Microbiological Research 206 (2018) 1–8

Contents lists available at ScienceDirect

Microbiological Research

journal homepage: www.elsevier.com/locate/micres

Distribution of apple and blackcurrant microbiota in Lithuania and the Czech Republic

Iglė Vepštaitė-Monstavičė[a], Juliana Lukša[a], Ramunė Stanevičienė[a], Živilė Strazdaitė-Žielienė[a], Vyacheslav Yurchenko[b,c], Saulius Serva[d,e], Elena Servienė[a,e,*]

[a] Laboratory of Genetics, Institute of Botany, Nature Research Centre, Akademijos str. 2, Vilnius LT-08412, Lithuania
[b] Life Science Research Centre and Institute of Environmental Technologies, Faculty of Science, University of Ostrava, Chittussiho 10, 70200 Ostrava, Czech Republic
[c] Biology Centre, Institute of Parasitology, Czech Academy of Sciences, Branišovska 1160/31, 370 05 Ceske Budejovice (Budweis), Czech Republic
[d] Department of Biochemistry and Molecular Biology, Institute of Biosciences, Vilnius University, Saulėtekio al. 7, Vilnius LT-10257, Lithuania
[e] Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Saulėtekio al. 11, Vilnius LT-10223, Lithuania

ARTICLE INFO

Keywords:
Metagenomic analysis
Microbiota
Apple
Blackcurrant

ABSTRACT

The microbial assemblies on the surface of plants correlate with specific climatic features, suggesting a direct link between environmental conditions and microbial inhabitation patterns. At the same time however, microbial communities demonstrate distinct profiles depending on the plant species and region of origin. In this study, we report Next Generation Sequencing-based metagenomic analysis of microbial communities associated with apple and blackcurrant fruits harvested from Lithuania and the Czech Republic. Differences in the taxonomic composition of eukaryotic and prokaryotic microorganisms were observed between plant types. Our results revealed limited geographic differentiation between the bacterial and fungal communities associated with apples. In contrast, blackcurrant berries harvested from different regions demonstrated high diversity in both bacterial and fungal microbiota structures. Among fungal and bacterial microorganisms, we identified both potentially beneficial (*Cryptococcus, Hanseniaspora*, *Massilia*, *Rhodotorula, Sphingomonas*) and phytopathogenic microorganisms (*Cladosporium*, *Pantoea*, *Phoma*, *Pseudomonas, Septoria, Taphrina*) indicating their important roles in ecological and evolutionary processes.

1. Introduction

Plants host many microorganisms that colonize the surface of fruits, leaves, flowers and stems, as well as within their tissues (Abdelfattah et al., 2016a). The distribution of microorganisms on fruits is defined by a continuum of factors, including plant species, geographic location, climatic conditions, ripening stage and the application of agrochemicals (Pretorius, 2000; Pinto et al., 2014, 2015). The microorganism community year-to-year is characterized by the appearance of many new patterns, indicating that the behavior of most of the strains is not perennial. Fungi and bacteria inhabiting the fruit surface may be transported from the soil to the plants by insects and other animal species (Valero et al., 2007; Stefanini et al., 2015). On the other hand, some microorganisms, particularly yeast, could be permanent residents on fruits employing the latter as depositorium for survival and propagation. Microorganisms naturally associated with fruits may be beneficial and induce resistance in the hosting plant (e.g. *Cryptococcus*, *Sphingomonas*) or phytopathogenic and responsible for significant economic losses (e.g. *Phoma*, *Pantoea*) (Coutinho and Venter, 2009; Liu et al., 2013; Abdelfattah et al., 2016a). The interactions between different microorganism species may influence the structure of microbial communities inhabiting the fruit surface and through either direct or indirect impact on the plant can mediate many ecological and evolutionary processes (Friesen et al., 2011; Alvarez-Perez and Herrera, 2013).

The fungal and bacterial communities can be very diverse and will be defined by the associated plant species (Pinto et al., 2014). However, geographic location and farming practice also significantly influence microbial diversity (Leff and Fierer, 2013). Until now, the biogeographic distribution of microbiota communities has been studied mainly on grapes, the essential resource for wine production (Setati et al., 2012; Pinto et al., 2015; Wang et al., 2015). Only a limited number of studies on microorganisms residing on plums, apples, pears, cherries, and strawberries have been reported (Janisiewicz et al., 2014; Abdelfattah et al., 2016a, 2016b; Clooney et al., 2016; Volschenk et al., 2016). Few of them were dedicated to comparison of fruit-associated fungal communities differing in location (Setati et al., 2012; Bokulich et al., 2014; Taylor et al., 2014).

* Corresponding author at: Laboratory of Genetics, Institute of Botany, Nature Research Centre, Akademijos str. 2, Vilnius LT-08412, Lithuania.
E-mail address: elena.serviene@botanika.lt (E. Servienė).

http://dx.doi.org/10.1016/j.micres.2017.09.004
Received 22 June 2017; Received in revised form 6 September 2017; Accepted 9 September 2017
Available online 14 September 2017
0944-5013/

The domesticated apple (*Malus pumila* Mill.) is a worldwide-grown major temperate fruit crop. Like many other fruits, apple is colonized by a number of different microorganisms and could be affected by several different phytopathogens (Teixidó et al., 1999; Graca et al., 2015). Current knowledge about the apple microbiota is limited and largely focused on species that cause disease and thus pose economic threats (Teixidó et al., 1999; Abadias et al., 2006). Another research focus is related to natural antagonists that could be used as biological control agents against phytopathogens (Piano et al., 1997). To date, only one comprehensive report on the fungal community associated with organic and conventionally grown apples in the state of Washington, USA, has been published (Abdelfattah et al., 2016b). It was demonstrated that the phylum Ascomycota was dominant on apples, followed by Basidiomycota and Chytridiomycota. Communities of fungal microorganisms differ depending on the parts of the apple fruit (e.g. *Cryptococcus* and *Alternaria* were most abundant on the stem and calyx; *Penicillium* – in peel and wounded flesh; while *Mycosphaerella* was found exclusively in the calyx). Bacterial communities associated with apples (in Colorado, USA) consisted of two most abundant groups – Microbacteriaceae and Sphingomonadaceae (Leff and Fierer, 2013). The apples used for that studies were purchased from a local supermarket or grocery store, thus analysis was conducted not immediately after collection. It is possible therefore that external conditions such as fruit storage and transportation as well as period of time before performing molecular analysis may have impacted the structure of microbiota due to decreasing survival of fruit-associated microorganisms or involving contaminating ones.

