RESEARCH

e-Xtra*

Deciphering the Rhizosphere and Geocaulosphere Microbiomes of Potato Following Inoculation with the Biocontrol Agent *Pseudomonas fluorescens* Strain LBUM223

Roxane Roquigny, Amy Novinscak, Geneviève Léger, Nathan Marcoux, David L. Joly, and Martin Filion,[†]
Department of Biology, Université de Moncton, Moncton, NB Canada

Accepted for publication 5 May 2018.

ABSTRACT

The phenazine-1-carboxylic acid (PCA)-producing *Pseudomonas fluorescens* strain LBUM223 shows biocontrol potential against *Streptomyces scabies*, which causes common scab of potato. To better characterize the impact of inoculating this specific biocontrol agent under field conditions, the microbiomes of the rhizosphere and the geocaulosphere of potato plants were characterized using next-generation sequencing. A single initial application or biweekly applications of LBUM223 were performed up to 11 weeks after planting. Rhizosphere and geocaulosphere soils (when potato tubers were produced) were sampled every 2 weeks. Following soil DNA extractions, 16S rRNA gene amplification and sequencing were performed using the Illumina MiSeq technology. The QIIME pipeline was used for data analyses. Results were generated from 45 rhizosphere and

27 geocaulosphere samples, for which 63,502 and 44,469 different operational taxonomical units were observed. Diversity comparisons between both datasets were performed. To our knowledge, this is the first time that the geocaulosphere microbiome is characterized and compared with the rhizosphere microbiome following inoculation with a specific microorganism. Eleven phyla accounted for 95% of the diversity, with Actinobacteria, Proteobacteria, Chloroflexi, and Acidobacteria being the most abundant ones. Overall, the results obtained suggest that *P. fluorescens* strain LBUM223 does not significantly alter the autochthonous rhizosphere nor geocaulosphere microbiomes.

Additional keywords: bacteriology, phyllosphere, soil ecology.

The rhizosphere is described as the narrow soil zone influenced by plant roots (Bakker et al. 2013; Raaijmakers et al. 2009). Diversity and abundance of the rhizosphere's autochthonous microorganisms, known as the rhizosphere microbiome, is known to be shaped by plant species, soil composition, and environmental conditions (Bakker et al. 2013; Tkacz et al. 2015). Plant roots release organic carbon favorable to soil microorganisms' proliferation (Bakker et al. 2013), which generally leads to plant root-specific microbiomes (Garbeva et al. 2008). In return, the composition of the rhizosphere microbiome impacts plant health (Raaijmakers et al. 2009; Rodríguez and Fraga 1999). Plant health is in part maintained thanks to the presence of specific beneficial microorganisms, such as rhizobacteria either promoting plant growth and/or suppressing plant pathogens

through different mechanisms, including antibiosis and induced systemic resistance. A good example is the control of *Gaeumannomyces graminis* var. *tritici* causing the take-all disease of wheat by 2,4-diacetylphloroglucinol (DAPG) and phenazine-1-carboxylic acid (PCA)-producing fluorescent *Pseudomonas* spp. (Pierson and Pierson 1996; Raaijmakers and Weller 1998; Thomashow and Weller 1988).

Numerous studies have so far described the composition of the plant rhizosphere microbiome associated with different plant species such as maize (Peiffer et al. 2013), sugar beet (Mendes et al. 2011), and potato (genetically modified or not) (Becker et al. 2008; Castillo et al. 2017; Heuer et al. 2002; İnceoğlu et al. 2011, 2013; Lottmann et al. 1999, 2000; Milling et al. 2005; O'Callaghan et al. 2008; Rasche et al. 2005; Smalla et al. 2001; Van Overbeek and Van Elsas 2008; Weinert et al. 2011). Proteobacteria and Actinobacteria are often described as the most abundant phyla inhabiting the rhizosphere of potato plants, and significant differences in phyla distribution and abundance are usually described when comparing the soil microbiomes associated with the rhizosphere and the bulk soil (Becker et al. 2008; İnceoğlu et al. 2011; O'Callaghan et al. 2008; Smalla et al. 2001). For some plant species, such as potatoes, another distinct soil zone rich in microbial

[†]Corresponding author: M. Filion; E-mail: martin.filion@umoncton.ca

Funding: This study was supported by Natural Sciences and Engineering Research Council and New Brunswick Innovation Foundation grants to M. Filion.

*The e-Xtra logo stands for "electronic extra" and indicates that one supplementary table is published online.

diversity and activity exists, and is defined as the geocaulosphere, which is the narrow soil zone surrounding the potato tubers (Lottmann et al. 1999). To date, very little is known about the composition of the geocaulosphere's microbiome and how it compares to the rhizosphere's microbiome (Lottmann et al. 1999, 2000; O'Callaghan et al. 2008).

