RESEARCH

e-Xtra*

Microbial Communities Associated with Storage Onion

Svetlana N. Yurgel,[†] Lord Abbey, and Nancy Loomer, Department of Plant, Food, and Environmental Sciences, Dalhousie University, Truro, NS, Canada; **Rosalie Gillis-Madden,** Perennia Food and Agriculture Inc., Kentville, NS, Canada; and **Melissa Mammoliti,** Department of Plant, Food, and Environmental Sciences, Dalhousie University, Truro, NS, Canada

Accepted for publication 10 January 2018.

ABSTRACT

Onion (*Allium cepa*) bulb rot is an important pathological problem worldwide, as it is caused by diverse pathogens, bacterial and fungal. The diversity and structure of microbial communities associated with apparently healthy and diseased onion bulbs were analyzed. The community-based approach allowed identification of a number of potential pathogenic microorganisms associated with onion bulbs affected by disease under storage environments. *Enterobacteriaceae*, *Burkholderia*, *Gluconobacter*, *Acinetobacter*, *Pseudomonas*, *Citrobacter*, *Nectriaceae*, *Botrytis*, *Wickerhamomyces*, *Penicillium*, and *Candida* were the most relatively abundant bacterial and fungal taxa in the diseased onion bulb dataset. It was found that these

taxa are also widely distributed in the bulbs without apparent disease symptoms. Additionally, several potential fermenters were detected in onion tissue. These data suggest that, in addition to abiotic factors, fermentation plays a role in the storage spoilage of onion bulbs. It was also shown that bacterial and fungal communities differ in their diversity and structure between individual onions affected by bulb rot. The identification of dinoflagellates *Symbiodinium* in onion bulbs suggested that this eukaryote might have a much broader spectrum of habitats and eukaryotic hosts.

Additional keywords: mycotoxins, postharvest pathology.

Onion (*Allium cepa* L.) is a member of the family *Alliaceae*, which includes garlic, shallot, and leeks. Global production of onion bulbs is estimated at approximately 88.5×10^6 tons, of which 0.24×10^6 tons are produced in Canada (FAOSTAT 2017). In spite of the importance of onion production to the agriculture and agri-food industry, postharvest loss to bulb rot diseases remains high. It has been suggested that as much as 20% of stored onion bulbs are lost to bulb rot disease alone in Nova Scotia (Nova Scotia onion growers, *personal communication* 2016). Infection of onion bulbs by pathogenic microbes and the associated loss of onion bulbs can be attributed to several pre- and postharvest factors such as genotypic characteristics, soil properties, climatic factors, and management practices (Abbey et al. 2004; Rasiukeviciute et al. 2016). Notable onion pathogens of importance are the gray mold fungi, *Botrytis* spp.; the black mold fungi, *Aspergillus* spp.; the blue mold fungi, *Penicillium* spp.; bacterial rot, *Erwinia* spp.; and bacterial rot,

Pseudomonas spp. (Christensen 1973; King and Schade 1984). In Nova Scotia, the biggest foliar fungal pathogens that growers base their fungicide programs around are *Peronospora destructor*, *Botrytis cinerea* and *Botrytis squamosa*, *Stemphylium vesicarium*, and *Alternaria porri*. The biggest soilborne fungal diseases that Nova Scotia growers combat would be *Fusarium oxysporum* f. sp. *cepae*, and to a lesser extent *Pyrenochaeta* (*Phoma*) *terrestris*, as well as damping-off early in the season caused by species of *Fusarium*, *Pythium*, and *Rhizoctonia*. It has been assumed that the primary bacterial rot organisms in Nova Scotia are *Burkholderia* spp., *Pantoea* spp., *Enterobacter* spp., *Pectobacterium* (*Erwinia*) *carotovora*, and *Pseudomonas* spp. (Nova Scotia onion growers, *personal communication*). Categorizing whether this is in fact the case will assist the industry in moving forward with proactive disease management strategies.

In contrast to the “one pathogen–one disease” concept often applied to plant–pathogen interactions, in natural environments, plants are usually infected by multiple pathogenic species (Abdel-Rahim et al. 2017; Fitt et al. 2006; Haapalainen et al. 2016; Lamichhane 2015). This concept suggests the natural occurrence of a complex plant-associated multispecies consortia in natural environments. Recent reports indicated that co-occurrence of multiple pathogens might increase the severity of plant diseases (Lamichhane and Venturi 2015). For example, several *Pantoea* spp. could coexist and coexpress their virulence in onion tissues (Vahling-Armstrong et al. 2015). In addition

[†]Corresponding author: S. N. Yurgel; E-mail: syurgel@dal.ca

This work was supported by Discovery Grant from the Natural Sciences and Engineering Research Council of Canada EGP 485260 to S. N. Yurgel.

