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Research article

High content analysis of sea buckthorn, black chokeberry, red and white currants microbiota – A pilot study

Juliana Lukša[a], Iglė Vepštaitė-Monstavičė[a], Vyacheslav Yurchenko[b], Saulius Serva[c], Elena Servienė[a,*]

[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, 710 00 Ostrava, Czech Republic
[c] Department of Biochemistry and Molecular Biology, Institute of Biosciences, Vilnius University, Saulėtekio al.7, Vilnius LT-10257, Lithuania

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ABSTRACT

The high potential of sea buckthorn, black chokeberry, red and white currants in healthy food industry boosted interest in the plant cultivation. The present study is the first work providing comprehensive information on microbial populations of these berries. Next Generation Sequencing allowed identification of eukaryotic and prokaryotic microorganisms prevalent on specific berries, including uncultivable microorganisms. Our study revealed the broad diversity of berries-associated bacterial and fungal microorganisms. Analysis of representative microbial OTUs showed a clear separation among inhabitants of sea buckthorn, black chokeberry and both currants, indicating plant-defined differences in the composition of the bacterial and fungal microbiota. Among the microorganisms distributed on tested berries, we documented potentially beneficial fungi and bacteria along with potential phytopathogens or those harmful for humans. Thus, plant microbiota appears to be highly relevant for the evaluation of the microbiota impact on food quality and human health.

1. Introduction

In recent years, the exploration of various plants and their products for improving human health grew steadily (Agbarya, Ruimi, Epelbaum, Ben-Arye, & Mahajna, 2014; Basu, Rhone, & Lyons, 2010; Boeing et al., 2012). Among the beneficial berries black chokeberry, sea buckthorn, and currants are particularly popular (Basu et al., 2010; Borowska & Brzóska, 2016; Chauhan, Negi, & Ramteke, 2007).

Hippophae rhamnoides L., the common sea buckthorn, is the most widespread species of the genus *Hippophae* common in Europe, Asia, and North America. Sea buckthorn is a popular garden and landscaping shrub preventing soil erosion and reducing pollution (Li, Du, & Guo, 2015). The berries of sea buckthorn have high contents of vitamins (C, E and K), carotenoids, flavanols, and sugars. They are used in food industry, medicine and cosmetics (Li & Schroeder, 1996; Patel, Divakar, Santani, Solanki, & Thakkar, 2012).

Black chokeberry, *Aronia melanocarpa* (Michx.) Ell., is widely planted in natural and domesticated environments of North America, Northern and Eastern Europe as ornamental plants. They are also used as a source for food or beverage production. Berries are loaded with essential phytonutrients, vitamins, antioxidants and bioactive agents, exhibiting antimicrobial, anticancer and antiviral activity (Baum, Howard, Prior, & Lee, 2016; Borowska & Brzóska, 2016; Liepiņa, Nikolajeva, & Jākobsone, 2013).

Red currant (*Ribes rubrum* L.) and white currant (the cultivar of *Ribes rubrum*) are native across Europe, Asia, and North America. These berries are usually cultivated for food and beverages production. Both fruits are rich in vitamin C and K, minerals, as well as organic acids and polyphenols, capable to boost the immune system, help fight infections, reduce a risk of heart disease and cancer (Mikulic-Petkovsek, Schmitzer, Slatnar, Stampar, & Veberic, 2012; Wojdyło, Oszmiański, Milczarek, & Wietrzyk, 2013).

The quality of fruits and berries along with the content of active components depend on the cultivation and climatic conditions during vegetation, application of agrochemicals, hydration and harvest time (Borowska & Brzóska, 2016; George & Cenkowski, 2007). Microorganisms colonizing the surface of fruits, leaves, stems or living within tissues have a major influence on plant development, adaptation and evolution, in turn affecting plant potential in food production (Abdelfattah, Wisniewski, Droby, & Schena, 2016; Barrow, Lucero, Reyes-Vera, & Havstad, 2008). The phytopathogenic microorganisms are responsible for significant economic losses, while others are considered beneficial by inducing resistance in the host (Abdelfattah, Wisniewski, Droby, & Schena, 2016; Droby, Wisniewski, Teixidó,

* Corresponding author.
E-mail address: elena.serviene@gamtc.lt (E. Servienė).