Known as ubiquitous bacteria inhabiting agricultural soils, and especially the rhizosphere of plants, many strains of *Pseudomonas* spp. are known as plant growth promoting rhizobacteria (Rameshkumar et al. 2012; Roquigny et al. 2017) and as biocontrol agents of soilborne pathogens (Huang et al. 2016; Xu et al. 2015). Recently, it has been demonstrated that *P. fluorescens* strain LBUM223 (hereafter named LBUM223) can alter the expression of key virulence genes in *Streptomyces scabies*, significantly reducing potato common scab disease symptoms under controlled (Arseneault et al. 2013; St-Onge et al. 2011) and field conditions (Arseneault et al. 2015). This effect is directly linked to phenazine-1-carboxylic-acid (PCA) production by LBUM223 (Roquigny et al. 2015; St-Onge et al. 2011).

Over the years, many studies have focused on the effect that inoculating plants with a given beneficial microorganism has on plant health and/or on a given plant pathogen. However, very few studies have looked at the impact that introducing a new organism in the rhizosphere, for biological control purpose, has on the indigenous soil microbial communities (Gao et al. 2012; Gilbert et al. 1996; Gilbert et al. 1993; Kozdrój et al. 2004). In this study, we investigated the impact that inoculating potato plants with LBUM223, a beneficial microorganism with strong biocontrol potential, has over time on the soil rhizosphere's and geocaulosphere's microbiomes of potato plants grown under field conditions.

MATERIALS AND METHODS

***P. fluorescens* strain LBUM223 inoculum.** LBUM223 was isolated from the rhizosphere of strawberry plants in Bouctouche, NB, Canada. Its genome was previously sequenced (Roquigny et al. 2015). LBUM223 was grown in tryptic soy broth (TSB) (BD, Franklin Lakes, NJ) with shaking at 25°C for 3 days. Bacterial concentration was determined by measuring absorbance (600 nm) and referring to a standard curve made by using serial dilutions of LBUM223 in TSB. Twenty milliliters of LBUM223 culture (2×10^9 CFU/ml) was either only applied once at tuber planting or reapplied biweekly throughout the potato growing season. Applications were made by directly pipetting the inoculum onto the seed tubers prior to covering with soil and to the base of each plant for biweekly applications.

Field trial and sampling. A field trial was performed in the same field as described in Arseneault et al. (2015, 2016) and the same procedure was used, except for the following modifications. Experiment was conducted from May to September 2015. A naturally infested field by *S. scabies* was divided into three blocks, each block containing randomized plots for each treatment: control (no application), single application of LBUM223, and multiple (biweekly) applications of LBUM223. Each plot contained 20 potato plants. One potato plant per plot was randomly selected for destructive sampling at each harvesting time (3, 5, 7, 9, and 11 weeks after planting). There were no geocaulosphere samples at time 3 and 5 weeks because no tubers were yet produced. At each harvesting time, plants were shaken to discard the nonadhering soil and remaining soil surrounding roots and tubers was manually collected, then flash-frozen in liquid nitrogen and labeled rhizosphere and geocaulosphere, respectively.

DNA extraction. Rhizosphere and geocaulosphere soil samples were treated as described in Arseneault et al. (2015). Briefly, soil

samples were lyophilized, and DNA was extracted from 0.5 g of soil using the method described by Griffiths et al. (2000). For each sample, DNA was resuspended in 50 µl of RNase-free water. DNA quantity and quality were verified by spectrophotometry (Nanodrop, ThermoScientific) and Quanti-It PicoGreen dsDNA assay (Molecular Probes, Eugene, OR).

16S rRNA gene amplification and sequencing. Using primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), the V4 region of the bacterial 16S rRNA gene was amplified by conventional PCR (Peiffer et al. 2013; Wen et al. 2017). A total of 72 amplicon samples (27 geocaulosphere soil samples and 45 rhizosphere soil samples: three treatments, three [geocaulosphere] or five [rhizosphere] sampling times, and three replicates per treatment/time) were submitted for sequencing on an Illumina MiSeq platform at the McGill University and Genome Quebec Innovation Centre (Montreal, Québec, Canada). Purified amplicons were pooled in equimolar concentrations and paired-end sequenced (2×150 bp).