*The e-Xtra logo stands for “electronic extra” and indicates that two supplementary figures and one supplementary table are published online.

to environmental factors and plant genotype, the intensity of disease progression could be influenced by the nature of the pathogen(s), such as virulence and aggressiveness, and biotic interactions between host–plant, plant microbiome and macroorganisms within the entire plant ecosystem (Borer et al. 2016). Therefore, a community-based approach, which considers a broad spectrum of plant-associated organisms is an important tool for the evaluation of plant–pathogen interactions and the development of new strategies for plant disease management. The application of a community-based strategy to help manage onion bulb rot diseases will require a comprehensive knowledge on the occurrence and variation in potential pathogen-causing diseases. This may differ with location and other aforementioned factors.

In this study, the diversity and structure of microbial communities associated with apparently healthy and diseased onion bulbs were analyzed. The onion bulbs were produced and stored under similar conditions and shared the same pre- and postharvest factors. This approach allowed the identification of a number of potential pathogenic microorganisms associated with onion bulbs, which showed the extent to which these microorganisms are widely distributed in the bulbs without apparent disease symptoms.

MATERIALS AND METHODS

Sample collection and processing. Stored bulbs of yellow onion cultivar Patterson were obtained from Nova Scotian onion grower in the Annapolis Valley. All agronomic and management practices were performed by the grower using standard practices on mineral soils. Standard practices plant onions on a raised bed to encourage proper drainage and include split-fertility applications as per soil test recommendations. Proper fertility management and good drainage are key pest mitigation practices recognized by industry. Regular pesticide applications and rotating active ingredients are employed as per scouting reports (<http://www.perennia.ca/wp-content/uploads/2015/12/Onions.pdf>). Typical pesticide applications would target field fungal diseases such as *Peronospora destructor* (Berk.) Casp., *Botrytis* spp., *Stemphylium vesicarium* (Wall.), and *Alternaria porri* (Ellis) Cif.; copper applications to manage bacterial diseases; as well as insecticide applications to manage *Thrips tabaci* (Lindeman) and *Delia* spp. Prior to harvest, maleic hydrazide is applied to prevent sprouting in storage. The onion bulbs were harvested in the fall of 2015 and stored in a local storage facility at an average temperature of 7°C for 3 months. Fifteen apparently healthy onion bulbs (further referred to as healthy bulbs and named H1 to H15) and 15 of those exhibiting bulb rot symptoms (further referred to as diseased bulbs and named D16 to D30) were randomly selected for microbiome profiling and evaluation of microbiome virulence. One 1-cm thick vertical longitudinal section of onion bulb was made with a sterile sharp knife and a cutting board. The cutting surface and knife were sterilized with 70% ethanol between samples. The sections of the bulbs were placed separately in labeled sterile bags and immediately frozen at –20°C. The cut bulbs were not sterilized and therefore introduction of minor exogenous microbial contamination was possible. After 2 weeks of storage at –20°C, the samples were thawed and macerated. Approximately 250 µl of juice and finely macerated onion tissues from each 1-cm bulb section was used for DNA isolation and sequencing. Approximately 9 ml of the onion juice and macerated tissue was thoroughly mixed with 1 ml of sterile glycerol. One milliliter of an aliquot of the mixture was then stored at –86°C for later use.

DNA extraction and sequencing. DNA extraction was carried out using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA) according to the manufacturer's protocol. DNA quality and concentration were measured using a NanoDrop 1000

spectrophotometer (Thermo Scientific, Waltham, MA). Five microliters of DNA sample was sent to the Dalhousie University CGEB-IMR (<http://cgeb-imr.ca/>) for V6 to V8 16S rRNA gene (16S), V4 18S rRNA gene (18S), fungal internal transcribed spacer (ITS)2 region (ITS) library preparation and sequencing. Samples were multiplexed using a dual-indexing approach and sequenced using an Illumina MiSeq with MiSeq Reagent Kit v3 (2 × 300 bp). All PCR procedures, primers, and Illumina sequencing details were as described in Comeau et al. (2016) and Yurgel et al. (2017).

Sequencing data processing. A Microbiome Helper standard operating procedure was used to process and analyze the sequencing data (Comeau et al. 2017). Briefly, overlapping paired-end reads were stitched together using PEAR v.0.9.6 (Zhang et al. 2014). Approximately 52.5, 94.5, and 97.9% of the 16S rRNA gene, 18S, and ITS reads, respectively, were successfully stitched. FASTX-Toolkit (v0.0.14) (Gordon and Hannon 2010) was run to filter out reads that had a quality score less than 30 at more than 10% of positions. In addition, reads shorter than 400 bp that did not contain matching 5' and 3' sequences were filtered out to the appropriate forward and reverse primers with BMap (v35.85) (Bushnell 2014). Lastly, USEARCH (v6.1) (Bushnell 2014; Edgar et al. 2011) was run to screen out chimeric reads using the options mindiv=1.5 and minh=0.2.

