Diversity of the spinach (*Spinacia oleracea*) spermosphere and phyllosphere bacterial communities

Gabriela Lopez-Velasco[1], Phyllis A. Carder[1,2], Gregory E. Welbaum[2] & Monica A. Ponder[1]

[1]Department of Food Science and Technology, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA; and [2]Department of Horticulture, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA

Abstract

The bacterial diversity of seeds, transmission of bacteria from seed to phyllosphere, and fate of seed-transmitted bacteria on mature plants are poorly characterized. Understanding the dynamics of microbial communities is important for finding bio-control or mitigation strategies for human and plant pathogens. Bacterial populations colonizing spermosphere and phyllosphere of spinach (*Spinacia oleracea*) seedlings and plants were characterized using pyrosequencing of 16S rRNA gene amplicons. Spinach seed microbiota was composed of three bacterial phyla: *Proteobacteria*, *Firmicutes* and *Actinobacteria*, belonging to > 250 different operational taxonomic units (OTUs). Seed and cotyledon bacterial communities were similar in richness and diversity. Richness of 3–4 leaf-stage of development plants increased markedly to > 850 OTUs classified within 11 phyla. Although some bacterial OTUs were detected on seeds, cotyledons and plants, the breadth of new sequences indicates the importance of multiple sources outside the seed in shaping phyllosphere community. Most classified sequences were from previously undescribed taxa, highlighting the benefits of pyrosequencing in describing seed diversity and phyllosphere bacterial communities. Bacterial community richness increased from 250 different OTUs for spinach seeds and cotyledons, to 800 OTUs for seedlings. To our knowledge this is the first comprehensive characterization of the spinach microbiome, complementing previous culture-based and clone library studies.

Introduction

Microbial communities populate plants at all stages of development (Andrews & Harris, 2000). Seeds can harbor significant levels of culturable microorganisms; typically 10^3–10^5 CFU g^{-1} for alfalfa (*Medicago sativa* L.), mung bean (*Vigna radiata* L. R. Wilczek) and onion (*Allium cepa* L.), and as many as 10^7 CFU g^{-1} on rice (*Oryza sativa* L.) seeds (Andrews *et al.*, 1979; Piernas & Guirad, 1997). Seed colonizing microorganisms may be benign, beneficial or pathogenic for humans (e.g. Shiga toxin-producing *Escherichia coli*) or plants (e.g. *Cladosporium*) (Hernandez-Perez & Du Toit, 2006; Rosenblueth & Martínez-Romero, 2006; EFSA, 2011). *Rhizobia* and methylotrophs are frequently associated with seeds of soybean (*Glycine max* L.) (Holland *et al.*, 1992) and bean (*Phaseolus vulgaris*) (Rosenblueth & Martínez-Romero, 2006; López-López *et al.*,

2010). The seed coat, embryo and endosperm of diverse crops such as alfalfa (Charkowski *et al.*, 2002), Norway spruce (*Picea abies* L. H. Karst) (Cankar *et al.*, 2005), cereals and cucurbits (Mundt & Hinkle, 1976) yield culturable bacteria. Vertical transmission of bacteria from seed to germinated seedlings is reported in various species of *Eucalyptus* (Ferreira *et al.*, 2008), strawberry (*Fragaria × ananassa* Duchesne) (Kukkurainen *et al.*, 2005), beans (López-López *et al.*, 2010), rice (Cottyn *et al.*, 2009), Norway spruce (Cankar *et al.*, 2005) and tomato (*Lycopersicon esculentum* L.) (Guo *et al.*, 2002).

The aerial surfaces of plants, particularly the leaves, support diverse microbial populations (Whipps *et al.*, 2008) that are affected by factors such as leaf age (Brandl & Amundson, 2008), moisture levels (Ercolani, 1991), the presence of various organic compounds that may be used as nutrients by microbes (Ruppel *et al.*, 2008), and

adverse factors such as UV radiation (Jacobs *et al.*, 2005). Recent studies utilizing culture-independent techniques revealed that microbial diversity of the phyllosphere is much greater than previously described with culture-dependent analyses (Yang *et al.*, 2001; Lambais *et al.*, 2006; Rastogi *et al.*, 2012). The role that seed microbiota play in the establishment of microbial communities on leaves at different stages of plant development is largely unexplored. Increased understanding of the bacteria commonly associated with all stages of plant maturation could aid the development of seed treatments and other biocontrol strategies using native microbes to enhance plant growth and suppress pathogens.