Sequence editing and community analysis. The open-source software Quantitative Insights into Microbial Ecology (QIIME) v1.9.1 was used, mainly using default parameters to analyze the 16S rRNA gene sequences (Caporaso et al. 2010). All raw fastq files were demultiplexed, quality filtered and screened for chimeras using usearch61 (Edgar 2010; Edgar et al. 2011) with the default parameters. Filtered sequences were then grouped into operational taxonomic units (OTUs) at 97% similarity level using usearch61 (Edgar 2010). A representative set of OTUs was generated from the most abundant method and used for assigning taxonomy using uclust and the Greengenes reference database (McDonald et al. 2012; Werner et al. 2012). All singleton OTUs were discarded. PyNAST (Caporaso et al. 2009) and the Greengenes core set (DeSantis et al. 2006) were used to perform sequence alignments and a phylogenetic tree was constructed from the aligned sequences using FastTree (Price et al. 2009). From taxonomy assignment, all samples were grouped in two datasets: rhizosphere and geocaulosphere, both analyzed the same way. Alpha diversity indices (Chao1 and Shannon) were calculated from the rarefaction curves using the QIIME pipeline (Caporaso et al. 2010). For the following steps, rarefied data, already normalized with the single rarefaction option, were used. Beta diversity indices were generated using weighted UniFrac distances (Lozupone et al. 2007). Three-dimensional principal coordinate analyses (PCoA) were visualized using EMPEROR (Vázquez-Baeza et al. 2013). The ADONIS, PERMANOVA, and ANOSIM statistical methods were used within QIIME to determine the significant differences existing between the different bacterial communities. SAS University Edition (SAS Institute Inc., Cary, NC) was used to compare rhizosphere and geocaulosphere datasets by using either nonparametric Wilcoxon or Student's *t* tests depending on the normality of the data. Pearson's correlations were also calculated within each dataset to evaluate phyla proportions between treatments for each time-point. For this analysis, the three replicates within treatment for each time were averaged for each phylum's proportion, and the comparisons were carried out on 12 phyla: the 11 most abundant phyla and the 12th was a group of 38 and 37 other phyla for rhizosphere and geocaulosphere datasets, respectively, representing 5% of the remaining diversity.

All sequences generated in this study are available in the NCBI sequence read archive under accession number SRP133575.

RESULTS

16S rRNA gene sequencing data analysis. From the 72 soils samples, a total of 3,069,254 high quality sequences were generated (Supplementary Table S1). For the 45 rhizosphere samples (five

times, three treatments, three replicates), the number of sequences obtained ranged from 30,200 to 62,165 sequences per sample, while the number of OTUs ranged from 10,685 to 17,452 per sample with a total count of 63,502 different OTUs (Table 1). For the 27 geocaulosphere samples (three times, three treatments, and three replicates), the number of sequences obtained ranged from 31,231 to 88,032 sequences per samples, while the number of OTUs ranged from 7,983 to 21,705 observed OTUs per sample with a total count of 44,469 different OTUs (Table 1). Rarefaction curves, showing the increasing number of OTUs detected as a function of the number of sequences obtained per sample, were obtained for the rhizosphere (Fig. 1A) and geocaulosphere samples (Fig. 1B). Although a significant portion of the diversity was captured with the sequencing efforts made, no plateau was observed in the rarefaction curves, suggesting that additional sequencing effort would have likely further increased the number of OTUs detected.

Diversity comparison between rhizosphere and geocaulosphere datasets. The average numbers of sequences and OTUs per sample were similar in the rhizosphere and in the geocaulosphere, showing no statistically significant differences (Table 1). While Shannon's diversity index was also similar between both datasets, Chao1's diversity index was significantly different, indicating that the rhizosphere samples were richer in diversity than the geocaulosphere samples, in line with the total count of the different OTUs found in the rhizosphere samples (63,502 OTUs) and the ones found in the geocaulosphere samples (44,469 OTUs) (Table 1).

Taxonomic composition of soil bacterial communities over time. Using the QIIME pipeline, taxonomic analyses were performed on the rhizosphere and geocaulosphere datasets, independently. Up to 49 different phyla were observed, but no sample contained all of them. In the rhizosphere dataset, four major phyla were present in all the samples: Actinobacteria, Proteobacteria, Chloroflexi and Acidobacteria (Fig. 2A), with an average value between samples of 21.5% ($\pm 2.6\%$), 21.1% ($\pm 1.8\%$), 16.9% ($\pm 1.1\%$), and 13.3% ($\pm 1.4\%$), respectively. In the geocaulosphere dataset, the same four major phyla were observed in similar proportions (Fig. 2B), 21.6% ($\pm 1.3\%$) of Actinobacteria, 20.6% ($\pm 1.6\%$) of Proteobacteria, 16.6% ($\pm 1.5\%$) of Chloroflexi, and 13.8% ($\pm 1.9\%$) of Acidobacteria.

More than 95% of the taxonomic diversity of the rhizosphere and geocaulosphere datasets was represented by 11 phyla across time points (Fig. 3). Indicated from the highest to the lowest represented, there were Actinobacteria, Proteobacteria, Chloroflexi, Acidobacteria, Planctomycetes, Cyanobacteria, Firmicutes, Gemmatimonadetes, Verrucomicrobia, Bacteroidetes, and Unassigned phylum. A total of 38 and 37 phyla could explain the remaining 5% diversity of the rhizosphere (Fig. 3A) and the geocaulosphere datasets (Fig. 3B), respectively.

Over time, only Cyanobacteria showed a population decline in both the rhizosphere and geocaulosphere datasets (Fig. 3). When specifically focusing on the families *Streptomycetaceae* and the *Pseudomonadaceae*, which contain *S. scabies* and *P. fluorescens*, respectively, no significantly changes were observed in their population proportion through time and between treatments (data not shown).

Fig. 1. Rarefaction curves representing observed operational taxonomic units (OTUs) per number of sequences per sample for **A**, rhizosphere and **B**, geocaulosphere datasets.