Operational taxonomic unit (OUT) picking and statistical analyses. Following these filtering steps, an open-reference OTU picking was run using QIIME wrapper scripts (Caporaso et al. 2010). Specifically, SortMeRNA v2.0-dev (Kopylova et al. 2012) was used for the reference OTU picking steps (with sortmerna_coverage=0.8) and sumacust v1.0.00 (Mercier et al. 2013) for the de novo OTU picking steps with 10% of the failures subsampled. Reference-based OTU picking was performed using representative subsets of the RDP (11 release) bacterial 16S rRNA (Cole et al. 2014) and Silva 111 (Glockner et al. 2017) 18S rRNA databases or UNITE (12_11 release) (Abarenkov et al. 2010) as the fungal ITS database. OTUs that contained fewer than 0.1% of the total sequences were filtered out in order to compensate for MiSeq run-to-run bleed-through (Comeau et al. 2016). The OTU tables were used to identify individual taxa whose relative abundances were associated with these variables. OTU tables were separated onto subtables with healthy and diseased samples for downstream analysis. Alpha-diversity (Shannon index) was then generated using QIIME.

Evaluation of virulence of microbial communities isolated from onion bulbs. Apparently healthy bulbs of onion cultivar Mountaineer were purchased from a local grocery store. The bulbs were surface-sterilized by saturating the surface with 70% ethanol and left for approximately 12 min to dry. The surface of the bench in the working area was disinfected with 70% ethanol and a Bunsen burner was set up to maintain an aseptic environment. The cutting surface and knife were also sterilized with 70% ethanol. Each of the onion bulbs was quarter cut in vertical longitudinally sections so that each piece contained a portion of the basal stem plate. The first quarter was placed in a plastic bag and sealed to serve as the noninoculated control treatment. The other three quarters were individually placed in plastic bags and labeled. Using a pipette, 0.1 ml of the thawed juice and crushed tissue mix that was previously prepared and frozen was placed on the cut surface of each quartered onion bulb tissue in the labeled bags and resealed. The cutting surface and knife were cleaned with 70% ethanol between samples. After the inoculation, the bagged samples were incubated at room temperature of approximately 20°C. Observations were made starting from 48 h and then every 24 h until 7 days after inoculation. Ratings were done on a scale of 1 to 5; where 1 = no visual sign of infection; 2 = may be infected and difficult to determine; 3 = visual signs of infection, not advanced, minor discoloration and some indication of cell breakdown; 4 = visual signs

of infection, advanced, obvious discoloration, indication of cell breakdown, and slight oozing; and 5 = visual signs of infection, very advanced, lots of discoloration, indication of cell breakdown, and oozing.

RESULTS

Bacterial communities associated with onion bulbs. A total of 262,339 high-quality 16S rRNA gene sequences were obtained from the healthy and the diseased onion bulb samples. Approximately 95 and 10% of high-quality 16S rRNA gene sequences obtained from the healthy and the diseased samples, respectively, were annotated as *Cyanobacteria* chloroplast and were filtered to remove plant-derived OTUs. In total, 3,604 (240 ± 368 per sample) and 175,808 ($11,720 \pm 9,008$ per sample) reads were retained in the samples obtained from healthy and diseased onions, respectively, which were distributed between 149 OTUs, at 99% identity. These OTUs comprised 53 bacterial taxa, grouped at genus level. There were 29 bacterial taxa common in both the healthy and the diseased datasets. The depth (the number of high-quality sequences) of the samples obtained from healthy bulbs was significantly ($P < 0.01$) lower than that of the diseased bulbs. This ranged between 39 and 1,484 reads for the samples obtained from healthy bulbs, and between 756 and 32,351 reads for the samples obtained from diseased bulbs (Supplementary Fig. S1).

Enterobacteriaceae (31%), *Pseudomonas* (26%), *Erwinia* (8%), and *Gluconacetobacter* (8%) were the most abundant high-quality 16S rRNA gene reads for bacteria in the healthy dataset (Fig. 1A). *Enterobacteriaceae* (38%), *Burkholderia* (31%), *Gluconobacter* (12%), *Acinetobacter* (5%), *Pseudomonas* (3%), and *Citrobacter* (1%) were the most relatively abundant high-quality 16S rRNA gene reads for bacterial taxa in the diseased dataset. *Enterobacteriaceae* OTUs were manually annotated using NCBI BLAST. *Rahnella* and *Rouxiiella* were the most abundant taxa represented by 83 and 94% of *Enterobacteriaceae* reads found in healthy and diseased datasets,

respectively (Supplementary Table S1). Together they were represented by 65 and 93% of *Enterobacteriaceae* reads found in the datasets for healthy and diseased onion bulbs, respectively. Only 3.6% of reads represented 24 OTUs unique to diseased datasets, and 78% of these reads, representing 3 OTUs, were annotated as *Acetobacteraceae*.

The diseased dataset was normalized to the depth of the smallest sample, 759 reads, which were distributed between 130 OTUs, at 99% identity. These OTUs comprised 50 bacterial taxa grouped at genus level. Bacterial communities differed in their diversity and structure within the dataset. Shannon diversity of bacterial communities ranged from 0.08 to 4.44 in the diseased samples, respectively (Fig. 2A). Considering bacterial communities associated with the diseased bulbs, most of the reads in samples D16 (99%), D17 (75%), D24 (70%), D28 (88%), and D29 (85%) were annotated as *Enterobacteriaceae*, while very little to none of the family was detected in samples D18, D23, and D25. *Gluconobacter* was a major bacterial group detected in samples D19 (72% of the reads) and D23 (98% of the reads) but it was not detected in samples D16, D28, and D29 (Fig. 2A).