Blackcurrant (*Ribes nigrum* L.) is a native temperate crop widely cultivated both commercially and domestically in the major part of Europe and northern Asia. Even in the USA, there is a growing interest in expanding *Ribes* production (Hummer and Dale, 2010). The berries are rich in polyphenols and vitamin C, thus are attractive for regulation of the gut and intestinal microbiota, protecting against anti-inflammatory degenerative disorders or even cancer in humans (Paredes-Lopez et al., 2010; Tabart et al., 2012). The blackcurrants or their extracts are also widely used in food and beverage manufacturing. The broad interest in growing and application of blackcurrants demand investigation of the microbial communities colonizing the surface of these berries. To the best of our knowledge, no reports on the blackcurrant fungal and bacterial microbiota have been presented thus far.

The objective of the present study was to identify the composition of the bacterial and fungal microbiota closely associated with apples and blackcurrants collected in Lithuania and the Czech Republic. The identification and quantification of fruit and berry microflora expanded current knowledge about the structure of plant-associated bacterial and fungal communities, and revealed the biogeographic distribution of microbiota on apples and blackcurrants, as well as provided valuable information on the impact of environmental factors on the distribution of these microbial populations.

2. Materials and methods

2.1. Ethics statement

The collection of samples was carried out on private land and the owner of the land gave permission to conduct the study on site. It did not involve endangered or protected species.

2.2. Sampling of the fruits and DNA extraction

The domesticated apples (*Malus pumila* Mill.) were aseptically collected in the late-August 2016 on the private farms located in the Vilnius region of Lithuania (GPS coordinates: 54°75′20.0″N, 25°27′99.6″E) and Ostrava region of the Czech Republic (GPS coordinates: 49°83′03.9″N, 18°17′47.3″E). Blackcurrants (*Ribes nigrum* L.) were sampled from the Ignalina region of Lithuania (GPS coordinates: 55°34′23.0″N, 26°16′46.8″E) and Ostrava region of the Czech Republic (GPS coordinates: 49°83′03.9″N, 18°17′47.3″E) in the mid-July 2016. The fruits were collected into sterile plastic bags and processed within 2–4 h after harvesting. Fruits of interest (300 g) were placed in 500 mL of sterile 0.05 M phosphate buffer pH 6.8 for 30 min (in the case of blackcurrants) and 2 h (for apples) with shaking at120 rpm. Outwashes were filtered through 420 μm filters, centrifuged at 12,000 g for 20 min, and precipitates were stored at −20 °C until subsequent analysis.

For metagenomic analysis, 40 mg of pellet per sample was used. DNA isolation from collected sediments was performed using a Genomic DNA purification kit (Thermo Fisher Scientific Baltics, Vilnius, Lithuania) in accordance with the manufacturer's instructions. The quantity and quality of extracted DNA were determined using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific).

2.3. Bacterial and fungal DNA amplification and amplicon library preparation

DNA samples from apples and blackcurrant microbiota were amplified using the primers specific for fungi and bacteria. For identification of fungal microorganisms, the ITS2 region of ribosomal DNA was amplified using ITS3-KYO2 (5′-GATGAAGAACGYAGYRAA-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) primers (Toju et al., 2012). For bacteria identification, the V3-V4 region of the 16S rRNA gene was amplified with primers S-D-Bact-0341-b-*S*-17 (5′-CCTACGGGNGGCWGCAG-3′) and S-D-Bact-0785-a-A-21 (5′-GACTACHVGGGTATCTAATCC-3′) (Klindworth et al., 2013). Amplicon libraries were prepared using modified Illumina adapters (www.illumina.com), validated on an Agilent Technologies Bioanalyzer DNA 1000 and sequenced using Illumina MiSeq V3 (2 × 300 bp) (Macrogen Inc., Seoul, Korea). All sequences obtained during this work are available at the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI), under accession number SRP108314.

2.4. Data processing and analysis

The bioinformatics pipelines, FLASH 1.2.11 (Magoc and Salzberg, 2011), CD-HIT-OTU 4.5.5 (Li et al., 2012), and QIIME v. 1.8 (Caporaso et al., 2010), were used to process and analyze the obtained sequence data. Preliminary processing of the data was performed using the default parameters of FLASH 1.2.11: sequences with a minimum quality score of 25 were filtered and paired-end reads were merged. Sequences were denoised, chimeric sequences were identified and filtered, and the remaining reads were clustered into the Operational Taxonomical Units (OTUs) with a minimum 97% similarity threshold, using the CD-HIT-OTU 4.5.5 (Li et al., 2012). The most abundant sequences in each OTU were used for the taxonomy assignments using the RDP (Ribosomal Database Project) (Wang et al., 2007; Cole et al., 2014) and the UNITE (Koljalg et al., 2013) databases as references. For downstream analysis, the OTU table was rarefied at an even depth to reduce biases in sequencing depth. Alpha diversity was calculated using observed species, Shannon, Good's coverage and Chao1 estimates (Caporaso et al., 2010). Weighted Unifrac algorithm was used to evaluate β-diversity (Lozupone and Knight, 2005). Principal coordinates analysis (PCoA), as implemented in QIIME v. 1.8, related the bacterial and fungal microbiota composition to sample types and examined the distance between different ecosystems.

2.5. Cultivable yeast enrichment and identification

The aseptically collected apple and blackcurrant fruits (30 g each) were kept in 5% dextrose solution for 15 days at a temperature of 22 °C. Serial dilutions were made in a Ringer solution (Merck, Kenilworth, United States), plated on YEPD-agar plates (1% yeast extract, 1% peptone, 2% dextrose, 2% agar) containing 50 μg/mL chloramphenicol

and incubated for 2–3 days at 25 °C. Randomly selected colonies with yeast-like morphology were used for molecular identification. DNA was isolated from fresh yeast culture (24 h) by using Genomic DNA purification kit (Thermo Fisher Scientific Baltics, Vilnius, Lithuania) in accordance with the manufacturer's instructions. For identification of yeast, the region between the 18S rRNA and 28S rRNA genes was amplified using ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) primers. The PCR was performed in a total reaction of 50 µL, consisting of 5 µL DreamTaq green buffer, 1 µL of 2 mM dNTP mix, 1 µL of each primer (10 µmol/L), 2.5 unit of Dream Taq DNA polymerase (all from Thermo Fisher Scientific Baltics, Vilnius, Lithuania), 1 µL of DNA template (5 ng) and sterile distilled water up to 50 µL. PCR amplification was carried out by Esco thermocycler, according to the following PCR conditions: an initial denaturation at 94 °C for 5 min, followed by 25 cycles of 94 °C for 1 min, 53 °C for 1 min 30 s and 72 °C for 2 min. The final extension was carried out at 72 °C for 10 min. The PCR products were digested with CfoI and HinfI enzymes and tested by 1% agarose gel electrophoresis. PCR products differing in restriction profiles were purified using a GeneJet PCR purification kit (Thermo Fisher Scientific Baltics, Vilnius, Lithuania), according to the manufacturer's instructions and sequenced using ITS1 and/or ITS4 primers at BaseClear (Leiden, Netherlands). The obtained sequences were compared with those found in the FASTA network service of the EMBL-EBI database (http://www.ebi.ac.uk/Tools/sss/fasta/nucleotide.html).