TABLE 1 Indicators of diversity in the rhizosphere and geocaulosphere sequence datasets								
Soil zone	Number of samples	Number of sequences	Average of sequences per sample ^a	Number of different OTUs	Average of OTUs per sample ^a	Shannon's diversity ^a	Chao1's diversity ^b	Estimated coverage ^c
Rhizosphere	45	1,928,972	42,866 ($\pm 1,243$)	63,502	13,593 (± 264)	12.0 (± 0.09)	23,141 ($\pm 4,193$)	52.5%
Geocaulosphere	27	1,140,282	42,232 ($\pm 2,249$)	44,469	12,850 (± 567)	12.2 (± 0.05)	20,604 ($\pm 4,831$)	57.6%
^a Calculated from both datasets. None of the comparisons were found to be statistically significant by Student's <i>t</i> tests or nonparametric Wilcoxon tests for sequences/sample and Shannon's diversity.								
^b Calculated from both datasets. Comparison between the rhizosphere and geocaulosphere datasets was found statistically significant by Student's <i>t</i> tests ($t = -2.35$; $P < 0.05$).								
^c Good's coverage (Good 1953).

Fig. 2. Relative abundances of bacterial phyla in A, rhizosphere and B, geocaulosphere datasets.

Impact of inoculating LBUM223 on microbiome composition.

Neither time, nor LBUM223 application mode (unique or biweekly) affected bacterial phyla composition. PCoA plots were generated from three principal differential factors. For the rhizosphere dataset, the 3 factors explained 30.17, 15.27, and 12.38% of the differences observed (Fig. 4A). Similarly, the factors for the geocaulosphere dataset explained 32.40, 18.68, and 10.78% of the differences observed (Fig. 4B). Within each dataset, Pearson's correlations were calculated from the proportions of 12 phyla (Fig. 3) for each treatment (average of replicates) at each time and compared with each other (Table 2). Correlations between treatments for each time ranged from 94.8 to 99.9% and from 95.9 to 99.4% for rhizosphere and geocaulosphere datasets, respectively, showing a close similarity between treatments at each time, within datasets.

DISCUSSION

Studying soil microbiome diversity alterations following inoculation with a beneficial microorganism with strong biocontrol potential is important to understand its impact on the autochthonous microbial communities. Here, we investigated the impact that inoculating potato plants with LBUM223 had over time on the soil rhizosphere's and geocaulosphere's microbiomes under field conditions. The rhizosphere is largely described as a high microbial activity zone when compared with the bulk soil, relying on exudation of nutrients by plant roots (Bakker et al. 2013; Berendsen et al. 2012). Surprisingly, the geocaulosphere is less studied, even though the tuber surface is the hotspot for pathogenic agents responsible of important potato diseases, such as potato common scab. Thus, some previously suggested that bacterial community structure between rhizosphere and geocaulosphere should be different because bacteria in the rhizosphere have access to nutrients that are excreted by the plant while nutrient availability at the tuber

surface might be mainly due to lesions and to cells decaying during tuber growth (Lottmann et al. 2000; Weinert et al. 2010). On the contrary, results here showed that rhizosphere and geocaulosphere microbiomes appeared similar when comparing their bacterial phyla composition. In another study, similar results were also

Fig. 4. PCoA plots from weighted UniFrac distance matrices for **A**, rhizosphere and **B**, geocaulosphere. Red spheres are for the control treatment, green spheres for single application, and blue spheres for multiple applications.

Fig. 3. Distribution of 12 phyla representing total diversity per treatment through time for **A**, rhizosphere and **B**, geocaulosphere datasets. Time (in weeks) increases from inner to outer circles (from weeks 3 to 11 for rhizosphere and from weeks 7 to 11 for geocaulosphere). The 12th phylum (Others) represents 5% of the remaining diversity consisting of 38 phyla for the rhizosphere and 37 phyla for the geocaulosphere.

observed for the fungal genera *Alternaria*, *Fusarium*, and *Rhizoctonia*, which were also detected in both the rhizosphere and the geocaulosphere (Fiers et al. 2010). Here, eleven phyla were found to represent 95% of the bacterial diversity at all times. The dominant ones were Actinobacteria, Proteobacteria, Chloroflexi, and Acidobacteria. Some members of these phyla were previously described as endophytic communities of potato roots, along with less abundant phyla, such as Verrucomicrobia and TM7 (Manter et al. 2010). The role played by most of these phyla is however still unknown and the mechanisms maintaining their diversity in the rhizosphere and the geocaulosphere are not fully understood. A mini-review summed up studies on the geocaulosphere and the different bacterial genera detected compared with those detected in the rhizosphere (Diallo et al. 2011). *Bacillus* spp., *Pseudomonas* spp., and *Streptomyces* spp., belonging to the Firmicutes, Proteobacteria, and Actinobacteria phyla, respectively, are among the major bacterial genera found in both microenvironments (Diallo et al. 2011). However, their proportions appeared different, in line with *Bacillus* strains more represented on tuber surface than in the rhizosphere, and paradoxically *Streptomyces* isolates being more present in the rhizosphere (Weinert et al. 2010). Here, the results showed that in addition to similar compositions, proportions of bacterial phyla communities were in a same range in both the rhizosphere and the geocaulosphere.

