Impact of Biochar Application to Soil on the Root-Associated Bacterial Community Structure of Fully Developed Greenhouse Pepper Plants[▽]

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Adding biochar to soil has environmental and agricultural potential due to its long-term carbon sequestration capacity and its ability to improve crop productivity. Recent studies have demonstrated that soil-applied biochar promotes the systemic resistance of plants to several prominent foliar pathogens. One potential mechanism for this phenomenon is root-associated microbial elicitors whose presence is somehow augmented in the biochar-amended soils. The objective of this study was to assess the effect of biochar amendment on the root-associated bacterial community composition of mature sweet pepper (*Capsicum annuum* L.) plants. Molecular fingerprinting (denaturing gradient gel electrophoresis and terminal restriction fragment length polymorphism) of 16S rRNA gene fragments showed a clear differentiation between the root-associated bacterial community structures of biochar-amended and control plants. The pyrosequencing of 16S rRNA amplicons from the rhizoplane of both treatments generated a total of 20,142 sequences, 92 to 95% of which were affiliated with the *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, and *Firmicutes* phyla. The relative abundance of members of the *Bacteroidetes* phylum increased from 12 to 30% as a result of biochar amendment, while that of the *Proteobacteria* decreased from 71 to 47%. The *Bacteroidetes*-affiliated *Flavobacterium* was the strongest biochar-induced genus. The relative abundance of this group increased from 4.2% of total root-associated operational taxonomic units (OTUs) in control samples to 19.6% in biochar-amended samples. Additional biochar-induced genera included chitin and cellulose degraders (*Chitinophaga* and *Cellvibrio*, respectively) and aromatic compound degraders (*Hydrogenophaga* and *Dechloromonas*). We hypothesize that these biochar-augmented genera may be at least partially responsible for the beneficial effect of biochar amendment on plant growth and viability.

Fears of global climate change, attributed mainly to the accumulation of anthropogenic greenhouse gases emitted by fossil fuel use, are driving the development of alternative energy sources based on renewable resources, including biomass. Pyrolysis, the direct thermal decomposition of biomass in the absence of oxygen to solid (biochar or charcoal), liquid (bio-oil), and gas (syngas) bioenergy coproducts, is one of the tools suggested to help drive this paradigm shift (41). Because the half-life of biochar in soil is estimated to range from hundreds to several thousand years, the amendment of soil with biochar is thought to have long-term carbon sequestration potential (58). Importantly, various types of biochar used along with organic and inorganic fertilizers have been reported to significantly improve soil tilth (22), crop productivity (24, 51), and the availability of nutrients to plants (37, 50). Improved crop response as a result of biochar amendment can be attributed to

its nutrient content and to several indirect effects, including the neutralization of phytotoxic compounds in the soil (56), the promotion of mycorrhizal fungi (57), and the alteration of soil microbial populations and functions (51).

Elad and colleagues (18) recently demonstrated that soil-applied biochar induces systemic resistance to the foliar fungal pathogens *Botrytis cinerea* (gray mold) and *Leveillula taurica* (powdery mildew) on sweet pepper and tomato and *Podosphaera aphanis* (powdery mildew) on strawberry plants (43), showing that biochar also has a positive impact on plant resistance to disease (18). Interaction between certain bacteria and plant roots can result in a phenomenon termed induced systemic resistance (ISR), where plants become resistant to selected pathogenic bacteria, fungi, viruses, insects, and nematodes. Both biological (virulent, avirulent, and nonpathogenic microorganisms) and chemical (methyl jasmonate, chitin, chitosan, laminarin, and alginate) elicitors can trigger ISR (26, 54). Species of soil microorganisms such as *Bacillus*, *Pseudomonas*, and *Trichoderma* are well-known to mediate ISR in numerous plant systems, including tomato, pepper, and bean plants (26, 28, 36). Phylogenetic characterization based on 16S rRNA gene analysis revealed that a large fraction of these isolates from

