Primed Soilless Growing Medium with Disaccharides Stimulated Microbial Biofilm Formation, and Increased Particle Aggregation and Moisture Retention during Muskmelon Transplant Production

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ABSTRACT. Priming commercial growing media and soils with dilute sugar solutions was investigated as a means of stimulating beneficial microflora to improve transplant productivity. Muskmelon (Cucumis melo) seedlings were grown in soilless growing medium primed with equal volumes of 50 mM sucrose or trehalose. After priming, the time when 50% of plants showed wilting symptoms was delayed 45 hours and the mean time that seedling xylem tension reached –1.0 MPa was delayed 70 hours compared with watered controls. Sucrose or trehalose priming improved water retention in the presence and absence of plants grown in sphagnum-based medium after an incubation period of ~24 h, but no improvement occurred when autoclaved medium or acid-washed sand were primed. Light micrographs of primed medium revealed positive staining of opaque material between organic-matter particles with alcian blue, a polysaccharide-specific stain. Sixteen bacterial colonies were cultured in liquid medium from leachate of positive-stained, primed, growing-medium samples and identified via 16S rRNA gene sequencing. Identified colonies were Curtobacterium pussillum, Paenibacillus lautus, Brevundimonas, and 13 Bacillus spp., including well-characterized biofilm producers. Increased soil-moisture retention was the result of a complex, glucose-based, hydrophilic, polysaccharide polymer of bacterial origin that was produced in liquid culture from extracts of primed medium.

Beneficial soil microorganisms play a key role in maintaining soil quality and health (Karlen et al., 1997; Pankhurst et al., 1997). Microbial activity in soils is stimulated by the release of carbon-rich material in the form of root border cells (Hawes and Brigham, 1992; Hawes et al., 1998), and/or the selective exudation of specific sugars, carboxylic acids, or amino acids (Grayston et al., 1998; Lugtenberg et al., 2001; Ryan et al., 2001; Siciliano et al., 1998) that encourage the development of cultivar-specific, plant-beneficial, microbial communities (Chanway et al., 1988; Lugtenberg et al., 2001; Marschner et al., 2004). During transplant production, selective exudation takes place only after seed germination and root system development. Without growing plants, soil microorganisms can survive for long periods of time as primarily dormant populations (Mondini et al., 2006). They respond rapidly to addition of fresh energy [i.e., sucrose (Kelliher et al., 2005) or glucose (Mondini et al., 2006)]. This new energy source enhances microbial growth and humification of soil organic matter, which in turn improves the mineral- and water-holding capacity of soil. This process is recognized in the literature as soil priming (reviewed in Blagodatskaya and Kuzyakov, 2008).

Some of the bacteria benefiting from the availability of fresh energy are also capable of activating defense responses, making plants more resistant to abiotic and biotic stresses (reviewed in Conrath et al., 2006). This phenomenon, referred to as biopriming, is being adapted to vegetative propagation of high-value crops via tissue culture (reviewed in Nowak and Schulaev, 2003). Plantlets inoculated with selected strains of endophytic bacteria can acquire resistance to transplanting shock, as well as to some fungal and viral diseases (reviewed in Kavino et al., 2007; Nowak and Schulaev, 2003).

Soils can be primed by using cover crops to include “primer plants,” which support establishment of soil microbial communities (Yunusa and Newton, 2003). Roots of plants used to prime soil penetrate deep into subsoil and secrete sugars to create and sustain symbioses with microbes that benefit plant growth and development (Wardle, 2002). However, organic compounds released from living plants or decomposing plant material may be insufficient to sustain or optimize the proliferation of beneficial soil microbes (Welbaum et al., 2004).