Culture-independent approaches have changed our understanding of the structure and diversity of microbial communities populating the phyllosphere, as well as giving new insight into the interactions of these microbes with plants and the environment (Whipps *et al.*, 2008). Pyrosequencing of the 16S rRNA gene allows simultaneous sequencing of up to 300 000 gene fragments without prior cloning (Ronaghi & Elahi, 2002; Liu *et al.*, 2007), enabling broader representation of community membership. The method was used to characterize microbial communities from deep sea environments (Sogin *et al.*, 2006; Huber *et al.*, 2007), intestinal tracts of humans and animals (Dowd *et al.*, 2008), soils (Roesch *et al.*, 2007; Urich *et al.*, 2008), fermented foods (Humblot & Guyot, 2009), rhizosphere (Jesus *et al.*, 2010) and the phyllosphere (Delmotte *et al.*, 2009; Lopez-Velasco *et al.*, 2011; Rastogi *et al.*, 2012). In this study, 454-pyrosequencing of 16S rRNA gene amplicons was used to compare the composition and richness of bacteria associated with the spermosphere and phyllosphere of spinach (*Spinacia oleracea*) 'Menorca' seeds, cotyledons and plant leaves.

Methods

Seeds for a savoy-leaf cultivar of spinach (Menorca; Seed-Way® LLC., Hall, NY) with an 86% germination rate were obtained and handled aseptically. Immediately upon opening the packet of seeds, 10 g (960–970 seeds) of the whole seed was removed and the remaining seeds reserved for planting. Seeds were ground using a sterile mortar and pestle for subsequent microbial DNA extraction. Seeds were not surface-disinfected prior to analysis in order to assess seed coat-associated bacteria. The remaining seeds were planted in 10.2 cm wide × 12.7 cm deep square plastic pots filled with a soilless potting medium (Metro-Mix 852; Sun Gro® Horticulture Ltd, Canada) and incubated in an ethanol disinfected growth chamber (model E-54B; Percival Scientific Inc., Boone, IO) at a constant temperature of 21 °C with a 12-h photoperiod. Light intensity

was 850 μmol photons m^{-2} s^{-1} measured 30 cm from the lamps using a PAR quantum sensor (Li-Cor LI-190 Quantum Sensor; LI-COR Biosciences, Lincoln, NE). Tap water was applied to the growing media beneath each plant as needed to maintain constant growth and to prevent moisture stress. Cotyledons or leaves were removed from randomly chosen plants by cutting at the base of the petioles using ethanol-sterilized scissors to achieve a combined weight of 10 g, an amount in previous experiments that provided adequate bacterial DNA for community analysis using denaturing gradient gel electrophoresis (DGGE; Carder, 2010). Cotyledons were harvested 35 days after planting, and leaves from the 3 to 4 leaf stage of development plants were harvested 60 days after planting. All leaves were stored at 4 °C and processed within 4 h after harvest.