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Spadaro, & Jijakli, 2016). Moreover, plant-associated microorganisms may cause foodborne diseases or may have relevant effect for human health by contributing to the diversity within gut microbiome or by stimulating immune response (Berg, Erlacher, & Grube, 2015; Higgins et al., 2018). The distribution of microorganisms on plants is defined by many factors, including plant species, ripening stage, climatic conditions and application of agrochemicals (Pinto et al., 2014, 2015, Pretorius, 2000). Changes in the planting regime alter microbial habitat and ecological niche, leading to the loss of microbial diversity and functional traits (Saleem & Moe, 2014).

According to metagenomic studies, the diversity of the microbiota on fruits and vegetables is high (100–1000 operational taxonomic units, OTUs) with only a few dominant groups (20–50 OTUs) (Leff & Fierer, 2013; Montesinos, Frances, Badosa, & Bonaterra, 2015). In the past, studies of the plant microbiota were based on isolation/culture techniques, thus missing on overall fungal microorganism and bacteria composition (Valero, Cambon, Schuller, Casal, & Dequin, 2007; Volschenk, du Plessis, Duvenage, & Korsten, 2016). The Next Generation Sequencing (NGS) and metagenomic approaches, complemented by the bioinformatics analyses, have made it possible to assay microbial communities, including the organism's refractory to cultivation. The NGS methods have gained increasing attention in recent years in studying of the microbial community dynamics at different points of food production chain, starting from cultivation of food sources, manufacturing and distribution (Abdelfattah, Wisniewski, Li Destri Nicosia, Cacciola, & Schena, 2016; Higgins et al., 2018; Leff & Fierer, 2013). High-throughput sequencing technologies have potential to advance effective application of microbial resources for improving food quality and safety (De Filippis, Parente, & Ercolini, 2018). Recent comprehensive NGS-based microbiome analyses were performed on several fruits and berries, such as grapes, apples, blackcurrants, strawberries, and oranges (Abdelfattah, Wisniewski, Droby, & Schena, 2016; Abdelfattah, Wisniewski, Li Destri Nicosia, et al., 2016; Clooney et al., 2016; Droby et al., 2016; Vepstaite-Monstavice et al., 2018). Nevertheless, our understanding of the diversity of the producer-associated microbial communities, the factors that influence the composition of these communities and the distributions of individual taxa across producer types, in particular among representatives of difficult-to-cultivate taxa, is still limited.

The broad interest in sea buckthorn, black chokeberry, red and white currants requires an investigation of the microbial communities, colonizing the surface of these berries. The goal of the current study was to identify the composition of bacterial and fungal microorganisms associated with the black chokeberry, sea buckthorn, red and white currants harvested in Lithuania. The high-throughput identification and quantification of microflora composition provided information relevant for plant disease management, increasing the yield of the desired crop, and uncovered the potential role of microbiota in berries-based food production.

2. Materials and methods

2.1. Ethics statement

The collection of berries was carried out on private lands with land owners' permission to conduct the study on sites. It did not involve endangered or protected species.

2.2. Sampling of the berries and DNA extraction

Sea buckthorn *Hippophae rhamnoides* L. berries were aseptically collected from the private farm located in the Vilnius region of Lithuania (GPS coordinates: 54°75′20.0”N, 25°27′99.6″E) in the mid-September 2016. Black chokeberry *Aronia melanocarpa* (Michx.) Ell. were collected from the Klaipeda region of Lithuania (GPS coordinates: 55°59′70.0”N, 21°59′60.7″E) in the mid-August 2016. Red currant (*Ribes rubrum*) and white currant (the cultivar of *Ribes rubrum*) were sampled from the Ignalina region of Lithuania (GPS coordinates: 55°34′23.0”N, 26°16′46.8″E) in the mid-July 2016. All plants did not receive any chemical treatment during growing and harvesting period. The samples were collected into sterile plastic bags and processed within 2–4 h after harvesting. The berries of interest (about 300 g) were placed in 500 mL of sterile 0.05 M phosphate buffer pH 6.8 for 30 min at room temperature with shaking at 120 rpm. Outwashes were filtered through 420 μm filters, centrifuged at 12,000 × *g* for 20 min, and pellets were stored at −20 °C until subsequent analysis.