Despite considerable research describing microbial population changes in soil and plant rhizosphere (reviewed in...
soil dry weight (fresh weight – dry weight) for 24 to 48 h and results are expressed as a percentage of the soil water contents were measured by drying samples at 98°C (176°C, 20 min steam cycle) before treatment to compare moisture retention. During and after priming, soilless medium, vermiculite, and wheat bran prilled with two glucose units, C₁₂H₂₂O₁₁, were separated by least significant difference (P < 0.05). The pressure-plate medium moisture retention values and means at 0.5 and 1.5 MPa. Analysis of variance was performed on different polysaccharide isolates. Chromatographic separation followed by trifluoroacetic acid hydrolysis) to release monosaccharides from solution culture by a two-step lysis (methanolysis of polysaccharides in solution and were incubated for centrifugation (7800 g, 15 min), washed with 5 mL of sterile water, and resuspended. The suspension was precipitated in 1% (w/v) KCL with ice-cold ethanol (three to four volumes), vigorously mixed in 5 mL of distilled water for 2 min, and insoluble material was removed by centrifugation (17,000 g, 15 min). Carbohydrate analysis and staining. Medium samples were suspended in phosphate buffer (pH 7) 1:100 (w/v) for 15 min and a 3-mL aliquot containing medium particles was transferred to a 140-mm-diameter petri plate. A 1% alcian blue solution in ethanol was diluted to 0.1% with water, and several drops were pipetted onto each sample. After 3 min, the stain was rinsed with phosphate buffer, drained, and viewed by light microscopy.

For carbohydrate analyses, polysaccharides (10 µg) were isolated from solution culture by a two-step lysis (methanolysis followed by trifluoroacetic acid hydrolysis) to release monosaccharides and uronic acids (Talaga et al., 2002). Quantitative external standard analyses were performed in duplicate on five different polysaccharide isolates. Chromatographic separation and data analysis were performed using a liquid chromatography system ( Dionex, Sunnyvale, CA) consisting of a LC30 oven, GP50 pumps, an ED50 pulsed amperometric detector, and PeakNet software ( Dionex). Separations were performed

Materials and Methods

**Plant material and soil analysis.** Muskmelon seeds (cv. Top Mark; Monsanto, St. Louis) were planted in black plastic transplant trays, [12 cells, 4 cm (length)× 3 cm (width)× 5.5 cm (depth), 60 cm³ per cell], equally filled with soilless growing medium (Sunshine Mix I, coarse sphagnum moss and perlite; Fissons, Vancouver, BC, Canada). For priming studies, equivalent volumes of vermiculite (particle diameter 1.5 mm, Tobacco Mix; Southern States, Richmond, VA), acid-washed sand (Sigma, St. Louis), or Hayter loam (fine-loamy, mixed, mesic Ultic Hapludalf) field soil collected near Blacksburg, VA, were sieved (2.4 mm), air dried for 2 d at room temperature, added to three 12-cell plastic trays per treatment, and tested in a growth chamber (Percival Scientific, Boone, IA). Soilless growing medium was autoclaved (134°C, 200 kPa, 20 min steam cycle) before treatment to compare moisture-retention patterns of steam-treated and nontreated samples. During and after priming, soilless medium, vermiculite, and soil water contents were measured by drying samples at 98°C for 24 to 48 h and results are expressed as a percentage of the soil dry weight (fresh weight – dry weight/dry weight × 100). Effects of priming with 50 mM sucrose solution on the water-retention capacity of three samples each of primed and nonprimed media were compared using a soil pressure plate (Soil Moisture Equipment, Santa Barbara, CA) after equilibration at 0.5 and 1.5 MPa. Analysis of variance was performed on pressure-plate medium moisture retention values and means were separated by least significant difference (P < 0.05). The Virginia Tech Soil Testing Laboratory (Blacksburg, VA) analyzed chemical properties of soilless growing media initially before treatments were applied.

In the initial growth-chamber study with air-dry soilless growing medium, muskmelon seeds were sown one per tray cell and hydrated with 20 mL of water or 15 or 50 mM trehalose solution and were incubated for 7 d without additional water. To assess the effect of priming on medium moisture retention, 50 mM sucrose or trehalose was added to air-dried soilless growing medium in replicates of four 12-cell trays each in a separate experiment. To evaluate concentration and possible toxic effects of sucrose, three 12-cell trays of soilless growing medium were watered or primed with 20 mL of 0, 1, or 10 to 90 mM sucrose in 10 mM increments. Moisture loss was quantified by weighing samples after watering air-dried medium. Wilting symptoms, loss of turgor causing leaves of four replications of 21-d-old plants (two to three true leaves) to drop to an angle at least 45° below the main stem, and seedling xylem tension were measured on four replications of 10-d-old plants each and three replications of 21-d-old plants (two- to three-leaf stage) using a pressure chamber (Soil Moisture Equipment, Santa Barbara, CA), to 300 h after solution application. Growth chamber experiments were conducted under a 16-h photoperiod, constant 25°C, and light intensity of 50 μE·m⁻²·s⁻¹, using two, three, or four replicates of 12 plants each organized in a completely randomized design.

