile control) *A. lentiformis* seeds served as the control. The number at the bottom of each bar represents the surviving plants out of five replicates (following thinning). Means with different letters are significantly different at $p < 0.05$.

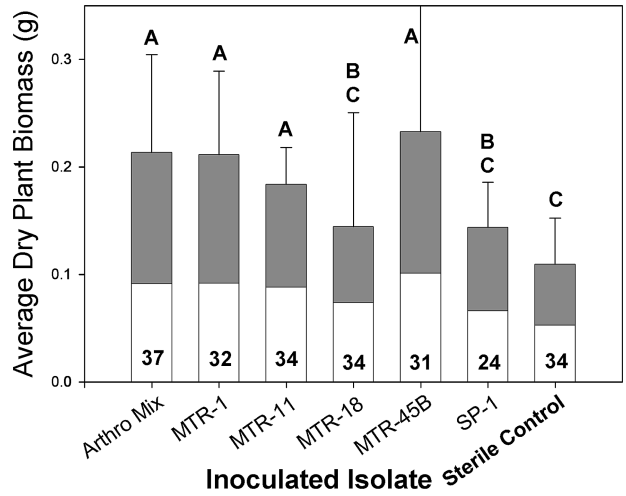

FIGURE 4. The effect of PGPB inoculation on the total average dry plant mass of buffalo grass grown in Klondyke tailings in absence of compost for 90 days (w/w) (mean + 1 SD for total plant biomass). Lower and upper bars represent the average dry root biomass and average dry shoot biomass, respectively, for each treatment. PGPB isolate IDs correspond to those given in Table S3. Surface-sterilized (sterile control) *A. lentiformis* seeds served as the control. The number at the bottom of each bar represents the surviving plants out of fifty seeds. Means with different letters are significantly different at $p < 0.05$.

There was no significant effect of the inoculated isolates on quailbush growth in the 0% compost treatment (data not shown). However, for the 10% compost treatment, plants inoculated with MTR 21A had significantly ($p < 0.05$) higher biomass (3.2 ± 1.1 g) than the uninoculated control (2.1 ± 0.4 g) (Figure 3). Isolate MTR 45B also increased the average biomass to 3.3 ± 2.5 but due to high variability in growth among replicates, this increase was not significantly different from the control. These results indicate that even over the longer term, inoculation has promise to aid in plant growth and establishment. However, it should be noted that only one of the four isolates, MTR 21A, that produced significantly better growth in the short term study was also effective in the longer-term study. Tolerance to low pH seemed to be an important factor in these longer studies; both MTR-21A and MTR-45B were able to grow at pH 4 (only five of the 21 isolates tested had this ability) (Table S3).

Results with buffalo grass showed that the addition of compost alone resulted in a 5-fold increase in total biomass from 0.71 ± 0.35 g to 3.7 ± 2.0 g. While several isolates inoculated into the 10% treatment increased biomass production by up to 1.5-fold, the increase was not statistically significant (data not shown). However, four of the six isolates tested, MTR-1, MTR-11, MTR45B, and a mix of MTR-44 and K4–10C (arthrobacter mix), doubled the growth of buffalo grass in the 0% compost treatment ($p < 0.5$) (Figure 4). All of these isolates except K4-10C, which was part of the mixed inoculum, showed tolerance to both Pb and Zn and all of these isolates showed growth at pH 5 (Table S3).

In summary, the establishment of a successful vegetative cover on mine tailings found in arid and semiarid environments is challenging and normally requires amendment with organic matter. The goal of this study was to determine whether the requirement for organic matter could be reduced by the use of PGPB to improve the economics of this remediation approach. The results of this study demonstrate that PGPB can successfully enhance growth of native plants in mine tailings from arid environments and at least partially replace the requirement for compost amendment. The impact of PGPB seems to be plant specific, e.g., buffalo grass responded more strongly to the PGPB at the 0% compost treatment, whereas quailbush responded more strongly in the 10% compost treatment. This study also suggests that very short-term (small pot) trials may not accurately identify successful PGPB, possibly due to poor persistence of the PGPB in the rhizosphere over a longer time scale. Finally, this study strongly indicates the importance of identifying PGPB that can persist in the tailings rhizosphere, e.g., exhibit metal and pH tolerance, in addition to providing plants with key PGP mechanisms. There is great likelihood that the performance of PGPBs could be enhanced through the use of PGPB mixtures and a better understanding of exactly what types of tolerance and PGP mechanisms are most important for optimal plant establishment.

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Supporting Information Available

Tables S1−S3. This material is available free of charge via the Internet at http://pubs.acs.org.

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