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16S rRNA Metagenomic Analysis of the Bacterial Community Associated with Turf Grass Seeds from Low Moisture and High Moisture Climates

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Abstract

Turfgrass investigators observed that plantings of grass seeds produced in moist climates produce seedling stands that show greater stand evenness with reduced disease compared to those grown from seeds produced in dry climates. In this study, we analyzed bacterial communities on/in seeds of three turf species from low moisture (LM) and high moisture (HM) climates. In result, climate appeared to structure the bacterial communities on/in seeds. LM seeds vectored mainly Proteobacteria (89%). HM seeds vectored a denser and more diverse bacterial community that included Proteobacteria (50 %) and Bacteroides (39%). Also, phyla Actinobacteria and Firmicutes took similar percentages on/in both LM and HM seeds. The greater diversity and density of seed vectored microbes in high moisture climates may benefit seedlings by helping them tolerate stress and fight disease organisms. However, this dense microbial community may also compete with seedlings for nutrients, slowing or modulating seed germination and seedling growth.

Introduction

All plants bear numerous microbes that may influence nutrition, development and stress responses of their hosts. Plant-associated microbes can either come from the surrounding environment, especially soil, or be transmitted via seeds. Environmental factors, such as pH and carbon/nitrogen ratio, may affect the structure of the microbial communities in soils, which further influences the structure of plant-associated microbes. However, little is known about the factors affecting seed microbes. As a source of plant-associated microbes, seed-vectored bacteria share a mutualistic association with the host seedlings. They played important roles in the development of host seedlings, impacting germination rates and seedling growth.

In this study, we investigated the bacterial community associated with cool-season turfgrass seeds produced in low moisture (LM) and high moisture (HM) climates. We also evaluated the potential influence of the bacterial community on seed germination rates and seedling growth rates.

Methodology

Seeds of twenty-seven cool-season turf cultivars were obtained from DLF Pickseed USA and used in this study. Based on the precipitation data collected from The National Oceanic and Atmospheric Administration (NOAA), the seeds were classified into LM seeds (annual precipitation < 750 mm) and HM seeds (annual precipitation >750 mm). 100mg of seeds of each turf cultivar were weighed out and washed with water for 3 times, 30 seconds each time to remove the dirt. The DNA extraction was conducted with DNeasy® PowerSoil® Kit following the manufacturer's instructions. The concentration of extracted DNA was measured and normalized to 5ng/μl for the library preparation. By using 12.5ng of the normalized DNA from turf seeds as the template, V3-V4 hypervariable regions of bacterial 16S rRNA gene were amplified to represent the bacterial community on/in turf seeds.

The analysis process is shown in Fig. 1.

Results

In total, 7,405,226 sequences were generated by Illumina MiSeq and analyzed with QIIME2 package. After being denoised and dereplicated with *dada2*, the remaining high-quality sequences were clustered into 310 OTUs. After the removal of mitochondrial and chloroplast genomes, 247 OTUs were used to represent the bacterial profile of turf seeds samples.

The bacterial community on/in turf seeds was composed of 5 phyla, 8 classes, 21 orders, 37 families and 69 genera. The bacterial community on/in HM seeds covered all discovered taxonomies, with 6644 sequences/sample. However, LM seeds only hosted part of them, 4 phyla, 8 classes, 9 orders, 10 families and 15 genera, with 2821 sequences/sample.

Regardless of the climate and turf species, bacterial communities at phylum level were dominated by Proteobacteria (51%) and Bacteroidetes (40%). Proteobacteria took 89% and 50% of the bacterial community on HM and LM climates seeds, respectively. Bacteroidetes was abundant in HM seeds (39%) but not LM seeds (2%). Actinobacteria (6%) and Firmicutes (3%) also comprised a portion of the bacterial community and exhibited no significant difference between the two climate types.