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biochar-amended soils were closely related to previously described plant growth-promoting and/or biocontrol agents (24, 43). Recent studies have applied both traditional isolation techniques and culture-independent molecular techniques to examine the impact of both pre-Columbian (terra preta) and modern pyrolysis-generated biochar application on soil microbial community structure. These studies showed that biochar amendment was generally characterized by higher soil pH levels and an increase in the relative abundance of members of the *Actinobacteria* and *Bacteroidetes* phyla (14, 33, 45). Although these studies shed light on the influence of charcoal on bulk soil microbiota, they did not explore the impact of biochar amendment on root-associated bacteria that potentially are involved in both plant growth promotion and priming that is responsible for induced plant resistance. The objective of this study was to assess the effect of soil biochar amendment (3%, wt/wt) on the root-associated bacterial community composition of mature sweet pepper (*Capsicum annuum* L.) plants, specifically focusing on biochar-induced phyla. Given that only a negligible fraction of the soil microbial community can be cultured (40), a molecular approach combining microbial fingerprinting techniques (denaturing gradient gel electrophoresis [DGGE] and terminal restriction fragment length polymorphism [T-RFLP]) with high-throughput 16S rRNA gene pyrosequencing was employed.

MATERIALS AND METHODS

Overview of biochar characteristics and greenhouse experimental setup. The greenhouse experimental setup and physicochemical properties of the biochar were described in detail previously (18, 24). Briefly, biochar was prepared from citrus wood in a traditional charcoal kiln (lump charcoal). Accounting for ash content (10.9%), the elemental composition of the biochar was 70.6% C, 0.6% N, 2.3% H, and 15.5% O, giving an O/C atomic ratio of 0.16, H/C ratio of 0.40, C/N ratio of 131, and H/O ratio of 2.4 (24). Prepared biochar was ground into a powder of 0.1- to 1.0-mm particles and mixed with an organic matter (OM)-poor sandy soil (Besor region, western Negev, Israel; OM, 0.4%; 92% sand, 1.5% silt, 6.5% clay) (18). Sweet pepper cv. Maccabi plants (Hazera Genetics, Berorim, Israel) were obtained from a commercial nursery (Hishitil, Ashkelon, Israel) 40 to 50 days after the initial seeding stage and transplanted into 1-liter pots containing sandy soil without or with biochar (3%, wt/wt). Plants were fertilized as described before, allowing for 25 to 50% drainage (18). Plants were maintained at 20 to 30°C in a pest- and disease-free greenhouse for 3 months prior to bulk soil and root-associated microbial DNA extraction.

DNA extraction. DNA was extracted from roots and bulk soil of sweet pepper plants grown in potting mixtures amended with either 0 (control) or 3% (wt/wt) biochar. Plant roots from two separate pots for each treatment (four pots total) were carefully removed and shaken vigorously to remove loosely adhered soil particles. Three samples of 20 g of roots from each pot containing tightly adhering soil were suspended in 90 ml of sterile 0.85% (wt/vol) sodium chloride (NaCl) containing 10 g glass beads (2 mm diameter; Sigma-Aldrich, St. Louis, MO) and shaken for 30 min at 250 rpm at room temperature to free root-associated bacterial cells. Root tissue samples then were removed from the suspension, and samples were centrifuged for 15 min at $6,500 \times g$, after which the supernatant was decanted. The derived pellet was highly enriched in root-associated bacteria.

For DNA extraction, six glass beads (0.11 mm diameter) were added to 0.5 g of root-associated bacterium-enriched pellets or to bulk potting mixture samples, and the mixtures were subjected to two 45-s cycles of bead beating (FastPrep; Bio 101, Holbrook, NY) in DNA extraction buffer (MoBio Laboratories Inc., Carlsbad, CA). Following this stage, the DNA was further extracted and purified from the lysate using the PowerSoil DNA extraction kit (MoBio Laboratories Inc., Carlsbad, CA) according to the manufacturer's instructions. An additional washing step with 70% ethanol was performed to remove residual contaminants. Extracted DNA was visualized by electrophoresis in 1% agarose gels and quantified spectrophotometrically by NanoDrop (NanoDrop Technologies, Wilmington, DE). Finally, extracted DNA was diluted to 10 ng ml⁻¹ prior to PCR amplification.