The composition of the rhizosphere microbiome of potato plants was previously described as dynamic and influenced by geographical locations, cultivars, plant genotypes (genetically modified or not), and plant growth developmental stages (Rasche et al. 2005; Smalla et al. 2001; Van Overbeek and Van Elsas 2008; Weinert et al. 2011). The geocaulosphere's microbiome was instead previously described as slightly influenced by plant genotypes (Weinert et al. 2010). We were expecting to observe a difference in bacterial composition throughout our experiment correlated to the potato growth developmental stages (from 3 to 11 weeks after tuber planting), similarly as what was observed in young, flowering and senescing potato plants in a field experiment (Van Overbeek and

Van Elsas 2008). Here, as no significantly difference was observed over time in the bacterial communities inhabiting the rhizosphere or the geocaulosphere, it suggests that the plant growth developmental stage did not play a significant role shaping the microbiome composition of the rhizosphere or the geocaulosphere. Our results are in part in accordance with a recent study, which concluded that the rhizosphere microbiome of potato plant could be divided in a "stable" core microbiome which is not influenced by vegetation stages and a "dynamic" one, which is (Pfeiffer et al. 2017). These results might explain the only significant trend, although minor, observed in terms of microbiome composition changes over time, which was the reduction of Cyanobacteria observed in both the rhizosphere and the geocaulosphere. In soil ecosystems, Cyanobacteria are mostly described as capable of nitrogen and carbon fixation, of nutrients release promotion and of reduction of water loss rate and soil erosion (Prasanna et al. 2014). These capacities make Cyanobacteria suitable organisms to positively impact soil fertility. Although Cyanobacteria are highly adaptive to varying environmental conditions (Oren 2014), it appears here that they could not maintain the same population levels over time.

Whether inoculation with LBUM223 was only at the planting stage (single) or reapplied biweekly (multiple), the autochthonous rhizosphere and geocaulosphere microbiomes were not significantly altered. Similar results were found by Piotrowska-Seget and Kozdroj (1999) where the introduction of HCN-producing *P. fluorescens* strains did not alter the soil bacterial diversity. The authors of this study concluded that as no significant effect was observed on the autochthonous soil microbial communities, this *P. fluorescens* strain could be safely used as a biocontrol agent against soilborne pathogens. Similar results were also obtained by Lottmann et al. (2000) when inoculating the biocontrol bacterium *P. putida* QC14-3-8 in the rhizosphere or in the geocaulosphere of wild-type or transgenic potato plants, constitutively expressing and secreting T4 lysozymes.

Although persistence of LBUM223 in both microenvironments was not studied here, the biweekly application must have prevented

TABLE 2
Pearson's correlations obtained when comparing the 12 phyla per treatment and time^a

Dataset	Week	Single application	Multiple applications
Rhizosphere	3	0.992 < 0.0001	0.991 < 0.0001
	Control		
	5	0.995 < 0.0001	0.994 < 0.0001
	7	0.990 < 0.0001	0.989 < 0.0001
	9	0.995 < 0.0001	0.984 < 0.0001
	11	0.973 < 0.0001	0.948 < 0.0001
	3	1.00	0.979 < 0.0001
	Single application		
	5	1.00	0.999 < 0.0001
	7	1.00	0.989 < 0.0001
Geocaulosphere	9	1.00	0.995 < 0.0001
	11	1.00	0.973 < 0.0001
	7	0.984 < 0.0001	0.991 < 0.0001
	Control		
	9	0.968 < 0.0001	0.974 < 0.0001
	11	0.979 < 0.0001	0.973 < 0.0001
	7	1.00	0.959 < 0.0001
	Single application		
	9	1.00	0.993 < 0.0001
	11	1.00	0.994 < 0.0001

^a For rhizosphere and geocaulosphere datasets, statistical analyses were performed with SAS University Edition. For each harvesting week, the first line contains the R^2 value followed by the associated P value.

a decreased population of LBUM223, as suggested in other studies (Arseneault et al. 2015; Gao et al. 2012) and demonstrated in a previous work performed in the same field under the same conditions (Arseneault et al. 2016). LBUM223 was quantified in both rhizosphere and geocaulosphere soils and the biweekly applications maintained a constant population of LBUM223 at approximately 10^7 bacteria/g of soil (Arseneault et al. 2016). Moreover, other studies showed that LBUM223 was capable of increasing potato yield and controlling common scab under field conditions using a biweekly application regime (Arseneault et al. 2013, 2015). In light of the results obtained in this study, we can conclude that the use of LBUM223 under field conditions appears safe as it does not significantly alter the autochthonous soil bacterial communities. Also, this study contributed to better understanding the microbial ecology of the potato rhizosphere and geocaulosphere. To our knowledge, this is the first study to compare the microbiomes inhabiting these two soil environments under field conditions and demonstrating their similarity over time.