3. Results

3.1. Diversity and richness of microbial communities

In this study, we assessed and compared the microbial communities of apples and blackcurrants sampled from Lithuania and the Czech Republic by DNA massive parallel sequencing of 16S rDNA for bacteria and ITS2 for fungal analysis. After quality evaluation, a total 1,466,580 high quality sequences were recovered (967,110 eukaryotic and 499,470 prokaryotic sequences). The clustering of the sequences generated a total of 1378 OTUs (940 [264 ± 95, hereafter median for 4 samples ± standard deviation] for fungal ITS2 and 438 [99 ± 24] for bacterial V3-V4) (Table 1). The total number of OTUs detected in individual samples varied from 85 to 328. Based on analysis of prokaryotic sequences, the highest number of OTUs was detected in blackcurrants sampled in Lithuania (Table 1). In agreement with OTU data, the Shannon's Diversity and the Chao1 estimates also revealed that blackcurrant berries had a higher bacterial diversity than apples. The analysis of eukaryotic sequences indicated that the Apple_CZ sample had the highest number of fungal OTUs, followed by Blackcurrant_CZ, Apple_LT and Blackcurrant_LT (Table 1).

The ratio between the number of the obtained and the expected OTUs (predicted by Chao1) was used to determine the coverage for the microbial communities. It was over 90% in all cases, indicating a good coverage. Rarefaction curve showed that the numbers of OTUs were saturated in all samples, making them suitable for further community analysis (Fig. 1).

Principal Coordinate Analysis (PCoA) performed with the representative OTUs showed a clear separation of apple and blackcurrant, indicating a difference in the composition of the bacterial and fungal microbiota (Fig. 2). The differences were mostly observed at the lower taxonomic levels (see Supplementary Tables S1 and S2 in the online version at DOI: http://dx.doi.org/10.1016/j.micres.2017.09.004). Bacterial microbiota of apples was clustered into a similar plot (Fig. 2A), while fungal microbiota of regionally distinct apples was slightly separated (Fig. 2B). The communities of prokaryotic and eukaryotic microorganisms on blackcurrants sampled in Lithuania and the Czech Republic were clearly separated in PCoA plots from each other and from apples (Fig. 2).

3.2. Composition of apple bacterial and fungal microbiota

The populations of fungal and bacterial microbiota at the phylum level were similar on both apples (Apple_LT and Apple_CZ) collected from the geographically distinct regions of Lithuania and the Czech Republic. The dominant phylum across the entire eukaryotic microorganism population was Ascomycota (86.3% and 86.8% in Apple_LT and Apple_CZ respectively), supplemented by Basidiomycota (12.5% and 11.2%) (Fig. 3A I). Regarding the bacterial population, Proteobacteria (95.8% on Apple_LT and 96.9% on Apple_CZ) dominated in both localities (Fig. 3A II, Table S2 in the online version at DOI: http://dx.doi.org/10.1016/j.micres.2017.09.004). The bacterial diversity was evident when we analyzed sub-phyla distribution (Figs. 3 B II, 4 A). Gammaproteobacteria dominated on both apple samples (81.6% in Apple_LT and 75.8% in Apple_CZ) (Fig. 3B II), represented by Enterobacteriaceae (48.9% in Apple_LT and 34.2% in Apple_CZ) and Pseudomonadaceae (32.6% and 41.3% respectively) (Fig. 4A). Fungal microorganisms mainly consisted of Saccharomycetes (70.2% in Apple_LT and 63.0% in Apple_CZ) (Fig. 3B I), which were not characterized at the genus (Fig. 4B) or at species level and were assigned to uncultured *Metschnikowiaceae* spp. (66.7% and 44.3% respectively) (see Supplementary Table S2 in the online version at DOI: http://dx.doi.org/10.1016/j.micres.2017.09.004). Slight differences of distribution of apple-associated fungal microorganisms were observed only at the genus level. From identified fungal microorganisms, *Cryptococcus* (5.7%), *Cladosporium* (4.2%) and *Hanseniaspora* (3.3%) dominated in Lithuania, while on apples sampled in the Czech Republic, *Hanseniaspora* represented the most abundant fraction (17.6%). Some genera were detected only in one location: for example, on apples grown in Lithuania, we observed *Stachybotrys*, while in the Apple_CZ sample – *Wickerhamomyces* (Fig. 4B). Same cultivable yeast species, such as *Issatchenkia terricola*, *Pichia fermentans*, *Torulaspora delbrueckii*, *Saccharomyces cerevisiae*, were weakly distinguished by NGS analysis (see Supplementary Table S2 in the online version at DOI: http://dx.

Table 1
Total sequences obtained for eukaryotic (ITS2) and prokaryotic (V3-V4) microbial community for apple and blackcurrant samples.

Samples	Target region	High quality reads	OTUs	Chao1	Coverage	Shannon diversity
Apple_LT	ITS2	251,647	225	225.13	0.9994	2.73
	V3-V4	117,934	90	91	0.989	2.31
Apple_CZ	ITS2	305,477	328	339.33	0.9666	2.91
	V3-V4	141,529	104	115	0.9043	2.59
Blackcurrant_LT	ITS2	128,244	85	92.5	0.9297	2.93
	V3-V4	117,227	150	160	0.9375	5.4
Blackcurrant_CZ	ITS2	281,742	302	306.23	0.9862	2.98
	V3-V4	122,780	94	96	0.9792	4.95
	Eukaryotic	967,110	940			
	Prokaryotic	499,470	438			
	Total	1,466,580	1,378			