**Bacterial isolation and polysaccharide production in culture.** Bacteria were isolated from soilless growing medium primed with 50 mM sucrose by mixing 1 g of medium with 15 mL of sterile water and collecting the supernatant. Serial dilutions (10⁻²–10⁵ times) of the supernatant were plated on tryptic soy agar (TSA) enriched with 50 mM sucrose. The opaque exudate that surrounded some colonies was tested for carbohydrate using alcian blue (C₅₆H₆₈Cl₄CuN₁₆S₄), which stains sulfated and carboxylated mucopolysaccharides, glycosaminoglycans, and sulfated and carboxylated glycoproteins (Powell et al., 1982). Prominent colonies were subcultured in 25 mL of semisynthetic liquid medium to assess polysaccharide production as described by Bruegger and Keen (1979) with potassium phosphate (0.05M) substituted for yeast extract and 50 g·L⁻¹ sucrose. After incubation at 25°C for 3 d with agitation at 90 rpm, cells were harvested by centrifugation (7800 g, 15 min), washed with 5 mL of sterile water, and resuspended. The suspension was precipitated in 1% (w/v) KCL with ice-cold ethanol (three to four volumes), vigorously mixed in 5 mL of distilled water for 2 min, and insoluble material was removed by centrifugation (17,000 g, 15 min).

**Carbohydrate analysis and staining.** Medium samples were suspended in phosphate buffer (pH 7) 1:100 (w/v) for 15 min and a 3-mL aliquot containing medium particles was transferred to a 140-mm-diameter petri plate. A 1% alcian blue solution in ethanol was diluted to 0.1% with water, and several drops were pipetted onto each sample. After 3 min, the stain was rinsed with phosphate buffer, drained, and viewed by light microscopy.
with a 4 × 250-mm column (Carbopac PA10, Dionex) and a 4 × 50-mm guard column (Talaga et al., 2002). Mass spectral analysis of the hydrolyzates was performed by direct-infusion electrospray ionization (Thermo Finnigan DecaXP; Thermo Scientific, San Jose, CA). Hydrolyzate was dissolved in methanol:water (1:1; v/v) and infused at a flow of 5 μL/min. Spray voltage was 4.5 kV (negative mode) with a capillary temperature of 225 °C. The mass range was 100 to 2000 m/z.

**Bacterial genetic analysis.** Genetic analysis of bacterial colonies (MIDI Laboratories, Newark, DE) from TSA was by cycle sequencing of the 16S rRNA amplification products with AmpliTaq FS DNA polymerase and dRhodamine dye terminators (Kolbert and Pershing, 1999; Patel et al., 2000). Two separate primer sets (5F and 531R) amplified 16S rRNA genes to produce 500-bp products. Amplification products were purified from excess primers and dNTPs (deoxyribonucleotide triphosphates) using molecular weight cut-off membranes (Microcon 100; Millipore, Bedford, MA) and were run on an agarose gel to control quality and quantity. Excess dye-labeled terminators were removed from sequencing reactions using a spin column (Sephadex G-50; Amersham Pharmacia Biotech, Piscataway, NJ). The sequencing products were collected by centrifugation, dried under vacuum, and frozen at −20 °C until loading. Sample electrophoresence was performed using a DNA Sequencer (ABI Prism 377; Applied Biosystems, Carlsbad, CA), and the data were analyzed with DNA editing and assembly software (Applied Biosystems). Edited and assembled DNA sequences were identified based on pairwise alignment algorithms and phylogenetic trees with MicroSeq Analysis Software and Sequence Database (Applied Biosystems).

**Results**

Priming commercial soilless growing medium with 50 mM trehalose reduced cumulative water loss between 25 and 260 h (Fig. 1A). At 25 h, there was only a small difference between trehalose-primed and nonprimed (watered) soilless growing medium. This difference increased and was largest between 100 and 150 h before reaching parity at 285 h (Fig. 1A).

The xylem tension of muskmelon seedlings grown in non-primed or trehalose-primed growing medium was similar until 125 h (Fig. 1B). After 125 h, the xylem tension of muskmelon seedlings in nonprimed growing medium abruptly increased. In contrast, the xylem tension of seedlings in trehalose-primed growing medium did not change between 125 and 150 h (Fig. 1B).