For metagenomic analysis, 40 mg of pellet per sample were 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 protocol. 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 sea buckthorn, black chokeberry, red and white currant microbiota were amplified using the specific primers: for fungal microorganisms ITS3-KYO2 (5′-GATGAAGAACGYAGYRAA-3′) and ITS4 (5′- TCCTCCGCTTATTGATATGC-3′) (Toju, Tanabe, Yamamoto, & Sato, 2012); for bacteria 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 library preparation was performed at Macrogen Inc. (Seoul, Korea) using modified Illumina adapters (Illumina, San Diego, USA). The validation of prepared library was performed on Agilent Technologies Bioanalyzer DNA 1000, the quantification of DNA library templates performed using qPCR according to the Illumina Protocols. Amplicons were sequenced at Macrogen Inc. (Seoul, Korea) using Illumina MiSeq V3 (2 × 300 bp).

2.4. Data processing and analysis

The bioinformatics pipelines, FLASH v1.2.11 (Magoc & Salzberg, 2011), CD-HIT-OTU v4.5.5 (Li, Fu, Niu, Wu, & Wooley, 2012), QIIME v1.8 (Caporaso et al., 2010), were used to process and analyze the sequence data. Preliminary processing of the data was performed using default parameters of FLASH v1.2.11: sequences with a minimum quality score of 25 were filtered and paired-end reads were merged. Sequences were denoised, chimeric sequences were identified and removed, and the remaining reads were clustered into the Operational Taxonomical Units (OTUs) with a minimum 97% similarity threshold, using the CD-HIT-OTU v4.5.5 (Li et al., 2012). The most abundant sequences in each OTU were used for the taxonomy assignments using the RDP for 16S rDNA (Cole et al., 2014; Wang, Garrity, Tiedje, & Cole, 2007) and the UNITE for ITS (Kõljalg et al., 2013) databases as references. The QIIME v1.8 (Caporaso et al., 2010) was used to generate rarefaction curves demonstrating richness of population. For a downstream analysis, the OTU table was rarefied at an even depth (unassigned sequences and chloroplasts were discarded, 62,652 number of reads used for 16S rRNA rarefaction and 134,622 number of reads - for ITS2). Alpha diversity was calculated using observed species, Shannon, Good's coverage and Chao1 estimates (Caporaso et al., 2010). Weighted UniFrac metrics were used to evaluate β-diversity and Principal coordinates analysis (PCoA) as implemented in QIIME v1.8 was used to relate the bacterial and fungal microorganism community composition to sample types (Lozupone & Knight, 2005). The heatmap was generated using ascendant hierarchical clustering based on Euclidian distances (in XLSTAT 2018.04.20).

2.5. Nucleotide sequence accession number

The sequencing data are available at the Sequence Read Archive

(SRA) of the National Center for Biotechnology Information (NCBI), under accession number SRP108325.

3. Results

3.1. Diversity and richness of microbial communities

Illumina MiSeq sequencing generated 455,374 high quality 16S rRNA gene amplicons and 862,565 ITS reads for the black chokeberry, sea buckthorn, red and white currants (Table 1). The clustering of the sequences generated a total of 1888 OTUs (498 for bacterial V3-V4 and 1390 for fungal ITS2) (Table 1). The total number of detected bacterial OTUs varied from 68 to 214, while that for fungal OTUs ranged from 217 to 491 in the tested berries samples. In both prokaryotic and eukaryotic sequences, the highest number of OTUs was observed in red currants and the lowest in white currants. In agreement with OTU data, the Shannon's Diversity and the Chao1 estimates also revealed that red currant berries had a higher bacterial and fungal microorganism diversity than other berries. 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 above 94% in all cases, indicating that a good coverage was achieved. Rarefaction curve showed that the numbers of OTUs were saturated in all samples and enough for further community analysis (Fig. S1).