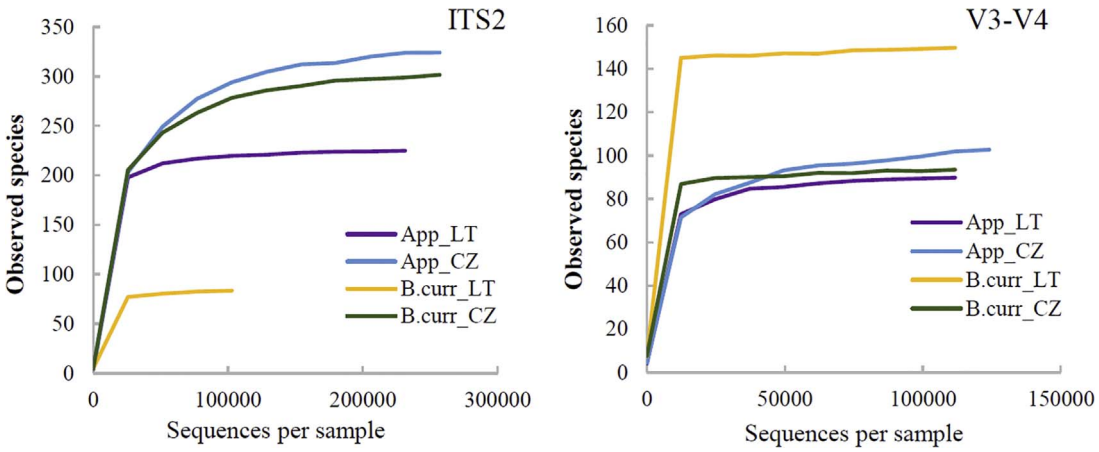

Fig. 1. Rarefaction curves at a genetic distance of 3% for each sample. ITS2 sequences from the analysis of the population of eukaryotic microorganisms and V3-V4 region sequences from the analysis of the community of prokaryotic microorganisms.

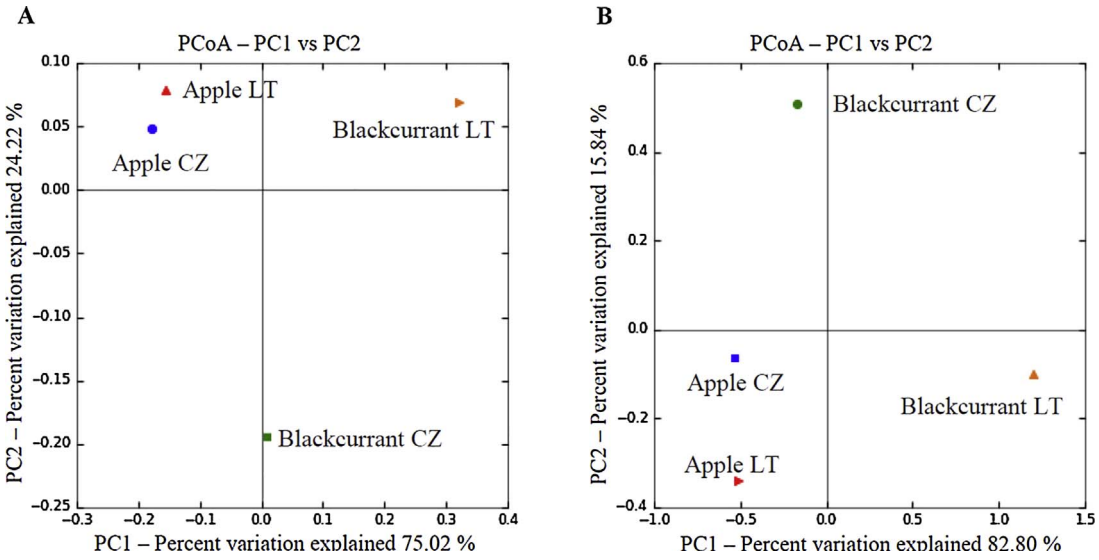

Fig. 2. Principal component analysis (PCoA) profiles based on the structure of bacterial (A) and fungal microorganisms (B) community.

doi.org/10.1016/j.micres.2017.09.004), but were detected after isolation from both apple samples by applying enrichment and cultivation techniques (see Supplementary Table S3 in the online version at DOI: http://dx.doi.org/10.1016/j.micres.2017.09.004).

3.3. Composition of blackcurrant bacterial and fungal microbiota

The distribution of fungal microorganisms at the phylum level was similar on blackcurrant berries located in both Lithuania and the Czech Republic. The dominant phylum across the entire eukaryotic microorganism population was Ascomycota (71.5% and 92.2% in Blackcurrant_LT and Blackcurrant_CZ respectively), though it also contained Basidiomycota (24.2% and 7.0%, respectively) and other unidentified fungi (Fig. 3A I**).** The distribution and abundance of bacteria varied depending on sampling geography (Fig. 3A II). Blackcurrants sampled in Lithuania were dominated by Firmicutes (35.4%), Proteobacteria (26.9%) and Actinobacteria (20.0%). However, on the blackcurrant berries harvested in the Czech Republic, the most abundant bacterial phylum was Proteobacteria (71.8%).

The broad diversity and variation of bacterial and fungal microorganisms among the blackcurrant samples harvested in Lithuania and the Czech Republic were evident at the family and genus level (Fig. 4). Among the dominant bacterial OTUs, Staphylococcaceae (27.1%), Flavobacteriaceae (7.2%) and Moraxellaceae (5.7%) were the most abundant families in Blackcurrant_LT, while the Blackcurrant_CZ sample was dominated by Enterobacteriaceae (20.4%), followed by Oxalobacteraceae (8.4%), Pseudomonadaceae (7.8%), Sphingomonadaceae (7.4%), Comamonadaceae (7.0%), Cytophagaceae (7.0%), Acetobacteraceae (6.1%), and Sphingobacteriaceae (5.4%) (Fig. 4A). The analysis of distribution of fungal microorganisms on blackcurrants harvested in Lithuania revealed that the most dominant genera were *Cladosporium* (45.4%) and *Cryptococcus* (15.2%), while the berries collected from the Czech Republic were dominated by *Hanseniaspora* (48.5%), followed by *Cladosporium* (5.2%) and *Rhodotorula* (1.5%) (Fig. 4B; see Supplementary Table S2 in the online version at DOI: http://dx.doi.org/10.1016/j.micres.2017.09.004).