Seeds, cotyledons or leaves were aseptically transferred into lab blender bags (Fisher Scientific, Atlanta, GA) containing 90 mL of 1% (w/v) sterile peptone water (Sigma-Aldrich Co., St. Louis, MO) supplemented with 1% (v/v) Tween-90 (PTW) (Fisher). Samples were stomached in a Bag Mixer lab blender (Interscience Laboratories, Rockland, MA) for 5 min to detach microbiota. Bacterial cells were collected from the suspension by centrifugation at 4000 *g* for 20 min at 4 °C, washed with 1 × phosphate-buffered saline, and resuspended in 100 μL of 1 × Tris-EDTA (TE) buffer. The bacterial cells were lysed by incubation with 300 μg of lysozyme (Fisher), 10 units of mutanolysin (Fisher) and 25 μg of achromopeptidase (Sigma-Aldrich) at 37 °C for 30 min. Lysates were treated with 25 units of proteinase K (Fisher) and incubated at 65 °C for 30 min. DNA was extracted from the lysed cells of each sample using the ZR soil microbe DNA kit™ (Zymo Research Co., Orange, CA) per manufacturer's instructions. Each extraction was performed in triplicate. A 270–300 bp nucleotide sequence of the V4 region of the 16S rRNA gene was amplified with primers used by Jesus *et al.* (2010) and Lopez-Velasco *et al.* (2011). Amplicons were generated as previously described by Lopez-Velasco *et al.* (2011). Libraries were prepared, enrichments titrated, and pyrosequencing performed using an LR70 sequencing kit and 70 × 75 PicoTiter-Plates performed with a Genome Sequencer FLX System (Roche, Branford, CT) by the core laboratory facility at the Virginia Bioinformatics Institute (Blacksburg, VA). Reads obtained from GS-FLX were preprocessed to identify sequencing errors and trimmed of linker sequences. Unique sequence taxonomic classification and operational taxonomic unit (OTU) assignment were performed using the Pyrosequencing pipeline of the Ribosomal Database Project (http://pyro.cme.msu.edu/) software tools (Cole *et al.*, 2009). Rarefaction indexes were calculated with 3% dissimilarity (http://pyro.cme.msu.edu/). OTU assignments,

estimates of richness (Chao1), and diversity (Shannon index [H']) were calculated at 3% dissimilarity. Evenness was calculated as $E = H'/H_{max}$; $H_{max} = \ln(\text{Chao1})$, where S is the total number of species in the sample, estimated with Chao1. Relative bacterial phylum abundance was calculated based on the total number of classified reads for each sample using the RDP classifier tool. Matches with an RDP confidence estimate below 60% were designated as unclassified bacteria (Wang *et al.*, 2007; Simon *et al.*, 2009). All sequences were deposited in the GenBank Sequence Read Archive (accession number SRP002353 http://www.ncbi.nlm.nih.gov/Traces/sra/sra.cgi).

Results

Pyrosequencing of the 16S rRNA gene yielded over 17 000 sequences per library. The numbers of unique sequences identified were greatest on the phyllosphere at the 3–4 leaf-stage plants (13 750) followed by cotyledons (6677), and seeds (6017) (Table 1, Fig. 1). These bacterial sequences represented members of 122 different OTUs, in this case different families and or genera. The majority of sequences were classified as bacteria; however, 85% of seed sequences and 65% of cotyledon sequences could not be further classified. In contrast, a much larger number of sequences (69%) from the leaves of 3–4 leaf growth stage plants were classified to family level or higher. Bacterial richness, as indicated by the Chao estimator, and diversity, as indicated by Shannon diversity index, were comparable for seeds and cotyledons. A marked increase in richness and diversity of phyllosphere bacteria was seen at the 3–4 leaf stage of development compared with seeds and cotyledons (Table 2). Seed-associated bacteria belonged to only three phyla: *Actinobacteria*, *Firmicutes* and *Proteobacteria*, and were classified into 16 families or genera (Table 3, Fig. 2). Three families or genera were exclusively detected in seeds and not in the other two stages of development (Table 3). Members of five bacterial phyla were detected on cotyledons, and bacteria belonging to 11 phyla were present on 3–4 leaf stage of development leaves (Table 3, Fig. 2). *Pro-*

teobacteria dominated bacterial communities at all three developmental stages, with the highest relative abundance of *Gammaproteobacteria* (Fig. 2). The relative abundance of *Firmicutes* declined post germination, with only decreases in abundance of the families *Staphylococcaceae* (0.5% and 0.03%), and *Bacillaceae* (1% and 0.15%) detected on cotyledons and the phyllosphere of 3–4 leaf stage of development plants, respectively. Bacterial 16S rRNA gene sequences belonging to the families *Enterobacteriaceae* and *Pseudomonadaceae* were present on seeds, cotyledons and leaves harvested at the 3–4 leaf growth stage, where they dominated the bacterial populations (Table 3). This study needs to be further expanded to demonstrate statistical difference among the relative abundance of each group and thus establish a quantitative comparison.