3.2. Characterization of sea buckthorn's bacterial and fungal microorganism communities

We proceeded to explore the taxonomic profiles of the sea buckthorn-associated bacterial and fungal microorganisms. In total, six bacterial phyla (35 families and 56 genera) and four fungal phyla (58 families and 108 genera) were identified. The dominant phylum across an entire prokaryotic microorganism population was Proteobacteria (71.1%) (Fig. 1A), mainly represented by Alphaproteobacteria and Gammaproteobacteria at the class level (Fig. 1B). Across the eukaryotic microorganism population, Ascomycota was the dominant phylum accounting for 89.4% of the total number of detected sequences, followed by Basidiomycota (8.2%) (Fig. 2A). The major group of OTUs within the Ascomycota belonged to the class Dothideomycetes, while the Basidiomycota was represented by members of the class Tremellomycetes (Fig. 2B).

At higher taxonomic resolution, bacterial community was mostly dominated by the families Enterobacteriaceae (31.4%), Microbacteriaceae (16.3%) and Pseudomonadaceae (14.1%) (Fig. 1C), exemplified by the most abundant genera *Pantoea* (16.8%), *Okibacterium* (14.4%) and *Pseudomonas* (14.1%), respectively (Fig. 1D, Table S1). Fungal microorganism community on sea buckthorn was dominated by Dothioraceae (78%), followed by Davidiellaceae (2.4%) and traces of Taphrinaceae and Saccharomycodaceae (Fig. 2C). At the genus level, the vast majority of sea buckthorn-associated fungal microorganisms (87.9%) were described as unidentified (Fig. 2D, Table S2).

3.3. Black chokeberry microbiota composition

Of the six bacterial phyla detected in the present study on black chokeberry, the dominant phyla were Proteobacteria (59.9%) and Bacteroidetes (27.7%). The rest of the community consisted of Actinobacteria (7.3%), Firmicutes (2.4%), and others (Fig. 1A). At the class level, prokaryotic microorganisms mainly belonged to Alphaproteobacteria, Cytophagia and Gammaproteobacteria (Fig. 1B). Among 33 bacterial families identified (Table S1), Sphingomonadaceae (27.7%) and Cytophagaceae (23.6%) were the most abundant (Fig. 1C), represented by the genera *Sphingomonas* (27.1%) and *Hymenobacter* (23.2%) (Fig. 1D). The rest of the community consisted of *Acinetobacter*, *Pseudomonas*, *Variovorax*, *Pantoea*, *Frondihabitans* and *Mucilaginibacter* (Fig. 1D; Table S1).

The fungal microorganism associated with black chokeberry belonged to three phyla, where Ascomycota (66.2%) and Basidiomycota (32.2%) were the most abundant (Fig. 2A, Table S2). The first of them was represented by Dothideomycetes and Taphrinomycetes, the second one - by Tremellomycetes, Microbotryomycetes and Exobasidiomycetes (Fig. 2B). Of the 34 fungal families identified on black chokeberry, Davidiellaceae (20.8%) dominated, along with Dothioraceae (7.9%) and Taphrinaceae (3.5%) (Fig. 2C). These families were represented by *Cladosporium* (20.8%) and *Taphrina* (3.5%). Representatives of Basidiomycota at the genus level were *Cryptococcus* (10.9%) and *Rhodotorula* (9.1%) (Fig. 2D). Other fungal microorganisms, such as *Phoma*, *Mrakiella*, *Lewia* and *Hanseniaspora*, were detected at low frequencies. About half of the black chokeberry-associated fungal microorganisms (45.6%) were unidentified at the genus level (Table S2).