4. Discussion

The distribution of microorganisms depends on plant species and may be affected by growing, ripening and storage conditions (Pinto et al., 2015). However, it is difficult to establish major and specific factors responsible for driving the divergence of bacterial and fungal communities (Leff and Fierer, 2013) since different factors have a cumulative effect. The current study evaluated the distribution of bacterial and fungal microorganisms found on apple fruits and blackcurrant berries, grown in distinct regions in Lithuania and the Czech Republic. None of the sampled plants received any chemical treatment

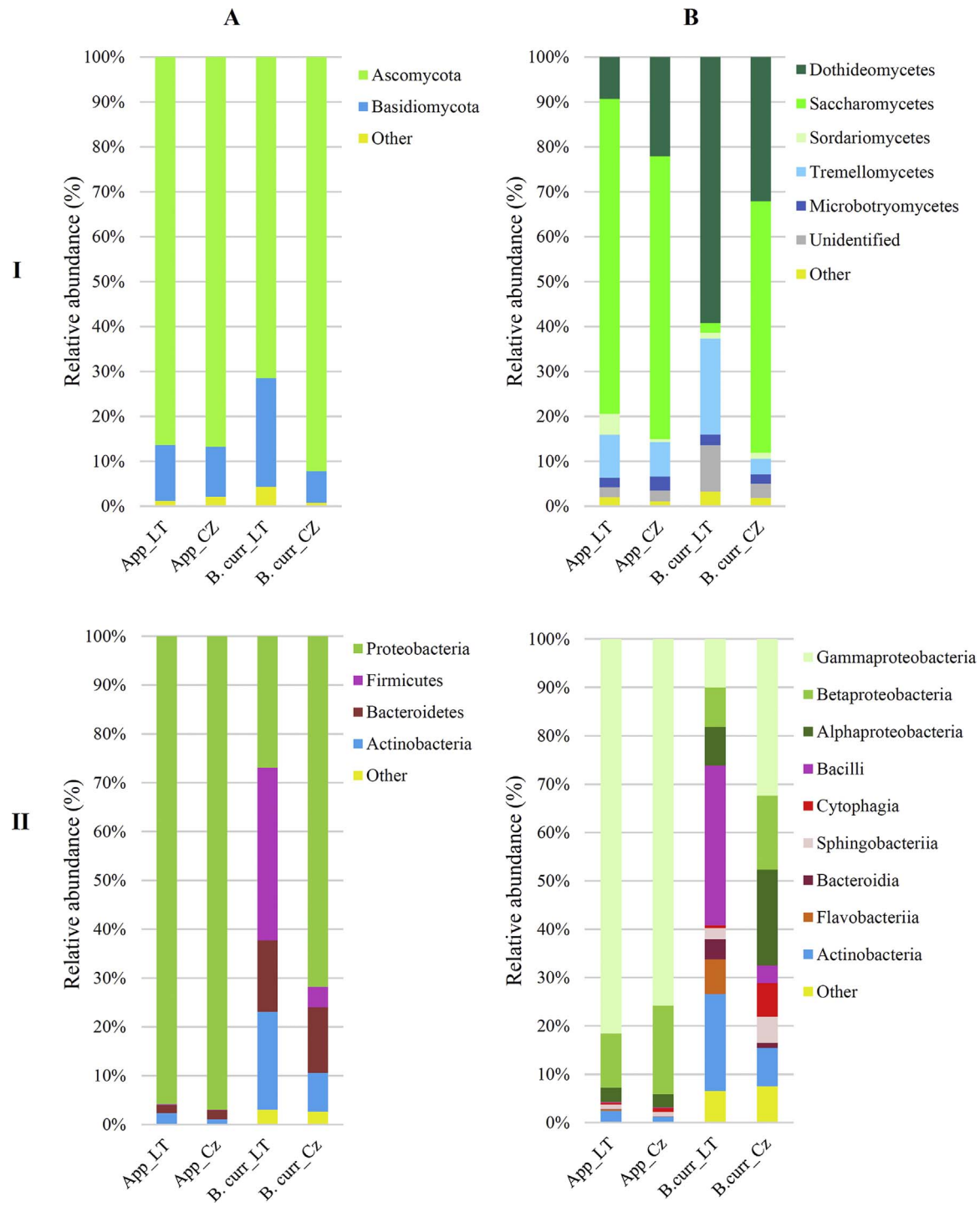

Fig. 3. Eukaryotic (I) and prokaryotic (II) microbial community distribution at the phylum (A) and class (B) level.
App_LT – apples sampled in Lithuania, App_CZ – apples collected in the Czech Republic; B. curr_LT – blackcurrant berries sampled in Lithuania, B. curr_CZ – blackcurrant berries collected in the Czech Republic.

and were analyzed immediately after sampling, thus minimizing external impact on the composition of microbiota.

We found that the bacterial communities from the apple surface were dominated by Gammaproteobacteria, mostly represented by the family Enterobacteriaceae. This is barely surprising, given that Gammaproteobacteria (as many other bacteria) is recognized initial degraders of organic matter contributing to the release of nutrients such as phosphorus and nitrogen (Sarr et al., 2017). However, our data differ from previous observations (Leff and Fierer, 2013), where the most abundant bacterial class on apples (purchased from a grocery store in Boulder, CO, USA) was Alphaproteobacteria, mostly the Sphingomonadaceae family with Enterobacteriaceae detected in lower frequency. By applying culture-dependent techniques, no Enterobacteriaceae were detected on apples that had been fresh-cut and purchased from different supermarkets of Spain (Abadias et al., 2008). In our case, irrespective of geographical location (samples from both Lithuania and the Czech Republic), more than two thirds of species were representatives of Gammaproteobacteria, such as *Pantoea* spp. and uncultured *Pseudomonas* (see Supplementary Table S1 in the online version at DOI: http://dx.doi.org/10.1016/j.micres.2017.09.004). Certain *Pantoea* or