Similarly, more plants wilted after 125 h in nonprimed growing medium (Fig. 1C). The time for 50% of muskmelon seedlings to show wilting symptoms was delayed 45 h in growing medium primed with 50 mM trehalose or water (Fig. 1C). Water control 15 mM Trehalose 50 mM Trehalose

Dried medium samples differed in appearance following priming with sucrose (Fig. 2, inset) or trehalose (not shown). Dried sucrose-primed soilless growing medium maintained tray-cell shape as a solid block when removed from cells, while dried nonprimed growing medium crumbled (Fig. 2).

The pH of nonprimed soilless growing medium was 6.7 and the percentage of organic matter was 67.3%, while the cation

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Fig. 1. Cumulative water loss from plug trays filled with equal volumes of soilless growing medium (Sunshine Mix I; Fissons, Vancouver, BC, Canada) primed with 20 mL of 50 mM trehalose or water in a growth chamber. Error bars represent ± se of four replications of 12 plants each for each time interval (A). Muskmelon seedling xylem tension time courses for 21-d-old plants (two- to three-leaf stage) measured for three plants at each time point with a pressure chamber after an initial application with 20 mL of 50 mM trehalose or water (B). Development of wilting symptoms of four replications of 21-d-old plants after 20-mL solutions of water or 50 mM trehalose were applied to individual tray cells. (C) Error bars represent ± se.

Fig. 2. Twenty-one-day-old muskmelon seedlings were grown in a growth chamber in soilless growing medium (Sunshine Mix I; Fissons, Vancouver, BC, Canada) for 72 h following a single treatment with 8 mL of water, or 15 or 50 mM trehalose. Similar results were obtained following treatment with sucrose. Two replications of 12 plants each are shown for each treatment and are representative of similar experiments conducted. Inset shows that medium treated with 50 mM sucrose retained cell shape when air-dried while watered media did not. Similar results were obtained following treatment with trehalose. (Please view this paper online at ashs.org to see the image in color.)
exchange capacity was 7.3 cmol·kg⁻¹. The mineral composition of nonprimed soilless growing medium was 15.5 mg·kg⁻¹, 55.0 mg·kg⁻¹, 801 mg·kg⁻¹, and 377 mg·kg⁻¹ for P, K, Ca and Mg, respectively (data not shown).

Between 75 and 150 h, the moisture content of growing medium was nearly identical in sucrose- or trehalose-primed medium, illustrating that the improvement in moisture retention could be obtained using either disaccharide (Fig. 3A). Plants were under less moisture stress in growing medium primed with sucrose or trehalose during the first 200 h compared with nonprimed medium that received only water (Fig. 3B). The time to reach −1.0 MPa was delayed 70 h in primed growing medium compared with nonprimed (Fig. 3B).

Growing medium primed with 10 mM or greater concentrations of sucrose maintained the highest water content from 48 to 120 h, while priming with 1 mM sucrose solution had the same effect as applying only water (Fig. 4A). Applications of 20 to 50 mM sucrose solutions resulted in the highest potting-medium-moisture contents over this period. Growing-medium-moisture contents, similar to the 50 mM treatments, were obtained using 60 to 90 mM sucrose solutions (not shown).

The moisture-retention patterns of soilless growing medium treated with water or primed with 50 mM sucrose were compared on a pressure plate. Soilless growing medium pressurized to 0.4 or 1.5 MPa retained significantly more water in primed compared with nonprimed samples treated with equal volumes of water (Table 1).

### Table 1. Soilless growing medium (Sunshine Mix I; Fissons, Vancouver, BC, Canada) water content after treatment with water or 50 mM sucrose and equilibration at 0.4 or 1.5 MPa on a pressure plate. Values represent three replications of each treatment. Media water content was calculated as a percentage of the soil dry weight (e.g., fresh weight − dry weight/dry weight × 100 after oven drying at 98°C).

| Soil treatment | Equilibration pressure | Water content (%) |
|---------------|------------------------|-------------------|
|               | 0.4 MPa | 1.5 MPa          |
| Water         |          |                  |
| 50 mM sucrose |          |                  |
| LSD         |          |                  |

*Least significant difference at *P* < 0.05.