Fungal communities associated with onion bulbs. In total, 857,051 high-quality ITS sequences were obtained from both the healthy and the diseased onion samples. Approximately, 99 and 90% of high-quality ITS sequences obtained from the diseased and the healthy samples, respectively, were annotated as *Allium cepa* 18S rRNA. These reads were filtered to remove plant derived OTUs. Overall, 3,404 (223 ± 227 per sample) reads for the healthy and 35,964 ($2,398 \pm 4,328$ per sample) reads for the disease datasets were retained. Comparatively, the depth of samples obtained from healthy bulbs was not significantly ($P > 0.05$) different from that of the diseased bulbs. The number of reads ranged between 25 and 803 for the healthy samples, and between 68 and 14,719 for the diseased samples, respectively. The reads were distributed between 40 OTUs at 99% identity, which comprised 22 fungal taxa, grouped at genus level (Fig. 1B). Xerophilic phylum *Basidiomycota*, *Wallemia* spp. were only detected in the healthy dataset; and yeasts, *Candida*,

Fig. 1. Microbial taxa identified in the study. **A**, Bacteria, 16S rRNA gene; **B**, fungi, ITS; and **C**, nonfungi eukaryotes, 18S rRNA. Nonnormalized samples.

Clavispora, *Meyerozyma*, and *Kazachstania* were found only in the diseased dataset. These taxa were represented by 5% of high-quality ITS sequences obtained from the diseased samples. *Botrytis*, *Penicillium*, *Saccharomyces*, *Wickerhamomyces*, and *Stemphylium* were the most relatively abundant fungal taxa in the healthy dataset and were represented by 21, 17, 15, 11, and 9% of the reads, respectively (Fig. 1B). *Nectriaceae*, *Botrytis*, *Wickerhamomyces*, *Penicillium*, and *Candida* were the most relatively abundant fungal taxa in the diseased dataset and were represented by 40, 25, 17, 9, and 3% of the reads, respectively (Fig. 1B).

The diseased dataset was normalized to the depth of the 68 reads (i.e., the depth of the smallest sample). These reads were distributed between 35 OTUs, at 99% identity, from 19 fungal taxa, grouped at genus level. Like the bacterial communities, the fungal communities differed in their diversity within the diseased dataset. Their Shannon diversity ranged from 0.11 to 3.62 for the diseased samples (Fig. 2B). In several diseased samples, a single fungal taxon was represented by the majority of the reads. For example, *Penicillium* was the most relatively abundant fungal taxon in samples D17 and D27, while *Botrytis*, *Nectriaceae*, and *Wickerhamomyces* were

Fig. 2. Relative abundances of major microbial taxa present in the microbial communities associated with diseased onions (D16 to D30). **A**, Bacteria, 16S rRNA gene; and **B**, fungi, ITS. Normalized samples. *, Shannon diversity.

represented by more than 90% of the reads in samples D30, D24, and D19, respectively. On the other hand, these fungi were relatively less abundant in some and were below detection in many other samples (Fig. 2B).

Eukaryotic communities associated with onion bulbs. A total of 718,138 high-quality 18S rRNA sequences were obtained from the diseased and the healthy samples. The reads were filtered to remove plant derived OTUs annotated as *Archaeplastida* and *Allium cepa*. A total of 9,785 18S reads were retained. The depth of the samples obtained from healthy bulbs was significantly ($P < 0.05$) less as compared with the diseased onion samples. The range was between 11 and 101 (35 ± 23 per sample) reads for the healthy samples, and between 23 and 3,487 (617 ± 1009 per sample) reads for the diseased samples. These reads were distributed between 31 eukaryotic OTUs including 25 fungal and 5 nonfungal OTUs, 3 *Nematoda Chromadorea*, 2 *Symbiodinium*, and 1 *Bombus* spp. across all samples at 99% identity. *Ascomycota*, *Saturnispora*, *Pichia*, *Hanseniaspora uvarum*, and *Galactomyces geotrichum* represented 15% of the reads in the diseased dataset. However, the reads were below detection limit in the healthy dataset. In total, nonfungal OTUs were represented by 944 reads. Only one sample, D26, was infected with *Nematoda Chromadorea* while OTU annotated as *Bombus* spp. was found in all the samples. OTUs annotated as *Symbiodinium* were detected in most samples with exception of H11, H14, and D19. While the number of reads annotated as *Symbiodinium* was relatively low across all the samples, this taxon was represented by 1 and 10% of all the 18S rRNA and nonfungal 18S rRNA reads, respectively. These OTUs were manually annotated and the correctness of the annotation was confirmed.