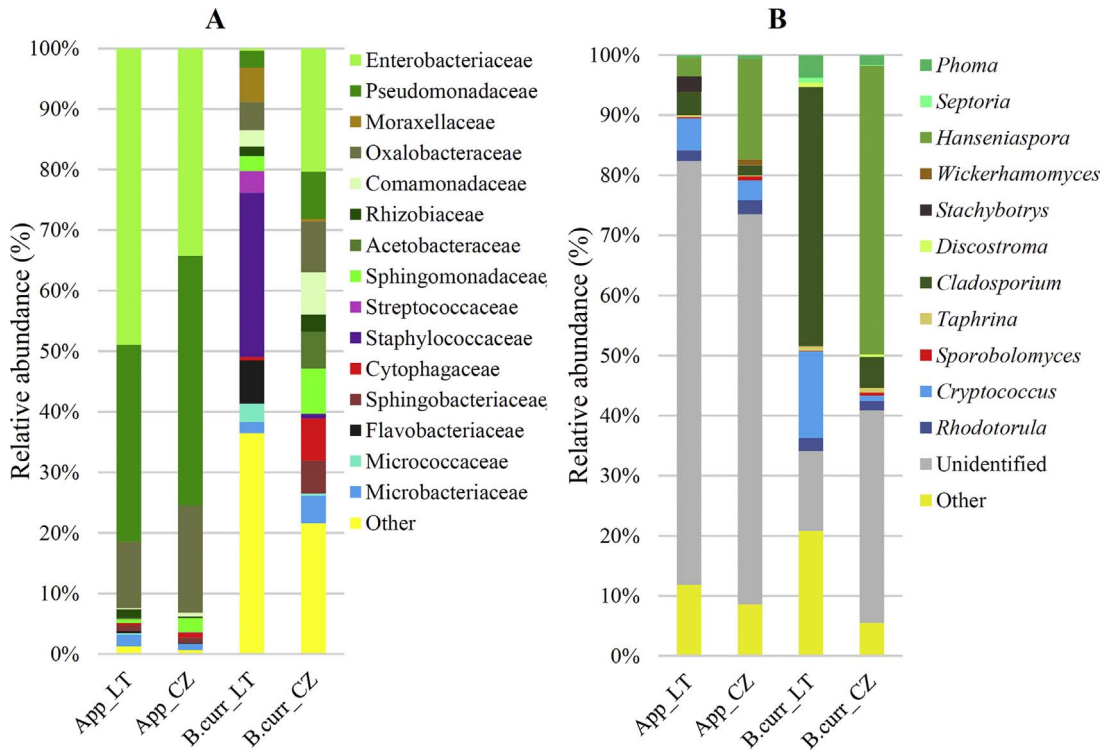

Fig. 4. Relative abundance of major bacterial families (A) and fungal microorganism genera (B) present on apples and blackcurrants sampled in Lithuania and the Czech Republic.

Pseudomonas species are well-known plant pathogens responsible for economic losses (Coutinho and Venter, 2009). On the other hand, they could produce antibacterial and antifungal agents protecting hosts from disease (Ligon et al., 2000; Enya et al., 2007). The rest of the bacterial population consisted of representatives of different genera, such as *Duganella*, *Massilia*, *Sphingomonas*, etc., which could be beneficial to plants due to their ability to induce plant resistance and promote growth (Kim et al., 1998; Ofek et al., 2012).

The bacterial community from the blackcurrant surface was more divergent in comparison to the apples. The microorganisms on blackcurrants located in Lithuania differed at the lower taxonomic level (family, genus or species) from berries harvested in the Czech Republic. Uncultured bacteria of *Staphylococcus* became more abundant on Lithuanian berries, followed by *Acinetobacter*, *Streptococcus*, *Flavobacterium*, etc. Uncultured *Tatumella* and *Pantoea* representing Enterobacteriaceae dominated on blackcurrants from the Czech Republic, followed by *Gluconobacter*, *Massilia*, *Lactobacillus*, *Methylobacterium,* etc (see Supplementary Table S1 in the online version at DOI: http://dx.doi.org/10.1016/j.micres.2017.09.004). These differences could be stipulated by distinct climatic conditions and the ripening stage of berries. Both potential plant pathogens and beneficial bacteria could be inferred among observed microorganisms. Taking into account that human pathogenic bacteria can adapt to plant hosts (Abdelfattah et al., 2016a), there is a chance that such kind of bacteria were also present on the berries tested in our study.

By focusing on the communities of fungal microorganisms, we identified Saccharomycetes to be the major class associated with apples in both locations and blackcurrants collected in the Czech Republic. Blackcurrant berries sampled in Lithuania were dominated by Dothideomycetes. Saccharomycetes were mainly represented by *Hanseniaspora uvarum* and uncultured *Metschnikowiaceae. Hanseniaspora* sp. (see Supplementary Table S2 in the online version at DOI: http://dx.doi.org/10.1016/j.micres.2017.09.004), characterized by low fermentative activity, have been frequently found on the surface of different fruits, e.g. grapes, strawberries or apples (Santo et al., 2012; Graca et al., 2015). *Metschnikowia* also includes species commonly found on the fruit surface and acting as biocontrol agents against different plant pathogens (Parafati et al., 2015). They can strongly antagonize the growth of various filamentous fungi and bacteria by depleting iron in the growth medium (Liu et al., 2013). Using metagenomic analysis, other members of Saccharomycetes, such as *Wickerhamomyces anomalus*, *Saccharomyces cerevisiae*, *Issatchenkia terricola* and *Pichia fermentans* were detected at low level (see Supplementary Table S2 in the online version at DOI: http://dx.doi.org/10.1016/j.micres.2017.09.004) or were notable only after culture enrichment (see Supplementary Table S3 in the online version at DOI: http://dx.doi.org/10.1016/j.micres.2017.09.004). Abundance of yeast is dependent on the fruit development stage and changes along with fruit ripening. Most likely, the low quantities of fermenting yeast found on the surface of our apples could be due to the early harvesting time, when the fruits were undamaged and accessibility to sugar sources was limited. On the other hand, *Hanseniaspora* sp., *Pichia* sp., *Metschnikowia pulcherrima*, *Saccharomyces cerevisiae* were documented on apples after enrichment of samples during only 15 days at fermenting conditions (Vadkertiova et al., 2012). *Wickerhamomyces* and *Pichia* spp. can live both outside and within fruit tissues and even low quantities, acting as biocontrol agents, could regulate the structure of plant microbiota (Vadkertiova et al., 2012; Muccilli et al., 2013; Abdelfattah et al., 2016b).

Cladosporium spp., as representatives of Dothideomycetes, were identified in all samples and in exceptionally high quantity on the blackcurrant berries sampled in Lithuania (see Supplementary Table S2 in the online version at DOI: http://dx.doi.org/10.1016/j.micres.2017.09.004). The abundant presence of this genus was not surprising, considering that it is ubiquitous fungi detected on the surface of different plants (Abdelfattah et al., 2016a, 2016b). In agreement with microbiota studies performed on strawberries (Abdelfattah et al., 2016a), grapes (Barata et al., 2012; Setati et al., 2012), apple, pear, and plum (Vadkertiova et al., 2012), *Cryptococcus* and pigmented yeast *Rhodotorula* were also found on the apples and currants in our experiments. *Cryptococcus* was recognized as a typical constituent of the yeast community and association with the early state of maturation

of fruits has been reported (Janisiewicz et al., 2010; Vadkertiova et al., 2012). These ubiquitous fungi have often been isolated from fruit washings and have been identified as biocontrol agents for management of the postharvest diseases (Liu et al., 2013). *Rhodotorula* sp. can be found on grapes during all ripening stages and produce biofilms on berry surfaces (Lederer et al., 2013).