Steam-treating growing medium before priming decreased moisture contents compared with untreated medium during the first 24 h (Fig. 4B). The moisture contents of primed and nonprimed growing medium were similar after 48 h, indicating that autoclaving negated the increased moisture content previously observed following sucrose priming. Only nonautoclaved growing medium primed with sucrose maintained higher moisture contents between 48 and 120 h (Fig. 4B).

To compare the priming response in soilless growing medium to other substrates, field soil and acid-washed sand were also primed with sucrose. When sand was primed with
equivalent volumes of water, or 10 or 50 mM sucrose solution, moisture contents after an incubation period were similar or lower than water controls (Fig. 5A). Hayter clay-loam soil primed with 50 mM sucrose had only slightly higher moisture content than the water control between 25 and 135 h after treatment (Fig. 5B). Vermiculite soil less medium for hydroponic production (tobacco mix) was primed with 50 mM sucrose and higher medium-moisture contents were obtained between 50 and 140 h compared with water alone (Fig. 5C).

Soilless growing medium primed with sucrose stained positively with alcian blue, a general stain for carbohydrates, when viewed under a microscope. Blue staining was detected among particles of perlite, peat, and organic matter in samples primed with sucrose solution, but not in those treated with water alone or autoclaved before priming (Fig. 6, A–C).

Bacteria were isolated from the supernatant of primed soilless medium and plated on TSA enriched with 50 mM sucrose. When colonies producing exudates that stained positively with alcian blue were subcultured on in liquid medium, they produced 2.2 to 3.3 mg dry weight per milliliter per day of putative polysaccharide that was qualitatively analyzed. The predominant neutral monosaccharide isolated from liquid-culture component of these polysaccharides was glucose (55%–65%) (Fig. 7). The remaining neutral sugars present were variable and depended on the isolate. Arabinose, galactose, mannose, glucosamine, and galactosamine were all detected, but comprised less than 10% of the neutral sugars (Fig. 7). The chromatographic analyses also revealed the presence of an unknown carbohydrate eluting at retention times.

Fig. 5. Comparison of the moisture contents of acid-washed sand in a growth chamber after addition of 8 mL of water or priming with 10 or 50 mM sucrose (A) Hayter clay-loam, field soil watered, or primed with 8 mL of 50 mM sucrose (B) or vermiculite soilless growing medium watered or primed with 8 mL of 50 mM sucrose (C).

Fig. 6. After watering or priming with 50 mM sucrose for at least 72 h, samples of soilless growing medium were dried and stained with alcian blue, a polysaccharide-specific stain. Alcian blue staining was apparent among particles of organic matter in sucrose-primed samples (A) ×3.2. Staining was absent in growing medium hydrated with only water (B) ×3.2. Staining was absent when growing medium was first autoclaved for 20 min then primed with 50 mM sucrose for 72 h (C) ×4.1. (Please view this paper online at ashs.org to see the image in color.)
Trehalose is a nonreducing disaccharide, α-D-glucopyranosyl-(1,1)-α-D-glucopyranoside, and is a common sugar in fungi, bacteria, and invertebrate animals (Almeida et al., 2007). Priming soilless growing medium with trehalose or sucrose slowed medium water loss and delayed wilting of muskmelon transplants (Figs. 1–3). Sugars regulate gene expression and trehalose, in particular, affects gene expression associated with carbohydrate metabolism and plant development (Aeschbacher et al., 2000; León and Sheen, 2003; Smeekens, 2000). Transgenic plants expressing the Escherichia coli or Saccharomyces cerevisiae genes for trehalose synthesis not only exhibit increased drought tolerance (Holmström et al., 1996; Pilon-Smith et al., 1998; Romero et al., 1997), but also exhibit strong developmental alterations, such as stunted growth (Goddijn et al., 1997; Romero et al., 1997).

Plant \( \psi_S \) measured by porometry and moisture sorption isotherms (Bell and Labuza, 2000) were not changed after soilless growing medium was primed with 50 mM sucrose (not shown). The mean \( \psi_S \) of sucrose-primed growing medium solution was –0.12 MPa measured by osmometry. This suggests that the delay in the onset of wilting resulted from improved medium retention of plant-available water and not plant-osmotic adjustment because priming increased moisture retention without plants present (Fig. 4). Oven drying and pressure-plate analysis confirmed that sucrose-treated growing medium held significantly more water compared with untreated samples (Fig. 3A; Table 1). Optimum sucrose-priming concentrations ranged from 30 to 50 mM (Fig. 2, 4A). Wilting and abnormal development were observed in muskmelon, marigold (Tagetes patula), and alysum (Labularia maritime) plants at sucrose concentrations >70 mM (Faltenevitch and Welbaum, 2008). Priming acid-washed sand (Fig. 5A) and autoclaved soilless growing medium (Fig. 4B) failed to improve moisture retention or cause positive staining with alcyan blue, suggesting that the positively stained matter was of microbial origin and not the direct effect of sucrose amendments (Fig. 6).