Discussion

Numerous studies report the presence of seed microorganisms but few have examined their presence of these microbes post germination (Mundt & Hinkle, 1976; Cankar *et al.*, 2005; Kukkurainen *et al.*, 2005; López-López *et al.*, 2010). In this study, pyrosequencing of 16s rRNA gene amplicons was used to examine the bacterial communities of spinach seeds and aerial tissues of spinach after germination (cotyledons) and plants (3–4 leaf stage). The large number of low-quality reads detected on seeds and cotyledons may reflect the presence of dead microorganisms, which contributed intact but poor quality DNA that was transferred from the seed coat to the cotyledons.

Bacterial community richness increased from *c.* 250 different OTUs for spinach seeds and cotyledons, to 800 OTUs for seedlings. The bacteria associated with the seeds belonged to only three phyla: *Actinobacteria*, *Firmicutes* and *Proteobacteria*, and were classified into 16 families or genera (Table 3, Fig. 2). Bacterial taxa such as *Enterobacteriaceae* (53.6% relative abundance), *Staphylococcaceae* (14.57%), *Pseudomonadaceae* (13.1%), *Bacillaceae* (6.4%) and *Rhizobiaceae* (3.7%) are commonly isolated from multiple seed species using culture-based studies, indicating that these are the dominant members of the seed bacterial community (Mundt & Hinkle, 1976; Rosenblueth & Martínez-Romero, 2006; Weiss *et al.*, 2007).

Bacterial richness, as indicated by the Chao estimator, and diversity, as indicated by the Shannon diversity index, were comparable for seeds and cotyledons; however, a marked increase in richness and diversity of phyllosphere bacteria was seen on 3–4 leaf stage of development plants compared with seeds and cotyledons (Table 2). *Proteobacteria* dominated bacterial communities at all three developmental stages, with the highest relative abundance of *Gammaproteobacteria* (Table 3). The relative abundance of *Firmicutes*, particularly OTUs

Table 1. Numbers of reads, unique sequences and trimmed reads for the spinach microbiome at different stages of development (seed, cotyledons and 3–4 leaf stage of development plants)

	Total reads	Sequences trimmed*	Unique	% of classified sequences[†]
Seed	17 341	2541	6017	34.93
Cotyledons	35 503	10 303	6677	15.08
Plant leaves	34 807	5849	13 750	69.26