3.4. Composition of red and white currant bacterial and fungal microorganism communities

The bacterial community profile analysis showed a total of eight phyla, 51 families and 82 genera distributed on red and white currants. Bacterial phyla, dominating on currant berries, were ascribed to Proteobacteria (56.2% on red and 57.5% on white currant, respectively), Bacteroidetes (28.4% and 26.6%, respectively) and Actinobacteria (11.5% and 13.8%, respectively) (Fig. 1A). At the class level, they were represented by Alpha-, Beta- and Gammaproteobacteria, along with Cytophagia and Actinobacteria (Fig. 1B). The broad diversity of bacteria was evident at the family and genus level (Fig. 1C, D). Most of the family-level OTUs belonged to Cytophagaceae (17.5% on red and 18.1% on white currant, respectively), Sphingomonadaceae (15.1% and 11.3%), Oxalobacteraceae (10% and 8.2%), Methylobacteriaceae (10.3% on both currants), Pseudomonadaceae (5.7% and 7.6%), Microbacteriaceae (7.8% and 10.4%) and Comamonadaceae (4.4% and 9.5%) (Fig. 1C). Dominating genera on both

Table 1
Summary of metagenomic surveys conducted on black chokeberry, sea buckthorn, red and white currant berries.

Samples	Target region	High quality reads	OTUs	Chao1	Coverage	Shannon diversity
Black chokeberry	V3–4	125,380	79	80.5	0.9814	4.15
	ITS2	230,205	236	238	0.9916	4.08
See buckthorn	V3–4	142,450	137	137.5	0.9964	3.20
	ITS2	262,515	446	471.6	0.9457	2.39
Red currant	V3–4	117,755	214	215.5	0.9930	5.64
	ITS2	224,754	491	494.2	0.9935	4.34
White currant	V3–4	69,789	68	69	0.9855	5.03
	ITS2	145,091	217	220	0.9864	4.11
	Prokaryotic	455,374	498			
	Eukaryotic	862,565	1390			
	Total	1,317,939	1888			