PCR and DGGE. PCR amplification for DGGE was performed on control and biochar-amended root-associated DNA samples using 20 ng of extracted DNA as a template with the general bacterial primer pair 907R and 341F with a 40-bp GC clamp attached to its 5' end (44). PCR mixtures (final volume of 50 µl) contained the following components: 1.5 U *Taq* DNA polymerase (DreamTaq; Fermentas Life Science, Vilnius, Lithuania), *Taq* buffer containing a final magnesium concentration of 2.5 mM, deoxynucleoside triphosphates (dNTPs) (20 nmol each), 12.5 mg bovine serum albumin, and 25 pmol of each primer. The PCR program consisted of an initial denaturation step of 95°C for 180 s followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 30 s. Cycling was completed with a final elongation step of 72°C for 5 min. PCR amplicons were checked by agarose gel electrophoresis (1%) and staining with ethidium bromide.

DGGE was performed in 6% (wt/vol) acrylamide gels containing a linear urea-formamide gradient ranging from 20 to 70% denaturant (with 100% defined as 7 M urea and 40% [vol/vol] formamide). Gels were run for 17 h at 100 V in the Dcode Universal Mutation System (Bio-Rad Laboratories, Hercules, CA). DNA was visualized after staining with Gelstar (Invitrogen Corporation, Carlsbad, CA) by UV transillumination (302 nm) and photographed with a Kodak KDS digital camera (Kodak Co., New Haven, CT).

Cluster analysis of DGGE community fingerprints. DNA fingerprints obtained from the 16S rRNA gene banding patterns on the DGGE gels were digitized using Fingerprinting II Informatix software (Bio-Rad Laboratories). The lanes were normalized to contain equal amounts of total signal after background subtraction. Clustering was determined by the unweighted pair group with mathematical averages (UPGMA) method.

T-RFLP analyses. The heterogeneity of 16S rRNA gene sequences from duplicate control and biochar-amended bulk soil and root-associated DNA samples was analyzed by terminal restriction fragment length polymorphism (T-RFLP) (17). An approximately 600-bp fragment of the 16S rRNA gene was amplified by PCR using the bacterial primers 341F, labeled with 6-carboxyfluorescein (6-FAM) at the 5' end, and 907R. PCR amplification consisted of an initial denaturation step of 95°C for 180 s followed by 25 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 30 s, and elongation at 72°C for 180 s. Cycling was completed with a final elongation step of 72°C for 10 min (8). PCR products from triplicate reactions were pooled and digested with 25 U mung bean nuclease (New England BioLabs, Inc.) in reaction buffer at 30°C for 1.5 h to remove single-stranded fragments that may bias the analyses (17). Nuclease-treated PCR products were purified using the Intron PCR purification kit (iNtRON Biotechnology, Gyeonggi-do, South Korea) and quantified spectrophotometrically by NanoDrop. Approximately 2-mg aliquots of each pooled PCR product were digested for 8 h at 37°C with *MseI* or *MspI* (New England BioLabs, Ipswich, MA) according to instructions provided by the manufacturer and purified by phenol-chloroform (1:1, vol/vol) extraction followed by ethanol precipitation. Finally, the purified DNA pellet was resuspended in nuclease-free double-distilled water. The size of individual terminal fragments (T-RFs) was determined by using an ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster City, CA). The peaks in each profile were related to specific fragment lengths based on a size marker (70-500 MapMarker; BioVentures, Murfreesboro, TN).