ACKNOWLEDGMENTS

We thank K. Nahar and the staff at the Potato Research Center (Agriculture and Agri-Food Canada in Fredericton, NB) for their technical assistance in the field.

LITERATURE CITED

- Arseneault, T., Goyer, C., and Filion, M. 2013. Phenazine production by *Pseudomonas* sp. LBUM223 contributes to the biological control of potato common scab. *Phytopathology* 103:995-1000.
- Arseneault, T., Goyer, C., and Filion, M. 2015. *Pseudomonas fluorescens* LBUM223 increases potato yield and reduces common scab symptoms in the field. *Phytopathology* 105:1311-1317.
- Arseneault, T., Goyer, C., and Filion, M. 2016. Biocontrol of potato common scab is associated with high *Pseudomonas fluorescens* LBUM223 populations and phenazine-1-carboxylic acid biosynthetic transcript accumulation in the potato geocaulosphere. *Phytopathology* 106:963-970.
- Bakker, P. A. H. M., Berendsen, R. L., Doornbos, R. F., Wintermans, P. C., and Pieterse, C. M. 2013. The rhizosphere revisited: Root microbiomics. *Front. Plant Sci.* 30:165.
- Becker, R., Behrendt, U., Hommel, B., Kropf, S., and Ulrich, A. 2008. Effects of transgenic fructan-producing potatoes on the community structure of rhizosphere and phyllosphere bacteria. *FEMS Microbiol. Ecol.* 66:411-425.
- Berendsen, R. L., Pieterse, C. M., and Bakker, P. A. 2012. The rhizosphere microbiome and plant health. *Trends Plant Sci.* 17:478-486.
- Caporaso, G. J., Bittinger, K., Bushman, F. D., DeSantis, T. Z., Andersen, G. L., and Knight, R. 2009. PyNAST: A flexible tool for aligning sequences to a template alignment. *Bioinformatics* 26:266-267.
- Caporaso, G. J., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Pena, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R., Tumbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J., and Knight, R. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* 7:335-336.
- Castillo, J. D., Vivanco, J. M., and Manter, D. K. 2017. Bacterial microbiome and nematode occurrence in different potato agricultural soils. *Microbiol. Ecol.* 74:888-900.
- DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., Huber, T., Dalevi, D., Hu, P., and Andersen, G. L. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl. Environ. Microbiol.* 72:5069-5072.
- Diallo, S., Crépin, A., Barbey, C., Orange, N., Burini, J. F., and Latour, X. 2011. Mechanisms and recent advances in biological control mediated through the potato rhizosphere. *FEMS Microbiol. Ecol.* 75:351-364.
- Edgar, R. C. 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26:2460-2461.
- Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., and Knight, R. 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27:2194-2200.
- Fiers, M., Chatot, C., Edel-Hermann, V., Le Hingrat, Y., Konate, A. Y., Gautheron, N., Guillery, E., Alabouvette, C., and Steinberg, C. 2010. Diversity of microorganisms associated with atypical superficial blemishes of potato tubers and pathogenicity assessment. *Eur. J. Plant Pathol.* 128:353-371.
- Gao, G., Yin, D., Chen, S., Xia, F., Yang, J., Li, Q., and Wang, W. 2012. Effect of biocontrol agent *Pseudomonas fluorescens* 2P24 on soil fungal community in cucumber rhizosphere using T-RFLP and DGGE. *PLoS One* 7:e31806.
- Garbeva, P., Van Elsas, J., and Van Veen, J. 2008. Rhizosphere microbial community and its response to plant species and soil history. *Plant Soil* 302:19-32.
- Gilbert, G. S., Clayton, M., Handelsman, J., and Parke, J. 1996. Use of cluster and discriminant analyses to compare rhizosphere bacterial communities following biological perturbation. *Microbiol. Ecol.* 32:123-147.
- Gilbert, G. S., Parke, J. L., Clayton, M. K., and Handelsman, J. 1993. Effects of an introduced bacterium on bacterial communities on roots. *Ecology* 74:840-854.