Pathogenic aggressiveness of microbial communities associated with healthy and diseased onion bulbs. On average, the extracts from the apparently healthy onion bulbs exhibited significantly less virulence compared with the extracts from the diseased onion bulbs. After 2 days postinoculation, rot severity was determined to be significantly ($P < 0.00001$) greater in onion bulbs inoculated with the diseased samples than in the bulbs inoculated with the healthy bulbs (Fig. 3). However, microbial communities from diseased bulb samples D23 and D27 exhibited low levels of

virulence and only very minor visual signs of infection were detected in the onion bulbs inoculated with extracts from these samples after 7 days of incubation (Supplementary Fig. S2). The incubation time and conditions of incubation might have an effect on the expression of disease symptoms in samples D23 and D27. For example, the lack of symptom expression could be linked to the prevalence of certain microorganisms, which probably did not have conducive environmental conditions during storage or required longer incubation period for disease development.

DISCUSSION

In this study, we employed a community-based approach to analyze microbiota associated with onion bulb disease under storage environments. We used a single variety of onion, which were exposed to the same pre- and postharvest factors. Each onion was considered as an individual ecological unit with the same genotype, i.e., Patterson, shared origin (place of cultivation), management practices, and storage location and conditions. Our goals were (i) to identify pathogenic communities in the onion bulbs exhibiting disease symptoms; and (ii) to evaluate the commonality of the identified pathogens in the apparently disease-free bulbs. In addition to an extension of our basic knowledge of plant-associated pathogenic communities and prevalence of plant-pathogens in environmental phytobiomes, the data presented in this study could help to identify the pathogens commonly present in Nova Scotia-produced onions and provide directions for management of onion bulb disease under storage environments in this area.

All microbial abundances reported in this study are relative and not absolute, a limitation for most 16S/18S microbiome studies. We also would like to emphasize that onion bulbs were not sterilized prior to DNA purification and it is therefore possible that some of the bacterial and eukaryotic species identified in this study could be present on the surface of the onions. This is especially important when considering microbial communities associated with apparently healthy bulbs. As a future research direction, it would be beneficial to assess microbial communities associated with external parts of the bulbs, which can clarify the mechanisms of microbial distribution in internal onion tissue.

Another important note is that a high difference in the occurrence of chloroplast derived sequences were observed in healthy and diseased onions, 95 and 10%, respectively. One possible explanation is that the internal healthy onion tissue had much lower microbial population, compared with the tissue of diseased onions. This resulted in presence of a low amount of microbial DNA template for PCR amplification and unspecific priming with plant chloroplast. A high imbalanced number reads obtained from microbial community sequencing for healthy and diseased onions did not allow direct comparison of diversity, community composition and taxa occurrence between health and diseased onions. Instead, the microbial communities from healthy tissue were used as a reference to evaluate the prevalence of pathogens in microbiota associated with apparently diseased onion bulbs.

Our first step was to look at the total microbiomes associated with healthy and diseased plant tissue. The results showed that *Enterobacteriaceae* were the most abundant bacteria in the onion tissue and comprised 31 and 38% of high-quality 16S rRNA gene reads in the healthy and diseased datasets, respectively. Members of the family *Enterobacteriaceae* such as *Pantoea*, *Dickeya* spp., and *Enterobacter cloacae* were associated with soft rot diseases of vegetables (Wakil and Oyindola 2011), and were also linked to onion bulb rot and decay (Vahling-Armstrong et al. 2015). However, manual annotation of OTUs, which were identified as *Enterobacteriaceae* in our study, showed that, while *Pantoea* was detected, *Rahnella* and *Rouxellia*

Fig. 3. Severity of onion bulb rot caused by inoculation of apparently healthy onions with extracts from healthy and diseased onion bulbs. Experiment was performed in three replicates (on three quarters of one onion). Average for inoculations with 15 extracts from healthy and diseased samples. Control: average development of disease on the noninoculated fourth quarters of the onions used for inoculation with extracts from 15 healthy and 15 diseased samples. Severity of disease development was rated on a scale of 1 to 5; where 1 = no visual sign of infection; 2 = very minor visual sign of infection; 3 = moderate visual sign of infection; 4 = advanced infection; and 5 = very advanced infection. Letters indicate significant differences between disease developments within each day of evaluation.

were the most abundant taxa in both healthy and diseased onion tissues. *Rahnella* has been identified as an organism occurring in rotting onions in New York State (Asselin et al. 2016). On the other hand, to the best of our knowledge, there are no previous reports identifying *Rouxiella* bacteria as a plant pathogen and this is the first study suggesting a role of *Rouxiella* in plant diseases. Further understanding of the role of these bacteria as onion pathogens may be beneficial to the onion industry.

Our analysis identified a number of bacterial and fungal plant pathogens, some of which were previously associated with onion diseases. *Burkholderia* (31% of total 16S rRNA gene reads) and *Pseudomonas* (3% of total reads) were among the most abundant OTUs identified in the diseased dataset. *Burkholderia* is a well-known onion pathogen that causes several onion diseases, such as slippery skin and sour skin (Burkholder 1950; Jacobs et al. 2008). *Pseudomonas* is a pectinolytic bacterium associated with onion bulb rot and decay (Vahling-Armstrong et al. 2015; Wakil and Oyinlola 2011). Of all the ITS reads from diseased dataset, 82% were annotated as *Nectriaceae*, *Fusarium*, and *Botrytis*. These fungal taxa belong to the top 10 most important plant-pathogenic fungi and are known pathogens causing bulb rot in onion (Coleman 2016; Dean et al. 2012; Williamson et al. 2007). *F. oxysporum* f. sp. *cepae* is a major concern for the Nova Scotia onion industry and controlling *Fusarium* basal rot in the field might play an even larger role in reducing storage losses to bulb rot than it was thought before.