The lag phase in moisture retention during the first 24 h after treatment was also consistent with a biological process (Fig. 4). Furthermore, the moisture-holding capacity of vermiculite-based growing medium and Hayter loam soil was only slightly increased by sucrose priming, illustrating that the dramatic improvement in soil-moisture retention in sphagnum-based soilless growing medium was not a universal response and was unique to Sunshine Mix I soilless growing medium (Fig. 5C).

Light micrographs of medium revealed alcyan blue staining for carbohydrate only in sugar-primed soilless growing medium, suggesting that improved soil-moisture holding capacity was related to the positive staining matter randomly scattered among medium particles (Fig. 6). The transformation of loose soilless growing medium into solid masses that resisted crumbling (Fig. 2, inset) is consistent with the observation that the carbohydrate-positive staining material was serving as hydrophilic glue to solidify particles when dried (Fig. 6A).

Thirteen of the 16 colonies cultured on agar medium supplemented with sucrose from soilless growing medium leachate were Bacillus spp. (Table 2, Fig. 8). Biofilm production by Curtobacterium pussillum, Paenibacillus lautus,

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**Table 2. Bacterial colonies isolated from soilless growing medium (Sunshine Mix I; Fisons, Vancouver, BC, Canada) and cultured in tryptic soy agar enriched with 50 mM sucrose. 16S rRNA amplification products were sequenced and database matches are listed below. Relationships among bacteria are summarized in Fig. 8.**

| Sample identifier | Gram stain | Appearance | 16S RNA match |
|-------------------|------------|------------|---------------|
| WBS1              | Unknown    | Unknown    | Curtobacterium pussillum |
| WBS2              | Positive   | Rod        | Bacillus subtilis subtilis |
| WBS3              | Positive   | Rod        | Bacillus subtilis subtilis |
| WBS4              | Positive   | Rod        | Bacillus subtilis subtilis |
| WBS5              | Positive   | Rod        | Bacillus pumilis |
| WBS6              | Positive   | Rod        | Bacillus amylophilaequetiens |
| WBS7              | Positive   | Rod        | Bacillus thuringiensis |
| WBS8              | Positive   | Rod        | Bacillus amylophilaequetiens |
| WBS9              | Positive   | Rod        | Bacillus thuringiensis |
| WBS10             | Positive   | Rod        | Bacillus megaterium |
| WBS11             | positive   | Rod        | Bacillus megaterium |
| WBS12             | Positive   | Rod        | Bacillus mojavensis |
| WBS13             | Positive   | Rod        | Bacillus mojavensis |
| WBS14             | Unknown    | Unknown    | Paenibacillus lautus |
| WBS15             | Unknown    | Unknown    | Bacillus mojavensis |
| WBS16             | Unknown    | Unknown    | Brevundimonas sp. |
| WBS17             | Unknown    | Unknown    | Bacillus circulans |

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**Fig. 7. Extracellular polysaccharides (EPS) chromatograms of neutral monosaccharides isolated from three liquid bacterial cultures from primed soilless growing medium (Sunshine Mix I; Fisons, Vancouver, BC, Canada). Glucose is the predominant monosaccharide, comprising between 55% and 65% of the total neutral sugars. Arabinose, galactose, mannose, glucosamine, and galactosamine were also detected, but variability in the levels of these monosaccharides prevented accurate quantification. EPS 1 and 4, and 2 and 5 were replicates of separate biological samples, while EPS 3 was an additional sample.**

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indicative of uronic acid that did not match galacturonic and glucuronic acid standards.

Sixteen bacteria, including Curtobacterium pussillum, Paenibacillus lautus, Brevundimonas, and 13 Bacillus spp., were cultured, isolated, and genetically identified from primed soilless growing medium (Table 2, Fig. 8). Eleven of the bacteria were gram-positive and rod-shaped (Table 2, Fig. 8).