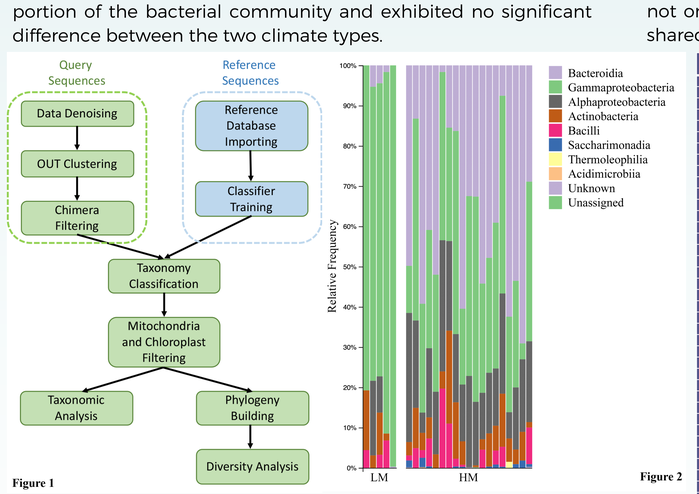

Figure 1

Figure 2

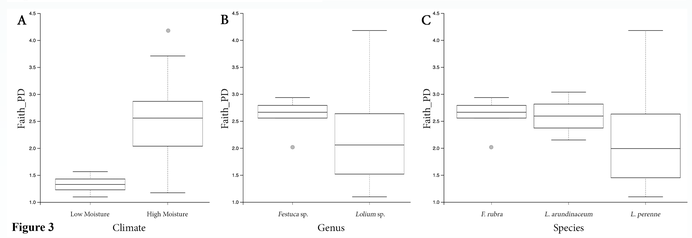

Figure 3

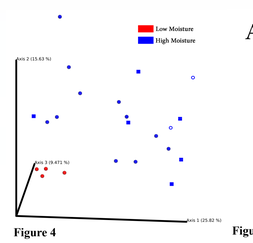

Figure 4

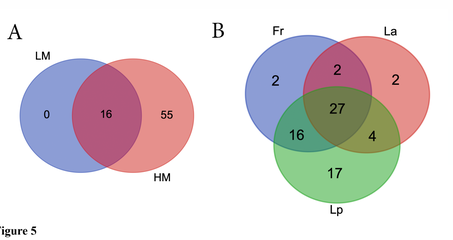

Figure 5

Results

Both HM and LM seeds shared some of the most abundant bacterial classes, i.e. Actinobacteria, Bacteroidia, Bacilli, Alphaproteobacteria and Gammaproteobacteria (Fig. 2). Compared to LM seeds, seeds from HM environment was richer in terms of Faith's phylogenetic diversity (Fig. 3).

At genus level, LM seeds harbored a significantly higher percentage of *Massilia*, *Pantoea*, and *Pseudomonas* compared to HM seeds (Table 1). In contrast, HM seeds harbored more of *Flavobacterium*, *Chryseobacterium*, *Pedobacter*, *Sphingomonas* and *Erwinia* (Table 1).

In the PCoA plot (Fig. 4), bacterial communities associated with turf seeds produced at LM conditions separated well from those in HM conditions. Also, the PERMANOVA test showed a significant difference between the two. However, no significant correlation was detected between different species of turf seeds and their bacterial profile.

The Venn diagram showed that LM seeds vectored no genus that HM did not. However, HM seeds harbored 55 genera that were not on/in LM seeds (Fig. 5A). Also, seeds of the three turf species shared 27 genera regardless of the climate (Fig. 5B).

Taxa level	Taxa name	Ave. Percentage		
		LM	HM	Combined
Phylum	Actinobacteria **	6%	7%	6%
	Bacteroidetes †	2%	39%	40%
	Firmicutes **	3%	4%	3%
	Patescibacteria	—	<1%	<1%
	Proteobacteria †	89%	50%	51%
Class	Actinobacteria **	6%	6 %	6%
	Bacteroidia	2%	39%	40%
	Bacilli **	3%	4%	3%
	Saccharimonadia	—	<1%	<1%
	Alphaproteobacteria †	6%	18%	15%
	Gammaproteobacteria †	83%	32%	36%
Order	Micrococcales **	6%	5%	5%
	Cytophagales	<1%	2%	2%
	Flavobacteriales †	<1%	11%	9%
	Sphingobacteriales †	1%	26%	21%
	Bacillales **	3%	4%	4%
	Saccharimonadales	—	<1%	<1%
	Rhizobiales	—	3%	2%
	Sphingomonadales †	6%	15%	13%
	Betaproteobacteriales †	30%	13%	17%
	Enterobacteriales †	24%	12%	15%
	Pseudomonadales †	29%	6%	11%
Family	Microbacteriaceae **	6%	5%	5%
	Hymenobacteraceae	<1%	1%	1%
	Flavobacteriaceae †	<1%	3%	3%
	Weeksellaceae †	<1%	7%	6%
	Sphingobacteriaceae †	1%	26%	21%
	Paenibacillaceae **	3%	4%	4%
	Rhizobiaceae †	—	2%	1%
	Sphingomonadaceae †	6%	15%	13%
	Burkholderiaceae †	30%	13%	17%
	Enterobacteriaceae †	24%	12%	15%
	Pseudomonadaceae †	29%	6%	11%
Genus	Curtobacterium **	2%	2%	2%
	Hymenobacter	<1%	1%	1%
	Flavobacterium †	<1%	3%	3%
	Chryseobacterium †	<1%	7%	6%
	Mucilaginibacter	—	<1%	<1%
	Pedobacter †	1%	25%	20%
	Paenibacillus **	3%	4%	4%
	Rhizobium*	—	2%	1%
	Sphingomonas †	6%	15%	13%
	Duganella †	5 %	3%	3%
	Massilia †	25%	9%	12%
	Erwinia †	<1%	3%	3%
	Pantoea †	23%	9%	12%
	Pseudomonas †	29%	6%	11%
Unassigned		<1%	<1%	<1%