Raw T-RFLP data were retrieved using Peak Scanner v1.0 (Applied Biosystems). The size (in base pairs) of each peak termed a T-RF was used to indicate an operational taxonomic unit (OTU), whereas the height of the peak was used to determine its relative abundance in the profile. True peaks (as opposed to background noise) were determined according to Abdo et al. (1) using T-rex (13) (<http://trex.biohpc.org>), and T-RF sizes were aligned to the nearest integer. In each sample, T-RF heights were normalized by the total height of T-RFs in that sample. The UPGMA clustering analysis based on Jaccard similarity then was performed using the PAleo-ecology STatistics (PAST) freeware package, version 2.03 (27) (<http://folk.uio.no/ohammer/past>). Multiresponse permutation procedures (MRPP) in PCORD 5.10 (MjM Software) were used for testing significant differences in T-RF population structure between the different experimental groups.

Pyrosequencing of bar-coded 16S rRNA amplicons. To assess the diversity and phylogenetic affiliation of the root-associated bacteria in the biochar-treated plants relative to the controls, triplicates of extracted DNA were pooled together, and bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) was performed on root-associated control and biochar-amended DNA samples in duplicate plants. The bTEFAP analyses were performed using universal eubacterial 16S rRNA gene primers 530F and 1100R as previously described (15, 16). The bTEFAP procedures were performed at the Research and Testing Laboratory (RTL, Lubbock, TX) using previously described protocols (51a).

FIG. 1. Effect of biochar on the bacterial community structure of bulk soil and roots from 3-month-old pepper plants. Shown is a cluster analysis of T-RFLP patterns generated by MseI digestion of partial 16S rRNA gene sequences. The dendrogram shows triplicate replicates of bulk soil and root-associated samples from duplicate pots. The UPGMA clustering analysis based on Jaccard similarity was performed using the PALEO-ecology STatistics freeware package PAST, version 2.03. Numbers (0 and 3) relate to the biochar concentration in the potting mixtures, root-associated samples were designated RA, and bulk soil samples were designated B; the numbers 1 and 2 represent the pot replicated for each treatment.

Pyrosequencing data analyses. Following sequencing, all failed sequence reads, sequences of less than 300 bp, low-quality sequence ends, and tags were removed, and sequences were depleted of any nonbacterial ribosome sequences and chimeras using Black Box Chimera Check custom software B2C2 (23) (Research and Testing Laboratory). The resulting high-throughput data sets were analyzed using Mothur (www.mothur.org) (49) as outlined at http://www.mothur.org/wiki/Costello_stool_analysis. The analysis involved the following steps, in brief. (i) A 50-bp-long moving window was slid across the reads, and its average Phred quality score was calculated; whenever mean quality dropped below 35, the read was trimmed to the end of the last window with a mean quality score higher than 35. In mock community experiments, P. D. Schloss (personal communication) found this strategy to decrease the error rate 10-fold compared to the rate after not trimming the sequences. In addition, we removed any sequence where the longest homopolymer (e.g., TTTT) was longer than 8 bases and if it contained an ambiguous base call (e.g., N'). (ii) Reads were aligned to the SILVA-compatible database of Mothur (www.mothur.org/w/images/9/98/Silva.bacteria.zip), containing nearly 15,000 sequences. (iii) Chimeras were removed using the native Mothur algorithm. (iv) Sequencing noise was reduced using a method based on that of Huse et al. (31). Since abundant sequences are likely to generate sequences that are less abundant and differ from the dominant sequence type by about 1 base every 100 bases, these noise sequences are merged with the abundant sequence type (31). The high-quality sequences were phylogenetically classified using the RDP-II Classifier (55) with a bootstrap confidence estimate threshold of 80%. The classifier, available at <http://sourceforge.net/projects/rdp-classifier>, assigns 16S rRNA gene sequences to the bacterial taxonomy of Garrity et al. (20); its classification is accurate, stable, independent of sequence alignments, and suitable for very large data sets (11, 39). Due to a lack of the normal distribution in the data, we used a nonparametric Mann-Whitney test to evaluate significant differences in abundances of phyla and genera as a result of biochar amendment at a confidence level of 95%.