*Reads that were trimmed due to low quality (size and sequence).
[†]Sequence reads that were classified according to the RDP database (cutoff 60%).
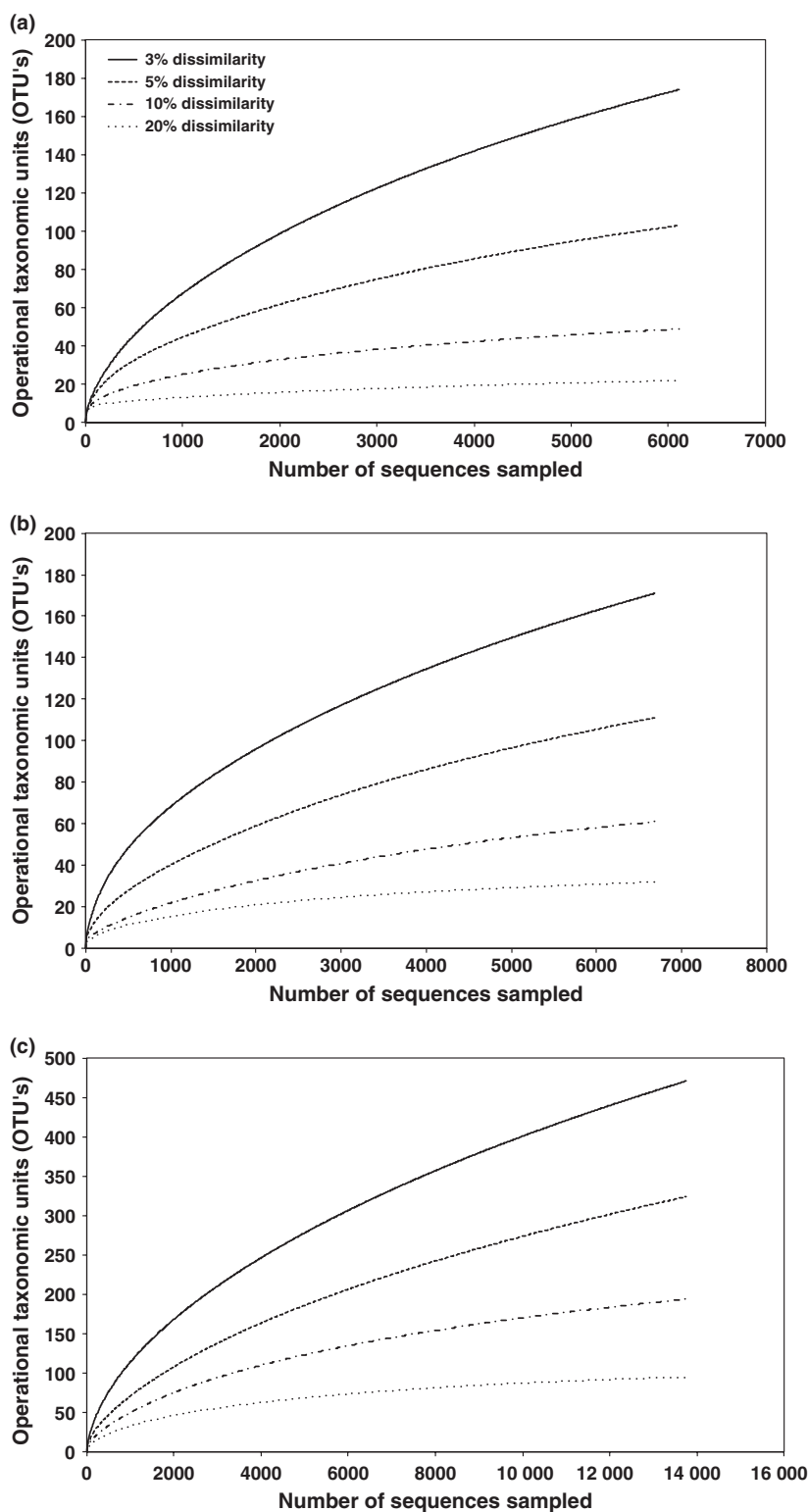
Fig. 1. Rarefaction curves for seed (a), cotyledon (b) and leaves of 3–4 leaf stage of development plants (c) indicating the observed number of OTU's within the 16S rRNA gene microbiomes at different levels of dissimilarity.

classified in the families *Staphylococcaceae* and *Bacillaceae*, decreased post germination (Table 3). In contrast, cotyledon communities increased in relative abundance of *Gammaproteobacteria*, *Betaproteobacteria*, *Planctomycetes* and *Bacteria-incertae-sedis* post germination. Increases in richness and diversity of the phyllosphere community of plants at the 3–4 leaf stage of development coincided with the appearance of members of seven additional phyla

Table 2. Richness and diversity estimators that predict the number of species in the microbiome of spinach at different development stages (seed, cotyledons and aerial surfaces of 3–4 leaf stage of development plants)

Sample	Chao 1 estimator*			Shannon index (*H*′)	Species evenness (*E*)[†]
	Lower limit	Chao1	Upper limit		
Seed	229	272	350	2.28 ± 0.44	0.41
Cotyledon	226	271	353	2.39 ± 0.46	0.42
Plants	745	863	1032	3.15 ± 0.51	0.46

*Richness was estimated with Chao1 and rarefaction curves represent values obtained at 3% of dissimilarity.

[†]The percentage of sequence reads per phylogenetic group was determined based on the number of reads per selected group divided by the total number of reads classified at a level beyond the Bacteria domain.