Table. Composition of bacterial community from LM and HM climate seeds.

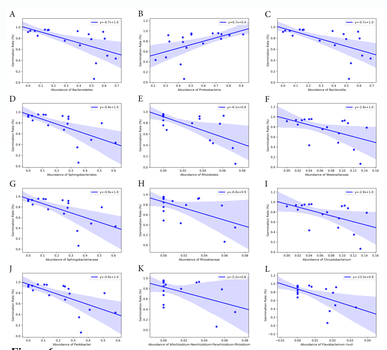

Figure 6

Figure 1. Graphical workflow of metagenomic analysis in our study.

Figure 2. Bar plot analysis illustrating the relative abundance and distribution of the OTUs assigned to class-level taxonomy. LM: low moisture; HM: high moisture.

Figure 3. Box plots depicting the Faith's Phylogenetic Diversity for different climate conditions (A), different genera (B), and different species (C).

Figure 4. PCoA Emperor plots based on Bray-Cuitis diversity matrix. Samples are scattered concerning their bacterial community. Species were represented by different shapes: ring – Loium arudinacea; sphere – Lolium perenne; square – Festuca rubra.

Figure 5. Venn diagrams showing the number of shared bacterial genera between different climate (A) and among different turf species (B). LM: low moisture; HM: high moisture; Fr: Festuca rubra; Lp: Lolium perenne; La: Loium arudinacea.

Figure 6. Correlation of seed germination rate with abundance of bacteria groups at different taxonomy levels. Phylum: A and B; Class: C; Order: D and E; Family: F – H; Genus: I – K; Species: L.

Results

Bacterial groups at different taxonomic ranks showed correlation with the seed germination rate and time (Fig. 6 & Fig. 7).

Proteobacteria exhibited a positive correlation with germination rate but a negative correlation with germination time. Phylum Actinobacteria also showed a negative correlation with the germination time but not a significant correlation with germination rate. Firmicutes showed some correlation with germination rate and germination time, but not significantly.

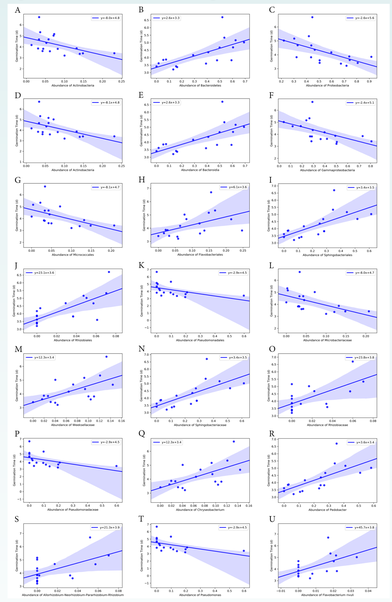

Figure 7. Correlation of average seed germination time with abundance of bacteria groups at different taxonomy levels. Phylum: A – C; Class: D – F; Order: G – K; Family: L – P; Genus: Q – T; Species: U.

Conclusion

We surveyed the bacterial community associated with seeds of several species of cool-season turfgrasses and identified the dominant bacterial groups of the communities at different taxonomic levels. Regardless of the moisture level during seed production and species of seeds, the core bacterial community in this study at genus level was composed of Pedobacter, Sphingomonas, Massilia, Pantoea, and Pseudomonas, most of which include many PGPB strains. Seeds produced in high moisture conditions maintained a more diverse and denser bacterial community compared to the seeds produced in low moisture conditions. The greater diversity and density of seed vectored microbes in high moisture climates may benefit seedlings by helping them tolerate stress but this dense microbial community may compete with seedlings for nutrients and slow early seedling growth.

Acknowledgements

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