16S rRNA gene amplicon pyrosequencing data accession number. The pyrosequencing-generated nucleotide sequences of the root-associated bacteria reported in this paper have been deposited in the NCBI Sequence Read Archive (SRA) database under project accession number SRA028937.1.

RESULTS AND DISCUSSION

The objective of this study was to assess the effect of biochar soil application on the structure and composition of bacterial communities in bulk soil and on the roots of 3-month-old sweet pepper plants. We focused on root-associated communities which most likely are involved in plant-microbial interactions. We hypothesize that the previously observed increase in plant growth and the induction of systemic resistance to plant disease (18, 24, 43) may be at least partially attributed to the proliferation of microbial elicitors that occurs as a result of biochar-stimulated shifts in the rhizosphere microbial community.

Molecular fingerprinting of bulk soil and root-associated bacterial communities. Culture-independent molecular fingerprinting analyses (T-RFLP and PCR-DGGE) of 16S rRNA gene fragments were performed to compare the community composition of root-associated and bulk soil bacteria in biochar-amended and nonamended (control) soils (Fig. 1). Hierarchical clustering of T-RFs digested with both MseI and MspI showed two distinct statistically significant (MRPP tests; $A > 0.09$; $P < 0.01$) root-associated and bulk soil clusters, demonstrating a strong rhizosphere effect on the bacterial community composition. Because the hierarchical clustering of the MseI- and MspI-digested T-RFLP patterns were very similar to each other, Fig. 1 shows the cluster analysis using MspI. Differentiation between microbial community composition in bulk soil and the rhizosphere is well documented in the literature and is attributed to carbon-rich root exudates, which can

FIG. 2. Effect of biochar on the bacterial community structure of bulk soil and roots from 3-month-old pepper plants. Shown is a cluster analysis of root-associated bacterial 16S rRNA gene DGGE ribotypes analyzed and clustered using the Fingerprint II software. The UPGMA tree is based on a Pearson correlation UPGMA matrix between the different DGGE patterns of different samples.

account for as much as 30% of a plant's fixed carbon (38). Bulk soil and root-associated T-RF clusters each were characterized by the presence of separate, statistically significant (MRPP tests; $A > 0.06$; $P < 0.01$) subclusters representing biochar-amended and control (nonamended) samples (Fig. 1). This was supported by UPGMA clustering analyses of 16S rRNA gene fragments using PCR-DGGE (Fig. 2), where root-associated bacterial communities also formed two distinct clusters depending on the biochar application. Collectively these techniques demonstrated that biochar treatment alters the bacterial community structures of both the rhizosphere and the bulk soil.

Pyrosequencing of bar-coded 16S rRNA amplicons. The pyrosequencing of bar-coded 16S rRNA amplicons is increasingly used for the comprehensive analysis of bacterial community composition in heterogeneous environments, including soil and the rhizosphere (2, 40, 47). We applied bTEFAP (bacterial tag-encoded FLX amplicon pyrosequencing) to evaluate root-associated bacterial community composition in biochar-amended and control plants, with the objective of identifying specific biochar-induced phyla potentially involved in induced plant resistance and plant growth promotion. Bacterial tag-encoded FLX amplicon pyrosequencing analysis was performed following the PCR amplification of root-associated DNA from duplicate 0 and 3% biochar-amended pots as described in Materials and Methods. A total of 20,142 OTUs were generated, with an average of 5,035 sequences per sample. A total of 1,134 and 2,611 OTUs for the control duplicates and 1,899 and 996 OTUs for the biochar-amended duplicates remained following quality control and filtering using the criteria detailed in Materials and Methods. These high-quality sequences were classified to the genus level and used for further phylogenetic analyses. The overwhelming majority of control (95.7%) and biochar-amended (92.5%) root-associated bacteria were associated with four primary phyla, *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, and *Firmicutes*, which represented $72\% \pm 7.4\%$ and $47\% \pm 2.5\%$, $12\% \pm 1.0\%$ and $30\% \pm 5.7\%$, $6.6\% \pm 3.8\%$ and $10.4\% \pm 2.1\%$, and $5.9\% \pm 2.9\%$ and $5.1\% \pm 0.2\%$ of the total classified phyla in the control and biochar-amended samples, respectively (Fig. 3). Approximately 75% of the sequences from both the control