**Discussion**

Thirteen of the 16 colonies cultured on agar medium isolated a unique bacterial community (Table 2, Fig. 8). Biofilm production by Curtobacterium pussillum, Paenibacillus lautus,
and *Brevundimonas* is less common than in *Bacillus* spp., which are common biofilm producers. For example, a complex glucose-based polysaccharide polymer is produced by *B. megaterium* (Bishop and White, 1993; Gandhi, et al., 1997). The polymer produced in solution cultures isolated from primed medium was predominately glucose, consistent with the hypothesis that *B. megaterium* contributed to biofilm production in primed soilless growing medium (Fig. 7). The optimal growth for *B. megaterium* in culture was obtained using 5% (w/v) date (*Phoenix dactylifera*) syrup or beet (*Beta vulgaris*) molasses supplemented with NH4Cl (Omar et al., 2001). *B. megaterium* has other documented agricultural benefits, such as an ability to solubilize inorganic phosphate (Cakmakci et al., 1992) and suppress *Rhizoctonia* root rot of soybean (*Glycine max*) (Zheng and Sinclair, 2000).

The production of biofilms has been characterized in other prokaryotes, including the *Pseudomonads* (Singh and Fett, 1995). The terrestrial cyanobacterium *Nostoc commune* excretes large amounts of glucan polysaccharide with unusual properties, including maintenance of secreted enzymes in an active state during long-term, air-dried storage, and accommodation of rapid biophysical and physiological changes upon rehydration (Helm et al., 2000).

The biofilm reported here has properties in common with glomalin, a fungal protein produced by vesicular–arbuscular mycorrhiza (Wright and Upadhyaya, 1996). Glomalin, produced by fungi in the order Glomales, may account for as much as 27% of total carbon in some soils (Rillig et al., 2001). Glomalin is thought to maintain soil structure and to promote water-holding capacity, proper aeration, and root growth in certain soils (Rillig et al., 2002). Soil microbial extracellular polysaccharides may be an important factor affecting soil structure in cultivated soils (Rillig et al., 2001; Roberson et al., 1995).

The benefits of sugar amendments to soils and compost have been reported for years (for example, Story, 1939). Molasses (syrup produced during sugar refining) has been used to hasten decomposition of compost and by agriculturalists as a soil amendment. Soils amended with molasses had greater moisture retention in a papaw (*Asimina triloba*) field study in Australia, although specific mechanisms were not investigated (Vawdrey et al., 2002). The possible benefits of directly priming soils with sucrose or other sugars with the express intent of stimulating beneficial soil microbes has not been fully evaluated (Schenck, 2001, Welbaum et al., 2004).

Because *Bacillus* is a gram-positive, nonpathogenic bacterium that forms resting spores and extracellular carbohydrates, the potential exists for priming certain soil types directly with specific carbon sources or indirectly with cover crops capable of simulating microbial colonies to improve soil characteristics through biofilm production. In the current study, the greatest effect occurred in commercial sphagnum-based soilless growing medium used primarily for greenhouse production of bedding plants and high-value crops. Adding sugar to sand,
field soil, or vermiculite-based growing medium produced a slight or no improvement in soil-moisture retention (Fig. 5). Similarly, inoculating field soils, or other brands of soilless growing media, with various combinations of bacteria from Table 2 and priming with sucrose did not increase moisture retention to the extent reported in Figs. 1, 3, and 4 (K. Woods, personal communication). This indicates that the Sunshine Mix 1 soilless growing medium composition (e.g., pH, mineral content, and high organic matter) along with its microbial composition offered unique conditions for biofilm production. Culture-dependent detection methods for fungi and bacteria may give a biased, incomplete picture of microbial communities (Pace, 1997) so that bacteria other than those in Table 2 may have been responsible for biofilm production because only 10% of the bacterial and fungal populations can be cultured (Kirk et al., 2004, Sakai et al., 2004). Nonculturable, DNA-based microbial detection techniques may be required to provide additional qualitative information about organisms contributing to biofilm production in soilless growing medium primed with sucrose or trehalose before biofilms can be produced in culture or other soils. This work illustrates that soilless growing media with certain microbial composition can be successfully primed with small concentrations of sucrose or trehalose to simulate biofilm production, thus improving moisture-holding capacity and transplant production under moisture stress conditions.

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