(*Acidobacteria, Chlamydiae, Chloroflexi, Deinococcus-Thermus*, OD1, TM7 and *Verrucomicrobia*); however, the community remained dominated by *Proteobacteria* (97% relative abundance) (Table 3). The most abundant sequences identified in this study belonged to members of *Enterobacteriaceae* and *Pseudomonadaceae*. These included mostly the genera *Pseudomonas* and *Pantoea*, which are commonly isolated using culture-based techniques, indicating these are dominant members of the phyllosphere (Ibekwe & Grieve, 2004; Ragaert *et al.*, 2007). The most abundant sequences in this study had previously been detected through analysis of the 16S rRNA gene in the phyllospheres of other plants, including mature spinach (Kadivar & Stapleton, 2003; Krimm *et al.*, 2005; Jackson *et al.*, 2006; Yang *et al.*, 2008; Yutthammo *et al.*, 2010; Lopez-Velasco *et al.*, 2011). Low abundances of *Acidobacteria, Chlamydiae, Chloroflexi, Deinococcus-Thermus*, OD1, *Planctomycetes, Gammaproteobacteria*, TM7 and *Verrucomicrobia* have also been detected previously in the phyllosphere of mature plants, including spinach (Jackson *et al.*, 2006; Yang *et al.*, 2008). In this study, members of these phyla were only detected after germination (Table 1), suggesting a non-seed origin. These bacteria possess a number of adaptations allowing for survival under a wide range of conditions, including exposure to UV light, which is also encountered on the phyllosphere (Hugenholtz *et al.*, 2001; Kulichevskaya *et al.*, 2007). *Verrucomicrobia*, an aerobic, heterotrophic group of slow-growing bacteria, is a prevalent member of the rhizosphere (Nunes da Rocha *et al.*, 2009), supporting transfer from soil to the phyllosphere. The presence, even in lower abundance on the leaf surface, may reflect a greater ecological importance of these poorly studied phyla.

Several spermosphere-inhabiting bacteria are capable of promoting plant growth and development by accelerating seedling emergence and outcompeting pathogens for

Table 3. Taxonomic identification of sequences detected in the microbiomes of seeds, cotyledons and aerial surfaces from 3 to 4 leaf-stage of development plants of spinach

Bacterial member	Relative abundance, %*,[†]		
	Seed	Cotyledon	3–4 leaf stage
Acidobacteria	–	–	0.11
Actinobacteria	1.76	1.04	0.77
Corynebacterium spp.	0.08	–	–
Sanguibacter spp.	0.08	–	–
Micrococcaceae	0.16	–	0.15
Other *Actinomycetales*	–	1.04	0.62
Bacteria_incertae_sedis	–	0.26	0.09
Chlamydiae	–	–	0.03
Chloroflexi	–	–	0.11
Deinococcus-Thermus	–	–	0.05
Firmicutes	22.49	1.82	0.79
Enterococcus spp.	1.44	–	0.03
Exiguobacterium	0.08	–	–
Bacillus spp.	0.72	–	0.02
Staphylococcus spp.	14.57	–	0.03
Other *Bacialles*	5.68	1.56	0.45
OD1	–	–	0.02
Planctomycetes	–	0.26	0.26

Bacterial member	Relative abundance, %		
	Seed	Cotyledon	3–4 leaf stage
Proteobacteria	76.37	95.91	97.13
Unclassified *Proteobacteria*	4.64	1.03	0.20
Subclasses			
Alphaproteobacteria	3.68	1.3	3.03
Rhizobiaceae	3.68	–	1.95
Hyphomicrobiaceae	–	0.52	0.29
Caulobacteraceae	–	0.52	0.12
Brucellaceae	–	–	0.11
Bradyrhizobiaceae	–	–	0.20
Methylobacteriaceae	–	–	0.04
Rhodospirillales	–	0.26	0.20
Sphingomonadales	–	–	0.14
Betaproteobacteria	0.16	8.51	1.17
Deltaproteobacteria	–	–	0.05
Gammaproteobacteria	67.89	85.07	92.68
Pseudomonadaceae	13.13	0.52	15.58
Enterobacteriaceae	53.64	82.47	74.25
Xanthomonadales	–	1.56	2.29
Unclassified	1.12	0.52	0.56
TM7	–	–	0.09
Verrucomicrobia	–	–	0.07

*Results are represented as relative abundance of the total classified bacteria.

