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

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A R T I C L E   I N F O

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A B S T R A C T

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, Pantoaea, 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 microorganisms 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; Stefansini 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, Pantoaea) (Goutinho 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; Cloney 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).

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The国内的苹果（Malus pumila Mill.）是广泛种植的主要温带水果。与其他水果相比，苹果被殖民化
为由不同种类的微生物和可能由不同种类的微生物组成，并受到不同
样体的疾病影响，并且形成局部的社区（Teixidó et al., 1999; Graca et al., 2015）。
当前对苹果微生物组的了解是有限的，而且主要集中在物种，而且可能对病原体形成局部的社区（Teixidó et al., 1999; Abadías et al., 2006）。另一个研究焦点
是相关研究集中于不同种类的微生物，可以作为用于生物化学研究
的微生物研究对象，对抗病原体的研究（Piano et al., 1997）。到该点，唯一的
一个报告对菌群的分布与相关的协会进行了全面的报告。所选的苹果种类
为在超市或食品店，因此分析必须在以后才进行。这是可能的，因为环境条件
如储存和运输以及时间对实验的影响可以影响到微生物组的结构
以降低与水果相关的微生物群落或涉及污染的原因。

黑醋栗（Ribes nigrum L.）是一种温带浆果，广泛种植于
欧洲和北亚。即使在美国，也有日益增长的兴趣
生产（Hummer and Dale, 2010）。该浆果的
维生素C和花青素含量高，因此是用于无污染
的食品和饮料制造。的

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

2.3. Bacterial and fungal DNA amplification and amplicon library
preparation

DNA samples from apples and blackcurrant microbe were am-
plified using the primers specific for fungi and bacteria. For identi-
fication of fungal microorganisms, the ITS2 region of ribosomal DNA
was amplified using ITS3-KY02 (5′-GATGAAACGCGYYGTTAGA-3′) and ITS4
(5′-TTCCTCGGCTATTGATATGC-3′) primers (Toju et al., 2012). For
bacteria identification, the V3-V4 region of the 16S rDNA gene was
amplified with primers S-d-Bact-0341-b-S-17 (5′-CTACGGGNGGC-
GWCCAG-3′) and S-d-Bact-0785-a-A-21 (5′-GACTACHVGGGTATCTAA-
ATGC-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 Il-
umina MiSeq V3 (2 × 300 bp) (Macrogen Inc., Seoul, Korea). All se-
quences 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 de-
fault 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 se-
quencing 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 im-
plemented in QIIME v. 1.8, related the bacterial and fungal microbiota
composition to sample types and examined the distance between dif-
frent 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′-TCCTCCGCTTATTGATATGC-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 CofI and Hinfl 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/ssa/tasta/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 individual samples varied from 85 to 328. Based on analysis of prokaryotic sequences, the highest number of OTUs was detected in black currants 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 Basidimicota (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-phyllum distribution (Figs. 3 B II, 4 A ). Gammamproteobacteria 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 level (Fig. 4B) or at species level and were assigned to uncultured Metschnikowiae 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>
<thead>
<tr>
<th>Samples</th>
<th>Target region</th>
<th>High quality reads</th>
<th>OTUs</th>
<th>Chao1</th>
<th>Coverage</th>
<th>Shannon diversity</th>
</tr>
</thead>
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<tr>
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<td>ITS2</td>
<td>251,647</td>
<td>225</td>
<td>225.13</td>
<td>0.9994</td>
<td>2.73</td>
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<tr>
<td></td>
<td>V3-V4</td>
<td>117,934</td>
<td>90</td>
<td>91</td>
<td>0.989</td>
<td>2.31</td>
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<tr>
<td>Apple_CZ</td>
<td>ITS2</td>
<td>305,477</td>
<td>328</td>
<td>339.3</td>
<td>0.9666</td>
<td>2.91</td>
</tr>
<tr>
<td></td>
<td>V3-V4</td>
<td>141,529</td>
<td>104</td>
<td>115</td>
<td>0.9043</td>
<td>2.59</td>
</tr>
<tr>
<td>Blackcurrant_LT</td>
<td>ITS2</td>
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<td>85</td>
<td>92.5</td>
<td>0.9297</td>
<td>2.93</td>
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<tr>
<td></td>
<td>V3-V4</td>
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<td>150</td>
<td>160</td>
<td>0.9375</td>
<td>5.4</td>
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<tr>
<td>Blackcurrant_CZ</td>
<td>ITS2</td>
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<td>302</td>
<td>306.23</td>
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<td>2.98</td>
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<td></td>
<td>V3-V4</td>
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<td>96</td>
<td>0.9792</td>
<td>4.95</td>
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<tr>
<td>Eukaryotic</td>
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<td></td>
<td></td>
<td>0.9862</td>
<td>2.98</td>
</tr>
<tr>
<td>Prokaryotic</td>
<td></td>
<td>499,470</td>
<td></td>
<td></td>
<td>0.9792</td>
<td>4.95</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1,466,580</td>
<td>1,378</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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...
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
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 (family, genus or species) from berries harvested in the Czech Republic, followed by Acinetobacter, followed by Staphylococcus, 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 Metschnikowiaaceae. 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, Issatchenka 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

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**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.
of fruits has been reported (Janisiewicz et al., 2010; Vadkertiova et al., 2016). 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 trend 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 microbiota (Redford et al., 2010).

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