Our results demonstrated differences in bacterial and fungal microbiota diversity across fruits and berries. The bacterial communities on apples were relatively uniform and similar to one another regardless of geographic location. The blackcurrant bacterial populations were divergent in the regional context and, in comparison with apples, demonstrated that both sampling location and plant species influenced bacterial community composition. A similar tendency was observed in the communities of fungal microorganisms, which were more similar on apples located in different regions than on blackcurrant berries. Our data on apples agree with several previous studies, where it has been demonstrated that the distribution of phyllosphere bacterial communities has minimal geographic differentiation (Redford et al., 2010). Likewise, our observed regional effect on blackcurrant microbiota is consistent with biogeographical correlation of grape wine microbial communities (Pinto et al., 2015). Microorganisms strongly differ across plant species, likely due to variations in metabolites, physical characteristics and symbiotic interactions with the host plant and other microbial inhabitants (Lindow and Brandl, 2003; Hunter et al., 2010). Shifts in the community composition could occur during the time of transport from the field to the grocery store and into hands of the final consumer (Leff and Fierer, 2013; Abdelfattah et al., 2016b). This could explain why our data on apple microbiota structure differ from previous studies conducted on apples purchased from the local supermarket. Moreover, whether conventional or organic farming, an exposure of plants to chemical treatments have been documented to alter the fungal and bacterial microbiota composition (Leff and Fierer, 2013; Abdelfattah et al., 2016b).

Author contributions

Investigation: IVM, JL, RS, ZSZ, VY
Bioinformatic analysis: JL, ES
Data curation and analysis: ES, SS, VY
Funding acquisition: ES
Writing – original draft: SS, ES
Writing – review & editing: SS, VY, ES

Acknowledgements

This study was supported by a grant from the Lithuanian Research Council (No. SIT-7/2015). The funders had no role in data collection and interpretation, or the decision to submit the work for publication. We would like to thank Tereza Ševčíková for help with apple and blackcurrant collection in the Ostrava region and Antanas Žilakauskis, Daniel Naumov, Dovilė Ežerskytė and, Vytautas Balnionis for help with sample collection in Lithuania and the laboratory analysis. Authors would like to thank Jonathan Robert Stratford for the English language review.

References

Abadias, M., Canamas, T.P., Asensio, A., Anguera, M., Vinas, I., 2006. Microbial quality of commercial 'Golden delicious' apples throughout production and shelf-life in Lleida (Catalonia, Spain). Int. J. Food Microbiol. 108 (3), 404–409.

Abadias, M., Usall, J., Anguera, M., Solsona, C., Vinas, I., 2008. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. Int. J. Food Microbiol. 123 (1–2), 121–129.

Abdelfattah, A., Wisniewski, M., Droby, S., Schena, L., 2016a. Spatial and compositional variation in the fungal communities of organic and conventionally grown apple fruit at the consumer point-of-purchase. Hortic. Res. 3, 16047.

Abdelfattah, A., Wisniewski, M., Li Destri Nicosia, M.G., Cacciola, S.O., Schena, L., 2016b. Metagenomic analysis of fungal diversity on strawberry plants and the effect of management practices on the fungal community structure of aerial organs. PLoS One 11 (8), e0160470.

Alvarez-Perez, S., Herrera, C.M., 2013. Composition, richness and nonrandom assembly of culturable bacterial-microfungal communities in floral nectar of Mediterranean plants. FEMS Microbiol. Ecol. 83 (3), 685–699.

Barata, A., Malfeito-Ferreira, M., Loureiro, V., 2012. The microbial ecology of wine grape berries. Int. J. Food Microbiol. 153 (3), 243–259.

Bokulich, N.A., Thorngate, J.H., Richardson, P.M., Mills, D.A., 2014. Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. Proc. Natl. Acad. Sci. U. S. A. 111 (1), E139–E148.

Caporaso, J.G., 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., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7 (5), 335–336.

Clooney, A.G., Fouhy, F., Sleator, R.D., O'Driscoll, Aisling, Stanton, C., Cotter, P.D., Claesson, M.J., 2016. Comparing apples and oranges?: next generation sequencing and its impact on microbiome analysis. PLoS One 11 (2), e0148028.

Cole, J.R., Wang, Q., Fish, J.A., Chai, B., McGarrell, D.M., Sun, Y., Brown, C.T., Porras-Alfaro, A., Kuske, C.R., Tiedje, J.M., 2014. Ribosomal database project: data and tools for high throughput rRNA analysis. Nucleic Acids Res. 42, D633–D642.

Coutinho, T.A., Venter, S.N., 2009. *Pantoea ananatis*: an unconventional plant pathogen. Mol. Plant Pathol. 10 (3), 325–335.

Enya, J., Shinohara, H., Yoshida, S., Tsukiboshi, T., Negishi, H., Suyama, K., Tsushima, S., 2007. Culturable leaf-associated bacteria on tomato plants and their potential as biological control agents. Microb. Ecol. 53 (4), 524–536.

Friesen, M.L., Porter, S.S., Stark, S.C., von Wettberg, E.J., Sachs, J.L., Martinez-Romero, E., 2011. Microbially mediated plant functional traits. Ann. Rev. Ecol. Evol. Syst. 42, 23–46.

Graca, A., Santo, D., Esteves, E., Nunes, C., Abadias, M., Quintas, C., 2015. Evaluation of microbial quality and yeast diversity in fresh-cut apple. Food Microbiol. 51, 179–185.

Hummer, K.E., Dale, A., 2010. Horticulture of Ribes. Forest Pathol. 40 (3–4), 251–263.

Hunter, P.J., Hand, P., Pink, D., Whipps, J.M., Bending, G.D., 2010. Both leaf properties and microbe–microbe interactions influence within-species variation in bacterial population diversity and structure in the lettuce (Lactuca species) phyllosphere. Appl. Environ. Microbiol. 76 (24), 8117–8125.

Janisiewicz, W.J., Kurtzman, C.P., Buyer, J.S., 2010. Yeasts associated with nectarines and their potential for biological control of brown rot. Yeast 27 (7), 389–398.

Janisiewicz, W.J., Jurick, W.M., 2nd Peter, K.A., Kurtzman, C.P., Buyer, J.S., 2014. Yeasts associated with plums and their potential for controlling brown rot after harvest. Yeast 31 (6), 207–218.

Kim, H., Nishiyama, M., Kunito, T., Senoo, K., Kawahara, K., Murakami, K., Oyaizu, H., 1998. High population of Sphingomonas species on plant surface. J. Appl. Microbiol. 85, 731–736.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glockner, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 41 (1), e1.

Koljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F., Bahram, M., Bates, S.T., Bruns, T.D., Bengtsson-Palme, J., Callaghan, T.M., Douglas, B., Drenkhan, T., Eberhardt, U., Duenas, M., Grebenc, T., Griffith, G.W., Hartmann, M., Kirk, P.M., Kohout, P., Larsson, E., Lindahl, B.D., Lucking, R., Martin, M.P., Matheny, P.B., Nguyen, N.H., Niskanen, T., Oja, J., Peay, K.G., Peintner, U., Peterson, M., Poldmaa, K., Saag, L., Saar, I., Schussler, A., Scott, J.A., Senes, C., Smith, M.E., Suija, A., Taylor, D.L., Telleria, M.T., Weiss, M., Larsson, K.H., 2013. Towards a unified paradigm for sequence-based identification of fungi. Mol. Ecol. 22 (21), 5271–5277.