[†]The percentage of sequence reads was calculated based on the total number of reads classified beyond the *Bacteria* domain using the RDP CLASSIFIER tool.

resources (Compant *et al.*, 2005; Long *et al.*, 2008); however, the functions and identities of the majority of seed-associated bacteria are currently unknown. Post ger-
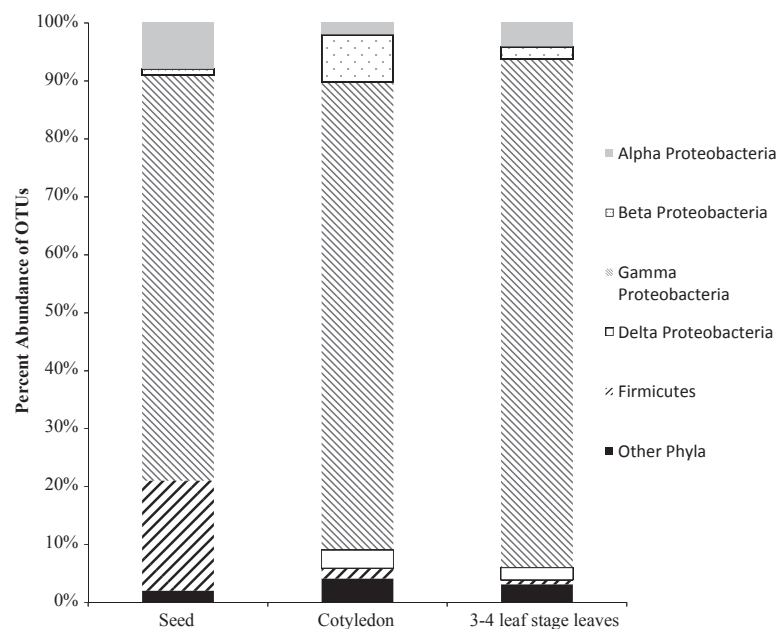
Fig. 2. Relative phylum/class abundance of classified bacterial sequences (> 1%) on seed, cotyledons and spinach leaves. (a) Percentage of relative abundance of the phyla *Firmicutes* and the classes within the dominant phyla, *Proteobacteria*.

mination, these bacteria may continue to enhance seedling growth and plant development by producing phytohormones, fixing atmospheric nitrogen and reducing plant disease, either through production of secondary metabolites or by inducing systemic resistance (Rosenblueth & Martínez-Romero, 2006). In this study, larger populations of methylotrophic bacteria (*Hyphomicrobiaceae*, *Methylobacteriaceae* and select *Betaproteobacteria*) were observed at seedling development. Methanol emission, related to leaf development, originates from methylotrophic bacteria and generally declines with increasing leaf age after expansion; this phenomenon corresponds with a decrease in methylotrophic bacteria on the 3–4 leaf stage of development plants (Nemecek-Marshall *et al.*, 1995). Although not measured in this study, methylotrophic bacteria produce phytohormones that may stimulate plant development in certain plant species; however, it is unknown whether these host–microbial interactions would also promote growth in spinach (Idris *et al.*, 2004). *Methylobacterium* spp., *Sphingmonas* spp. and *Pseudomonas* spp. were recently described as the most active members of the soybean phyllosphere (Delmotte *et al.*, 2009) and are frequently isolated from the phyllosphere of other plants, suggesting these bacteria may play an important role on the phyllosphere of spinach. The pyrosequencing approach taken in this study did not examine phyllosphere community function. The product size (250 bp) prevented the identification of the majority of bacteria below the genera level. Therefore, this approach targeting a conserved sequence in the 16S rRNA gene could not discriminate between pathogens and beneficial bacteria. Fungi or

viruses, neither of which would be detected with the primers used in this study, cause the majority of seed-borne spinach diseases.