FIG. 3. Distribution of root-associated bacterial phyla detected by the pyrosequencing of bar-coded 16S rRNA gene amplicons. Bars show taxonomic assignments of 16S rRNA sequences that could be classified to the phylum level using the RDP-II Classifier tool with an 80% confidence level. Relative fractions of the most abundant phyla are indicated. Asterisks indicate significant differences in the relative abundance of groups in the biochar versus control samples using the Mann-Whitney test at a confidence level of 95%.

and the biochar-amended samples belonged to the *Proteobacteria* and *Bacteroidetes* phyla, similarly to community trends documented in other previously described soil environments (4, 40, 47). The relative abundance of the *Bacteroidetes* phylum was substantially higher in the biochar-amended root-associated community, whereas the relative abundance of the *Proteobacteria* phylum was much greater in the control samples (12 versus 30% and 72 versus 48% for the control and biochar samples, respectively) (Fig. 3). Traditional isolation techniques conducted in this study using the selective medium HA-Agar (29) (data not shown) and previously conducted studies using both molecular and culture-dependent techniques (33, 43, 45) have indicated that the relative abundance of *Actinobacteria* is induced in biochar-amended soils. Nonetheless, pyrosequencing analysis did not show significantly higher abundance of this phylum in the biochar samples (Fig. 3).

Although no significant differences were observed in the levels of genus richness between the biochar-amended and control samples (averages of 195 and 172 unique genera for duplicate samples, respectively), the composition of selected genera significantly differed between the two treatments. Comparative analysis of pyrosequencing data identified several genera with significantly different levels of abundance in the control and biochar-amended samples (Fig. 4).

Flavobacterium appeared to be the genus most significantly affected by biochar. The relative abundance of this group was $4.2\% \pm 2.9\%$ of the total genus-defined root-associated OTUs in control samples and it was $19.6\% \pm 4.5\%$ in the 3% biochar-amended samples (Fig. 4), explaining to a great extent the substantially higher levels of *Bacteroidetes* detailed in Fig. 3. Multiple bacterial community characterization techniques, including classical plate isolation (21), 16S rRNA gene fragment clone library screening (34), and pyrosequencing (3, 40), have demonstrated the substantial abundance of *Flavobacterium*

FIG. 4. Relative abundance of root-associated bacterial genera in the control and biochar-amended soils identified using the pyrosequencing of bar-coded 16S rRNA gene amplicons. Asterisks indicate significant differences in the relative abundance of groups in the biochar versus control samples using the Mann-Whitney test at a confidence level of 94.2% (*), 95% (**), and 99% (***).

strains in plant root environments, which in some cases may exceed 5% of the total bacterial population.

Members of the *Flavobacterium* genus are widely distributed in nature, where they play a role in mineralizing various types of organic matter (carbohydrates, amino acids, proteins, and polysaccharides). They often possess an arsenal of extracellular enzymes, such as proteinases and chitinases, which enable them to degrade bacteria, fungi, insects, and nematode constituents (9), and often they produce secondary metabolites, including a wide range of antibiotics (12, 42). Most *Flavobacterium* species are characterized by gliding motility that is facilitated by a novel unique secretion system (48) that enables rapid movement of up to 5 mm s⁻¹ on solid surfaces (32). Interestingly, this secretion system also is responsible for the transport of extracellular chitinase. Certain *Flavobacterium* isolates have been shown to have biocontrol capabilities. For example, selected *Flavobacterium* isolates from sunflowers, apples, and bananas were highly antagonistic toward the soil-borne fungal pathogens *Sclerotium rolfii*, *Lasiodiplodia theobromae*, *Colletotrichum musae*, and *Phytophthora cactorum*, which can infect a wide range of agricultural and horticultural crops (5, 25, 30). In addition, some flavobacterial strains possess ISR-inducing capacities, potentially attributed to oligomeric elicitors generated from macromolecule degradation (6, 7). We hypothesize that the gliding motility, wide spectrum of extracellular enzymes, and antibiotic production capacity enhances the rhizosphere competence of these flavobacteria, potentially explaining their ubiquitous presence on plant roots and their potential role in induced plant growth and disease resistance. Nonetheless, it still is not clear why the relative abundance of this genus increased so significantly on the biochar-amended roots.