Lederer, M.A., Nielsen, D.S., Toldam-Andersen, T.B., Herrmann, J.V., Arneborg, N., 2013. Yeast species associated with different wine grape varieties in Denmark. Acta Agr. Scand. B-S P 63 (1), 89–96.

Leff, J.W., Fierer, N., 2013. Bacterial communities associated with the surfaces of fresh fruits and vegetables. PLoS One 8 (3), e59310.

Li, W., Fu, L., Niu, B., Wu, S., Wooley, J., 2012. Ultrafast clustering algorithms for metagenomic sequence analysis. Brief. Bioinform. 13 (6), 656–668.

Ligon, J., Hill, D., Hammer, P., Torkewitz, N., Hofmann, D., Kempf, H., van Pee, K., 2000. Natural products with antifungal activity from Pseudomonas biocontrol bacteria. Pest. Manag. Sci. 56, 688–695.

Lindow, S.E., Brandl, M.T., 2003. Microbiology of the phyllosphere. Appl. Environ. Microbiol. 69 (4), 1875–1883.

Liu, J., Sui, Y., Wisniewski, M., Droby, S., Liu, Y., 2013. Review: utilization of antagonistic yeasts to manage postharvest fungal diseases of fruit. Int. J. Food Microbiol. 167 (2), 153–160.

Lozupone, C., Knight, R., 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. 71 (12), 8228–8235.

Magoc, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27 (21), 2957–2963.

Muccilli, S., Wemhoff, S., Restuccia, C., Meinhardt, F., 2013. Exoglucanase-encoding genes from three Wickerhamomyces anomalus killer strains isolated from olive brine. Yeast 30 (1), 33–43.

Ofek, M., Hadar, Y., Minz, D., 2012. Ecology of root colonizing Massilia (Oxalobacteraceae). PLoS One 7 (7), e40117.

Parafati, L., Vitale, A., Restuccia, C., Cirvilleri, G., 2015. Biocontrol ability and action mechanism of food-isolated yeast strains against Botrytis cinerea causing post-harvest bunch rot of table grape. Food Microbiol. 47, 85–92.

Paredes-Lopez, O., Cervantes-Ceja, M.L., Vigna-Perez, M., Hernandez-Perez, T., 2010. Berries: improving human health and healthy aging, and promoting quality life—a review. Plant Food. Hum. Nutr. 65 (3), 299–308.

Piano, S., Neyrotti, V., Migheli, Q., Gullino, M.L., 1997. Biocontrol capability of Metschnikowia pulcherrima against Botrytis postharvest rot of apple. Postharvest Biol. Technol. 11 (3), 131–140.
Pinto, C., Pinho, D., Sousa, S., Pinheiro, M., Egas, C., Gomes, A.C., 2014. Unravelling the diversity of grapevine microbiome. PLoS One 9 (1), e85622.
Pinto, C., Pinho, D., Cardoso, R., Custodio, V., Fernandes, J., Sousa, S., Pinheiro, M., Egas, C., Gomes, A.C., 2015. Wine fermentation microbiome: a landscape from different Portuguese wine appellations. Front. Microbiol. 6, 905.
Pretorius, I.S., 2000. Tailoring wine yeast for the new millennium: novel approaches to the ancient art of winemaking. Yeast 16 (8), 675–729.
Redford, A.J., Bowers, R.M., Knight, R., Linhart, Y., Fierer, N., 2010. The ecology of the phyllosphere: geographic and phylogenetic variability in the distribution of bacteria on tree leaves. Environ. Microbiol. 12 (11), 2885–2893.
Santo, D.E., Galego, L., Gonçalves, T., Quintas, C., 2012. Yeast diversity in the Mediterranean strawberry tree (Arbutus unedo L.) fruits' fermentations. Food Res. Int. 47 (1), 45–50.
Sarr, P.S., Sugiyama, A., Begoude, A.D., Yazaki, K., Araki, S., Nawata, E., 2017. Molecular assessment of the bacterial community associated with Cassava (Manihot esculenta Crantz) cultivation in Cameroon. Microbiol. Res. 197, 22–28.
Setati, M.E., Jacobson, D., Andong, U.C., Bauer, F.F., 2012. The vineyard yeast microbiome, a mixed model microbial map. PLoS One 7 (12), e52609.
Stefanini, I., Dapporto, L., Legras, J., Calabretta, A., Di Paola, M., De Filippo, C., Viola, R., Capretti, P., Polsinelli, M., Turillazzi, S., Cavalieri, D., 2015. Role of social wasps in Saccharomyces cerevisiae ecology and evolution. Proc. Natl. Acad. Sci. U. S. A. 109, 13398–13403.
Tabart, J., Franck, T., Kevers, C., Pincemail, J., Serteyn, D., Defraigne, J.O., Dommes, J., 2012. Antioxidant and anti-inflammatory activities of Ribes nigrum extracts. Food Chem. 131 (4), 1116–1122.
Taylor, M.W., Tsai, P., Anfang, N., Ross, H.A., Goddard, M.R., 2014. Pyrosequencing reveals regional differences in fruit-associated fungal communities. Environ. Microbiol. 16 (9), 2848–2858.
Teixidó, N., Usall, J., Viñas, I., 1999. Efficacy of preharvest and postharvest *Candida sake* biocontrol treatments to prevent blue mould on apples during cold storage. Int. J. Food Microbiol. 50 (3), 203–210.
Toju, H., Tanabe, A.S., Yamamoto, S., Sato, H., 2012. High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. PLoS One 7 (7), e40863.
Vadkertiova, R., Molnarova, J., Vranova, D., Slavikova, E., 2012. Yeasts and yeast-like organisms associated with fruits and blossoms of different fruit trees. Can. J. Microbiol. 58 (12), 1344–1352.
Valero, E., Cambon, B., Schuller, D., Casal, M., Dequin, S., 2007. Biodiversity of *Saccharomyces* yeast strains from grape berries of wine-producing areas using starter commercial yeasts. FEMS Yeast Res. 7 (2), 317–329.
Volschenk, Q., du Plessis, E.M., Duvenage, F.J., Korsten, L., 2016. Effect of postharvest practices on the culturable filamentous fungi and yeast microbiota associated with the pear carpoplane. Postharvest Biol. Technol. 118, 87–95.
Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73 (16), 5261–5267.
Wang, C., Garcia-Fernandez, D., Mas, A., Esteve-Zarzoso, B., 2015. Fungal diversity in grape must and wine fermentation assessed by massive sequencing, quantitative PCR and DGGE. Front. Microbiol. 6, 1156.