- Good, I. J. 1953. The population frequencies of species and the estimation of population parameters. *Biometrika* 40:237-264.
- Griffiths, R. I., Whiteley, A. S., O'Donnell, A. G., and Bailey, M. J. 2000. Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA-and rRNA-based microbial community composition. *Appl. Environ. Microbiol.* 66:5488-5491.
- Heuer, H., Kroppenstedt, R. M., Lottmann, J., Berg, G., and Smalla, K. 2002. Effects of T4 lysozyme release from transgenic potato roots on bacterial rhizosphere communities are negligible relative to natural factors. *Appl. Environ. Microbiol.* 68:1325-1335.
- Huang, H., Sun, L., Bi, K., Zhong, G., and Hu, M. 2016. The effect of phenazine-1-carboxylic acid on the morphological, physiological, and molecular characteristics of *Phellinus noxius*. *Molecules* 21:613.
- Inceoglu, Ö., Al-Soud, W. A., Salles, J. F., Semenov, A. V., and van Elsas, J. D. 2011. Comparative analysis of bacterial communities in a potato field as determined by pyrosequencing. *PLoS One* 6:e23321.
- Inceoglu, Ö., van Overbeek, L. S., Salles, J. F., and van Elsas, J. D. 2013. Normal operating range of bacterial communities in soil used for potato cropping. *Appl. Environ. Microbiol.* 79:1160-1170.
- Kozdrój, J., Trevors, J., and Van Elsas, J. 2004. Influence of introduced potential biocontrol agents on maize seedling growth and bacterial community structure in the rhizosphere. *Soil Biol. Biochem.* 36:1775-1784.
- Lottmann, J., Heuer, H., de Vries, J., Mahn, A., Düring, K., Wackernagel, W., Smalla, K., and Berg, G. 2000. Establishment of introduced antagonistic bacteria in the rhizosphere of transgenic potatoes and their effect on the bacterial community. *FEMS Microbiol. Ecol.* 33:41-49.
- Lottmann, J., Heuer, H., Smalla, K., and Berg, G. 1999. Influence of transgenic T4-lysozyme-producing potato plants on potentially beneficial plant-associated bacteria. *FEMS Microbiol. Ecol.* 29:365-377.
- Lozupone, C. A., Hamady, M., Kelley, S. T., and Knight, R. 2007. Quantitative and qualitative β diversity measures lead to different insights into factors that structure microbial communities. *Appl. Environ. Microbiol.* 73:1576-1585.
- Manter, D. K., Delgado, J. A., Holm, D. G., and Stong, R. A. 2010. Pyrosequencing reveals a highly diverse and cultivar-specific bacterial endophyte community in potato roots. *Microbiol. Ecol.* 60:157-166.
- McDonald, D., Price, M. N., Goodrich, J., Nawrocki, E. P., DeSantis, T. Z., Probst, A., Andersen, G. L., Knight, R., and Hugenholtz, P. 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J.* 6:610-618.
- Mendes, R., Kruijt, M., de Bruijn, I., Dekkers, E., van der Voort, M., Schneider, J. H., Piceno, Y. M., DeSantis, T. Z., Andersen, G. L., and Bakker, P. A. 2011. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science* 332:1097-1100.
- Millington, A., Smalla, K., Maidl, F. X., Schlöter, M., and Munch, J. C. 2005. Effects of transgenic potatoes with an altered starch composition on the diversity of soil and rhizosphere bacteria and fungi. *Plant Soil* 266:23-39.
- O'Callaghan, M., Gerard, E. M., Bell, N. L., Waipara, N. W., Aalders, L. T., Baird, D. B., and Conner, A. J. 2008. Microbial and nematode communities associated with potatoes genetically modified to express the antimicrobial peptide magainin and unmodified potato cultivars. *Soil Biol. Biochem.* 40:1446-1459.
- Oren, A. 2014. Cyanobacteria: Biology, ecology, and evolution. Pages 145-166 in: *Cyanobacteria: An Economic Perspective*. N. K. Sharma, A. K. Rai, and L. J. Stal, eds. John Wiley & Sons, Sussex, UK.
- Peiffer, J. A., Spor, A., Koren, O., Jin, Z., Tringe, S. G., Dangl, J. L., Buckler, E. S., and Ley, R. E. 2013. Diversity and heritability of the maize rhizosphere microbiome under field conditions. *P. Natl. Acad. Sci.* 110:6548-6553.