Environmental parameters are believed to influence the development of the phyllosphere microbiota but several recent studies support an additional limited role in vertical seed transmission. Predictable patterns in bacterial communities on *Pinus ponderosa* needles have been observed across different geographic locations supporting this theory (Redford *et al.*, 2010). Bacterial community profiles of *Chenopodium album* and *Stellaria media* seeds isolated from soils of different types and locations were highly conserved and did not resemble the soils from which they were obtained (van Overbeek *et al.*, 2011). The translocation of bacterial endophytes from seed to immature and mature plant tissues has been demonstrated with wheatgrass (*Elymus trachycaulus* and *Agropyron fragile*) (Ringelberg *et al.*, 2012) and rice (Hardoim *et al.*, 2012). In this study, several identical sequences classified as *Pseudomonadaceae* (3), *Enterobacteriaceae* (5), unclassified *Firmicutes* (1), and unclassified *Gammaproteobacteria* (2) were detected on spinach seeds, cotyledons and seedlings. Recent studies in maize suggest that the seed-associated bacterial community varies in relation to plant host phylogeny; however, *Clostridium* and *Paenibacillus* were associated with seeds of 10 different maize cultivars from different geographic locations (Johnston-Monje & Raizada, 2011). In rice seeds, 45% of the bacterial community from one generation of seeds was also present in the second generation (Hardoim *et al.*, 2012). The recovery of closely related bacteria on seeds

from different generations across different niches and in different types of plants has resulted in the hypothesis introduced by Hardoim *et al.* (2012) that there is a core microbiota of plant-adapted bacteria that may use seeds for their dissemination. This study identifies *Rhizobium* spp., *Methylobacterium* spp., *Panotea* spp., *Pseudomonas* spp. and *Microbacterium* spp. sequences that are suggested to be plant niche-adapted. Since this study examines the bacterial community of only one cultivar of spinach, it is not possible to determine how well the concept of core-plant microbiome applies to spinach as a species. This study also used new-crop high quality seeds with high viability, which may reflect the reduced richness and reduced numbers of culturable bacteria (10^4 CFU g^{-1}). Leaked electrolytes from aged or damaged seeds could serve as a bacterial food source, resulting in a larger, more diverse population on seeds and seedlings. In addition, characterization of other sources such as soil and water used for irrigation is needed to understand their role in shaping the microbial community. Future studies should use molecular epidemiological methods such as repetitive element palindromic PCR (REP-PCR), single nucleotide polymorphism analysis or pulse-field gel electrophoresis to compare bacterial isolates from seed, cotyledon and seedlings. These techniques can determine whether bacteria isolated from different plant tissues or stages of development are the same strains, which would provide additional evidence for vertical transmission of bacteria from seed to the phyllosphere. Future studies should also include multiple growing conditions, as the soil type, temperature, plant cultivar, and seed quality are all known to influence the establishment of endophytes (Rosenblueth & Martínez-Romero, 2006). Previous studies within our laboratory have demonstrated that PCR-DGGE patterns of spinach are highly similar between plants grown in a field or a growth chamber, but that unique amplicons were observed in both conditions (Carder, 2010). No attempt was made in this study to distinguish endophytes (bacteria within the plant tissue) from any surface-associated bacteria, as the intent was to determine the potential for all bacteria, including those surface-associated bacteria, to be transferred to the aerial tissues. The large abundance of *Staphylococcaceae* on seeds may reflect the presence of contamination from the hands of workers, as these bacteria are known to be common members of the skin microbiota (Grice & Segre, 2011). The majority of these sequences were not detected on aerial surfaces of the plants, suggesting they would have minimal influence on the plant. However, introduction of bacteria to the seeds from worker hands or equipment may have important ramifications for human health. Seed-mediated transmission of human pathogens to sprouts has been well documented (EFSA, 2011). This study expands the knowledge of seed-inhabiting bacterial communities and supports the concept that seeds contribute to bacterial colonization of the phyllosphere with major population shifts occurring post germination. These bacteria represent an untapped reserve for bacterial isolates naturally present on the seed, which survive storage and are transferred to plant tissues with potential for plant growth promotion and bio-control of pathogens.

Acknowledgements

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