The abundance of the fermenting bacteria *Gluconobacter*, *Acinetobacter*, and *Citrobacter* (Cheng et al. 2014; Li et al. 2017; Saichana et al. 2015; Thanh et al. 2016) and the fermenting yeasts *Wickerhamomyces* and *Candida* (Dashko et al. 2014) might be an indication of fermentation processes in the diseased tissue. Potential fermenters such as *Saccharomycetales* were relatively more abundant in the diseased dataset as compared with their healthy counterpart. *Gluconobacter* is known for its ability to ferment sugars and sugar derivatives (Saichana et al. 2015). *Citrobacter* was previously identified as one of the predominant bacteria in onion fermentation (Cheng et al. 2014). While *Acinetobacter* is considered as a food spoilage genus (Doulgeraki et al. 2012), it was recently identified as one of the genera involved in soybean and Vietnamese alcoholic fermentation (Kim et al. 2009; Li et al. 2017). Additionally, the low virulence of samples D23 and D27 could be explained by the fact that the majority of bacteria found in these samples were already known or are potential fermenters. Acetic acid bacteria, *Gluconobacter* was represented by 97% of reads in sample D23 (Fig. 2B), and *Acinetobacter* and *Citrobacter* were represented by 51 and 15% of reads in sample D27, respectively. These suggested that the apparent spoilage of samples D23 and D27 might be primarily as a result of fermentation, which was probably slowed down under normal aerobic conditions. Approximately, 73% of reads obtained from virulent sample D19 were also annotated as *Gluconobacter*. However, 16% of the reads in this sample were annotated as *Burkholderia*, a bacterial pathogen affecting onion bulbs, which might cause accelerated disease progression (Jacobs et al. 2008).

There was a substantial overlap between the composition of microbial communities associated with apparently diseased and healthy onions. For instance, 96% of 16S rRNA rRNA gene and 95% of ITS reads, obtained from diseased datasets, represented OTUs common for communities associated with both apparently diseased and healthy onions. None of the OTUs unique to the diseased dataset were annotated as potential plant pathogens. Fermenter *Acetobacteraceae* was represented by 76% of the reads that were unique to diseased 16S rRNA gene dataset. It was also found that all the reads that were unique to the diseased ITS dataset were annotated as fermented yeasts *Saccharomycetales*, which further confirm an increase in fermentation in the diseased onion bulbs.

Our data indicate that bacterial and fungal communities differ in their diversity and structure between individual onions affected by bulb rot. Within every field, there are differences in microclimates. Whereby plants have access to different amounts of fertility and moisture due to native soil variations, population fluctuations, or are subjected to differing disease and insect pest pressure due to wind patterns, topography, insect colonization habits, pesticide spray coverage, degree of mechanical damage, and so forth (Gent et al. 2004; Hildebrand and Sutton 1982; Shelton et al. 2006). These microclimates can also affect onion growth stage, which can also have an impact on plant susceptibility in the presence of pathogens (Stumpf et al. 2017). Clearly bulb rot is due to numerous different species, and exacerbated by interactions between species. Our analysis did not delve into which species might have been the primary colonizer, and which species might be opportunistic or a secondary colonizer, nor whether any of the species may have an antagonistic affect, all of which would have had an effect on community diversity and structure.

Soils are natural habitats for roundworm (*Nematoda Chromadorea*) and bumble bees (*Bombus* spp.) are native to Nova Scotia (Cameron et al. 2011). These could explain the presence of the OTUs annotated as these organisms in the onion bulb samples. However, it was unexpected to detect endosymbiotic dinoflagellates *Symbiodinium* as a part of the onion microbiome, since *Symbiodinium* is usually associated with animals found exclusively in aquatic (freshwater and marine) environments (Peixoto et al. 2017; Stat et al. 2006). *Symbiodinium* OTUs were found in most onion bulb samples and the correctness of their annotation was verified manually. This is the first report that is suggesting that *Symbiodinium* might have much broader spectrum of habitats (terrestrial) and eukaryotic hosts (plants).

In summary, the community-based approach allowed us to identify a number of potential pathogenic microorganisms associated with onion bulbs. We found that these microorganisms are also widely distributed in the bulbs without apparent disease symptoms. In addition to pathogens, several potential fermenters were detected in onion tissue. These data suggest that the fermentation plays a role in the storage spoilage of onion bulbs. The identification of dinoflagellates *Symbiodinium* in onion tissue suggests that this eukaryote might have much broader spectrum of habitats (terrestrial) and eukaryotic hosts (plants). Future work could document the occurrence of the identified bacterial and fungal communities over the course of the growing season in an effort to document which species may be the root cause of bulb rot.