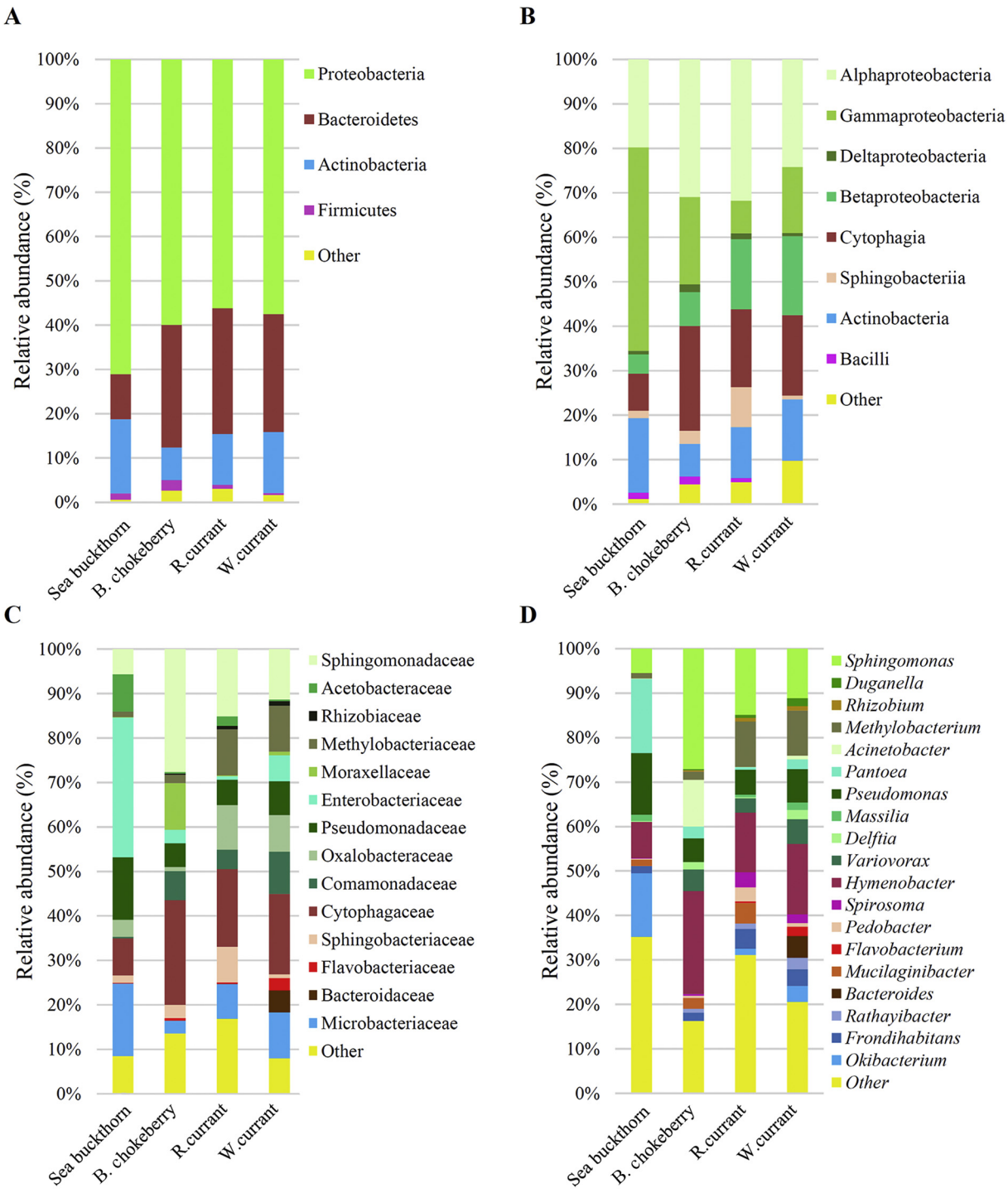


Fig. 1. Prokaryotic microbial community distribution on sea buckthorn, black chokeberry, red and white currant. A – phylum level, B – class, C – family, D – genus. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

currants analyzed were *Hymenobacter*, *Sphingomonas* and *Methylobacterium*, followed by *Pseudomonas*, *Frondihabitans* and *Variovorax* (Fig. 1D). Some genera were observed on red currant berries only, such as *Mucilaginibacter* (4.6%), or exceptionally on white currants, such as *Bacteroides* (4.9%) (Table S1).

The fungal microorganisms distributed on red and white currants belonged to two phyla, 59 families and 106 genera. Similarly to the sea buckthorn and black chokeberry, the dominant phylum was Ascomycota (68.3% on red and 57.3% on white currant, respectively), followed by Basidiomycota (28.2% and 39.7%) (Fig. 2A). The most abundant class was Dothideomycetes (53.1% and 51.5% on red and white currants, respectively) (Fig. 2B), represented by Dothioraceae and Davidiellaceae families (Fig. 2C). Some families, as Glomerellaceae (9.1%), were detected on red currant only (Fig. 2C), represented by *Colletotrichum* (Table S2). The most dominant genera on both currants were *Cladosporium* (17.3% on red and 20.7% on white currant) and *Cryptococcus* (11.5% and 21%, respectively), followed by *Phoma* (4.8% and 8.3%) and *Rhodotorula* (1.6% and 3%) (Fig. 2D). Unidentified at the genus level fungal microorganisms were present on both plants (40.2% and 31.8% for red and white currant, respectively) (Table S2).