Besides *Flavobacterium* strains, which are well-known for their ability to rapidly digest insoluble chitin (42), we also observed the significant induction of other hydrolytic enzyme-

producing genera, such as *Chitinophaga* (*Bacteroidetes*) and *Cellvibrio* (*Betaproteobacteria*). The relative abundance of these genera was 0.05% ± 0.06% of total root-associated OTUs in control samples versus 0.5% ± 0.3% in the biochar-amended samples and 0.06% ± 0.03% in control samples versus 1.6 ± 0.2% in biochar-amended samples, respectively (Fig. 4). We suggest that these biopolymer-degrading bacteria mineralize chitin in the outer shells and cell walls of rhizosphere-associated arthropods and fungi (35). Oligomeric products of chitin degradation are well-known elicitors of ISR (46), and this may partially explain the induced resistance observed in the biochar-amended experiments (18). However, it currently is not clear why these chitin-degrading genera were induced in the biochar-amended samples.

Additional biochar-stimulated genera not affiliated with plant growth stimulation or induced plant resistance in the literature included *Hydrogenophaga* and *Dechloromonas*, whose relative abundance was 0.2% ± 0.02% in control samples versus 0.7% ± 0.16% in the biochar-amended samples and 0.06% ± 0.08% in control samples versus 2.2% ± 1.6% in the biochar-amended sample, respectively (Fig. 4). *Hydrogenophaga* spp. were shown to dominate biphenyl catabolism in a horseradish (*Armoracia rusticana*) rhizosphere contaminated with polychlorinated biphenyls (PCBs), which are naturally present in coal tar, crude oil, and natural gas (53). In addition, *Hydrogenophaga* spp. can utilize the aromatic contaminant 4-aminobenzenesulfonate (4-ABS) as the sole carbon, nitrogen, and sulfur source under aerobic conditions (19). *Dechloromonas* spp. are found in soil environments, where they can oxidize toluene, benzoate, and chlorobenzoate, generally with no detrimental effects on adjacent plants (10). Previously, the chemical analyses of the biochar used in this study revealed that it is enriched with an array of aromatic compounds, such as phenol, methyl-phenol, and dihydroxybenzenes (24). This may explain the enrichment of these aromatic compound degraders in the biochar-amended samples.

Biochar amendment appeared to be antagonistic toward the *Pseudoxanthomonas* genus (*Gammaproteobacteria*), where the average number of characterized OTUs was 1.6% ± 0.5% versus 7.3% ± 7.0% of the total characterized genera in the control samples (Fig. 4). Several *Pseudoxanthomonas* species are known opportunistic plant pathogens that are able to attack a diverse array of economically important crops (52).

Conclusions. This study shows a clear shift in the root-associated microbial community structure of sweet pepper plants grown in biochar-amended soil, characterized by a substantial induction of several chitin- and aromatic compound-degrading genera. We suggest that physical and chemical factors (biochar-associated organic compounds) collectively are responsible for the observed community shift and that induced bacterial communities are at least partially responsible for the induced growth and plant resistance phenomena observed in previously described experiments (18, 24). Current research is focusing on assessing the influence of specific biochar components (the biochar carbon skeleton, particle size, specific biochar-associated organic fractions, etc.) on the bacterial community composition in correlation with the observed plant physiology (in-

duced disease resistance and growth promotion) and on assessing the potential role of biochar-amended soil-associated flavobacterial isolates in plant growth and disease resistance.

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