LITERATURE CITED

- Abarenkov, K., Henrik Nilsson, R., Larsson, K. H., Alexander, I. J., Eberhardt, U., Erland, S., et al. 2010. The UNITE database for molecular identification of fungi—Recent updates and future perspectives. *New Phytol.* 186:281-285.
- Abbey, L., Joyce, D. C., Aked, J., Smith, B., and Marshall, C. 2004. Electronic nose evaluation of onion headspace volatiles and bulb quality as affected by nitrogen, sulphur and soil type. *Ann. Appl. Biol.* 145:41-50.
- Abdel-Rahim, I. R., Abdel-Hafez, S. I. I., and Abo-Elyousr, K. A. M. 2017. Onion purple blotch symptoms, at Assiut Governorate (Egypt), caused by synergistic association between *Alternaria porri* and *Stemphylium vesicarium*. *J. Plant Dis. Prot.* 124:195-200.
- Asselin, J. A. E., Bonasera, J. M., and Beer, S. V. 2016. PCR Primers for detection of *Pantoea ananatis*, *Burkholderia* spp., and *Enterobacter* sp. from onion. *Plant Dis.* 100:836-846.
- Borer, E. T., Laine, A. L., and Seabloom, E. W. 2016. A multiscale approach to plant disease using the metacommunity concept. *Annu. Rev. Phytopathol.* 54: 397-418.
- Burkholder, W. H. 1950. Sour skin, a bacterial rot of Onion bulbs. *Phytopathology* 40:115-117.
- Bushnell B. 2014. BBMap: A fast, accurate, splice-aware aligner. Report Number LBNL-7065E.