- Pfeiffer, S., Mitter, B., Oswald, A., Schlöter-Hai, B., Schlöter, M., Declerck, S., and Sessitsch, A. 2017. Rhizosphere microbiomes of potato cultivated in the High Andes show stable and dynamic core microbiomes with different responses to plant development. *FEMS Microbiol. Ecol.* 93:fiw242.
- Pierson, L. S., III, and Pierson, E. A. 1996. Phenazine antibiotic production in *Pseudomonas aureofaciens*: Role in rhizosphere ecology and pathogen suppression. *FEMS Microbiol. Lett.* 136:101-108.
- Piotrowska-Seget, Z., and Kozdroj, J. 1999. Indigenous microflora responses to introduction of cyanogenic strains of *Pseudomonas fluorescens* into soil. *Acta Microbiol. Pol.* 48:73-78.
- Prasanna, R., Sood, A., Ratha, S. K., and Singh, K. P. 2014. Cyanobacteria as a "green" option for sustainable agriculture. Pages 145-166 in: *Cyanobacteria: An Economic Perspective*. N. K. Sharma, A. K. Rai, and L. J. Stal, eds. John Wiley & Sons, Sussex, UK.
- Price, M. N., Dehal, P. S., and Arkin, A. P. 2009. FastTree: Computing large minimum evolution trees with profiles instead of a distance matrix. *Mol. Biol. Evol.* 26:1641-1650.
- Raaijmakers, J. M., Paulitz, T. C., Steinberg, C., Alabouvette, C., and Moenne-Loccoz, Y. 2009. The rhizosphere: A playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant Soil* 321:341-361.
- Raaijmakers, J. M., and Weller, D. M. 1998. Natural plant protection by 2, 4-diacetylphloroglucinol-producing *Pseudomonas* spp. in take-all decline soils. *Mol. Plant-Microbe Interact.* 11:144-152.
- Rameshkumar, N., Ayyadurai, N., Kayalvizhi, N., and Gunasekaran, P. 2012. Genotypic and phenotypic diversity of PGPR fluorescent pseudomonads isolated from the rhizosphere of sugarcane (*Saccharum officinarum* L.). *J. Microbiol. Biotechnol.* 22:13-24.
- Rasche, F., Hödl, V., Poll, C., Kandeler, E., Gerzabek, M. H., Van Elsas, J. D., and Sessitsch, A. 2005. Rhizosphere bacteria affected by transgenic potatoes with antibacterial activities compared with the effects of soil, wild-type potatoes, vegetation stage and pathogen exposure. *FEMS Microbiol. Ecol.* 56:219-235.
- Rodríguez, H., and Fraga, R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol. Adv.* 17:319-339.
- Roquigny, R., Arseneault, T., Gadkar, V. J., Novinscak, A., Joly, D. L., and Filion, M. 2015. Complete genome sequence of biocontrol strain *Pseudomonas fluorescens* LBUM223. *Genome Announc.* 3:e00443-e15.
- Roquigny, R., Novinscak, A., Biessy, A., and Filion, M. 2017. Pseudomonadaceae: From biocontrol to plant growth promotion. Pages 39-68 in: *Rhizotrophs: Plant Growth Promotion to Bioremediation*. Springer, Singapore.
- Smalla, K., Wieland, G., Buchner, A., Zock, A., Parzy, J., Kaiser, S., Roskot, N., Heuer, H., and Berg, G. 2001. Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: Plant-dependent enrichment and seasonal shifts revealed. *Appl. Environ. Microbiol.* 67:4742-4751.
- St-Onge, R., Gadkar, V. J., Arseneault, T., Goyer, C., and Filion, M. 2011. The ability of *Pseudomonas* sp. LBUM223 to produce phenazine-1-carboxylic acid affects the growth of *Streptomyces scabies*, the expression of thaxtomin biosynthesis genes and the biological control potential against common scab of potato. *FEMS Microbiol. Ecol.* 75:173-183.
- Thomashow, L. S., and Weller, D. M. 1988. Role of a phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Gaeumannomyces graminis* var. *tritici*. *J. Bacteriol.* 170:3499-3508.
- Tkacz, A., Cheema, J., Chandra, G., Grant, A., and Poole, P. S. 2015. Stability and succession of the rhizosphere microbiota depends upon plant type and soil composition. *ISME J.* 9:2349-2359.
- Van Overbeek, L., and Van Elsas, J. D. 2008. Effects of plant genotype and growth stage on the structure of bacterial communities associated with potato (*Solanum tuberosum* L.). *FEMS Microbiol. Ecol.* 64:283-296.
- Vázquez-Baeza, Y., Pirrung, M., Gonzalez, A., and Knight, R. 2013. EMPoror: A tool for visualizing high-throughput microbial community data. *Gigascience* 2:16.
- Weinert, N., Meincke, R., Gottwald, C., Heuer, H., Schlöter, M., Berg, G., and Smalla, K. 2010. Bacterial diversity on the surface of potato tubers in soil and the influence of the plant genotype. *FEMS Microbiol. Ecol.* 74: 114-123.
- Weinert, N., Piceno, Y., Ding, G.-C., Meincke, R., Heuer, H., Berg, G., Schlöter, M., Andersen, G., and Smalla, K. 2011. PhyloChip hybridization uncovered an enormous bacterial diversity in the rhizosphere of different potato cultivars: Many common and few cultivar-dependent taxa. *FEMS Microbiol. Ecol.* 75:497-506.
- Wen, C., Wu, L., Qin, Y., Van Nostrand, J. D., Ning, D., Sun, B., Xue, K., Liu, F., Deng, Y., and Liang, Y. 2017. Evaluation of the reproducibility of amplicon sequencing with Illumina MiSeq platform. *PLoS One* 12: e0176716.
- Werner, J. J., Koren, O., Hugenholtz, P., DeSantis, T. Z., Walters, W. A., Caporaso, J. G., Angenent, L. T., Knight, R., and Ley, R. E. 2012. Impact of training sets on classification of high-throughput bacterial 16S rRNA gene surveys. *ISME J.* 6:94-103.
- Xu, S., Pan, X., Luo, J., Wu, J., Zhou, Z., Liang, X., He, Y., and Zhou, M. 2015. Effects of phenazine-1-carboxylic acid on the biology of the plant-pathogenic bacterium *Xanthomonas oryzae* pv. *oryzae*. *Pestic. Biochem. Physiol.* 117: 39-46.