- Cameron, S. A., Lozier, J. D., Strange, J. P., Koch, J. B., Cordes, N., Solter, L. F., and Griswold, T. L. 2011. Patterns of widespread decline in North American bumble bees. *Proc. Natl. Acad. Sci. USA* 108:662-667.
- Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., et al. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* 7:335-336.
- Cheng, L., Luo, J., Li, P., Yu, H., Huang, J., and Luo, L. 2014. Microbial diversity and flavor formation in onion fermentation. *Food Funct.* 5:2338-2347.
- Christensen, C. M. 1973. Loss of viability in storage microflora. *Seed Sci. Technol.* 1:547-562.
- Cole, J. R., Wang, Q., Fish, J. A., Chai, B., McGarrell, D. M., Sun, Y., et al. 2014. Ribosomal Database Project: data and tools for high throughput rRNA analysis. *Nucleic Acids Res.* D1:D633-D642.
- Coleman, J. J. 2016. The *Fusarium solani* species complex: ubiquitous pathogens of agricultural importance. *Mol. Plant Pathol.* 17:146-158.
- Comeau, A. M., Douglas, G. M., and Langille, M. G. 2017. Microbiome Helper: A custom and streamlined workflow for microbiome research. *mSystems* 2:e00127-16.
- Comeau, A. M., Vincent, W. F., Bernier, L., and Lovejoy, C. 2016. Novel chytrid lineages dominate fungal sequences in diverse marine and freshwater habitats. *Sci. Rep.* 6:30120.
- Dashko, S., Zhou, N., Compagno, C., and Piskur, J. 2014. Why, when, and how did yeast evolve alcoholic fermentation? *FEMS Yeast Res.* 14: 826-832.
- Dean, R., Van Kan, J. A., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D., et al. 2012. The Top 10 fungal pathogens in molecular plant pathology. *Mol. Plant Pathol.* 13:414-430.
- Doulgeraki, A. I., Ercolini, D., Villani, F., and Nychas, G. J. 2012. Spoilage microbiota associated to the storage of raw meat in different conditions. *Int. J. Food Microbiol.* 157:130-141.
- 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.
- FAOSTAT. 2017. Food and agriculture data—Crops. Food and Agricultural Organization of the United Nations, Rome, Italy.
- Fitt, B. D., Huang, Y. J., van den Bosch, F., and West, J. S. 2006. Coexistence of related pathogen species on arable crops in space and time. *Annu. Rev. Phytopathol.* 44:163-182.
- Gent, D. H., Schwartz, H. F. 2004. Distribution and incidence of *Iris yellow spot virus* in Colorado and its relation to onion plant population and yield. *Plant Dis.* 88:446-452.
- Glockner, F. O., Yilmaz, P., Quast, C., Gerken, J., Beccati, A., Ciuprina, A., et al. 2017. 25 years of serving the community with ribosomal RNA gene reference databases and tools. *J. Biotechnol.* 261:169-176.
- Gordon, A., and Hannon, G. 2010. FASTX-Toolkit: FASTQ/A short-reads preprocessing tools. http://hannonlab.cshl.edu/fastx_toolkit
- Haapalainen, M., Latvala, S., Kuivainen, E., Qiu, Y., Segerstedt, M., and Hannukkala, A. O. 2016. *Fusarium oxysporum*, *F. proliferatum* and *F. redolens* associated with basal rot of onion in Finland. *Plant Pathol.* 65:1310-1320.
- Hildebrand, P. D., and Sutton, J. C. 1982. Weather variables in relation to an epidemic of onion downy mildew. *Phytopathology* 72:219-224.
- Jacobs, J. L., Fasi, A. C., Ramette, A., Smith, J. J., Hammerschmidt, R., and Sundin, G. W. 2008. Identification and onion pathogenicity of *Burkholderia cepacia* complex isolates from the onion rhizosphere and onion field soil. *Appl. Environ. Microbiol.* 74:3121-3129.
- Kim, T. W., Lee, J. H., Kim, S. E., Park, M. H., Chang, H. C., and Kim, H. Y. 2009. Analysis of microbial communities in doenjang, a Korean fermented soybean paste, using nested PCR-denaturing gradient gel electrophoresis. *Int. J. Food Microbiol.* 131:265-271.
- King, A. D. J., and Schade, J. E. 1984. Alternaria toxins and their importance in food. *J. Food Prot.* 47:886-901.
- Kopylova, E., Noe, L., and Touzet, H. 2012. SortMeRNA: Fast and accurate filtering of ribosomal RNAs in metatranscriptomic data. *Bioinformatics* 28:3211-3217.
- Lamichhane, J. R. 2015. Bacterial diseases of crops: elucidation of the factors that lead to differences between field and experimental infections. Pages 227-246 in: *Advances in Agronomy*. Elsevier.
- Lamichhane, J. R., and Venturi, V. 2015. Synergisms between microbial pathogens in plant disease complexes: A growing trend. *Front. Plant Sci.* 6:385.
- Li, Z., Rui, J., Li, X., Li, J., Dong, L., Huang, Q., et al. 2017. Bacterial community succession and metabolite changes during doubanjiang-meju fermentation, a Chinese traditional fermented broad bean (*Vicia faba* L.) paste. *Food Chem.* 218:534-542.
- Mercier, C., Boyer, F., Bonin, A., and Coissac, E. 2013. SUMATRA and SUMACLUSt: Fast and exact comparison and clustering of sequences. <http://metabarcoding.org/sumatra>
- Peixoto, R. S., Rosado, P. M., Leite, D. C., Rosado, A. S., and Bourne, D. G. 2017. Beneficial microorganisms for corals (BMC): Proposed mechanisms for coral health and resilience. *Front. Microbiol.* 8:341.
- Rasiukeviciute, N., Suproniene, S., and Valiuskaite, A. 2016. Effective onion leaf fleck management and variability of storage pathogens. *Open Life Sci.* 11:259-269.
- Saichana, N., Matsushita, K., Adachi, O., Frebort, I., and Frebortova, J. 2015. Acetic acid bacteria: A group of bacteria with versatile biotechnological applications. *Biotechnol. Adv.* 33:1260-1271.
- Shelton, A. M., Zhao, J. Z., Nault, B. A., Plate, J., Musser, F. R., and Larentzaki, E. 2006. Patterns of insecticide resistance in onion thrips (Thysanoptera: Thripidae) in onion fields in New York. *Insect. Resist. Manage.* 99: 1798-1804.
- Stat, M., Carter, D., and Hoegh-Guldberg, O. 2006. The evolutionary history of symbiodinium and scleractinian hosts—Symbiosis, diversity, and the effect of climate change. *Plant Ecol. Evol. Syst.* 8:23-43.
- Stumpf, S., Gitaitis, R., Coolong, T., Riner, C., and Dutta, B. 2017. Interaction of onion cultivar and growth stages on incidence of *Pantoea ananatis* bulb infection. *Plant Dis.* 101:1616-1620.
- Thanh, V. N., Thuy, N. T., Chi, N. T., Hien, D. D., Ha, B. T., Luong, D. T., Ngoc, P. D., and Ty, P. V. 2016. New insight into microbial diversity and functions in traditional Vietnamese alcoholic fermentation. *Int. J. Food Microbiol.* 232:15-21.
- Vahling-Armstrong, H. P., Dung, J. K. S., Humann, J. L., and Schroeder, B. K. 2015. Effects of postharvest onion curing parameters on bulb rot caused by *Pantoea agglomerans*, *Pantoea ananatis* and *Pantoea allii* in storage. *Plant Pathol.* 65:536-544.
- Wakil, S. M., and Oyindola, K. A. 2011. Diversity of pectinolytic bacteria causing soft rot disease of vegetables in Ibadan, Nigeria. *J. Appl. Biosci.* 38:2540-2550.
- Williamson, B., Tudzynski, B., Tudzynski, P., and van Kan, J. A. 2007. Botrytis cinerea: the cause of grey mould disease. *Mol. Plant Pathol.* 8:561-580.
- Yurgel, S. N., Douglas, G. M., Comeau, A. M., Mammoliti, M., Dusault, A., Percival, D., et al. 2017. Variation in bacterial and eukaryotic communities associated with natural and managed wild blueberry habitats. *Phytophormes* 1:102-113.
- Zhang, J., Kobert, K., Flouri, T., and Stamatakis, A. 2014. PEAR: A fast and accurate Illumina paired-end reAd mergeR. *Bioinformatics* 30:614-620.