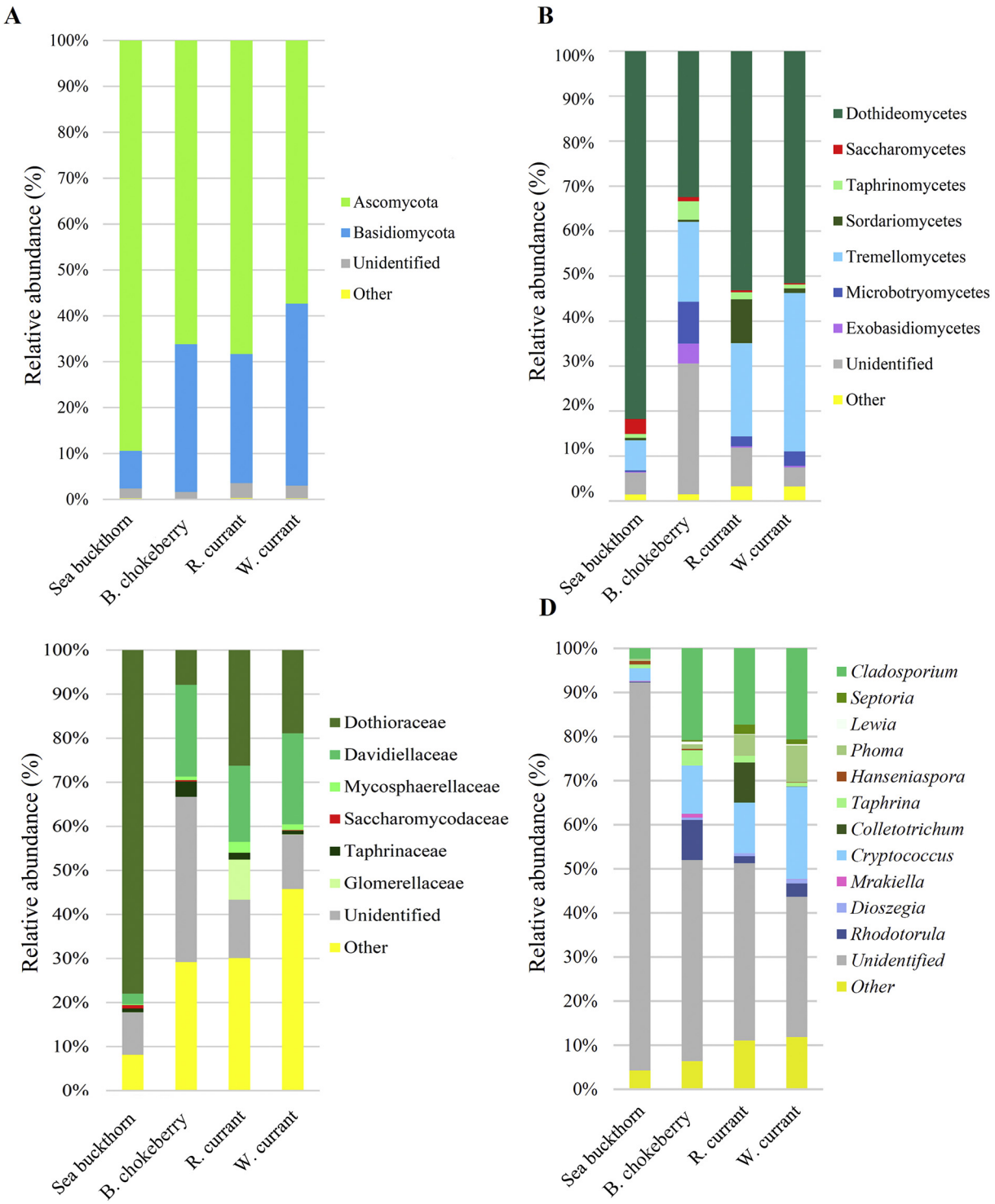


Fig. 2. Fungal microorganism community distribution on sea buckthorn, black chokeberry, red and white currant. A – phylum level, B – class, C – family, D – genus. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.5. Comparison of bacterial and fungal microorganism communities on sea buckthorn, black chokeberry, red and white currants

The Principal Coordinate Analysis (PCoA), performed with the representative OTUs, displayed a clear separation of sea buckthorn, black chokeberry and both currants, indicating a difference in the composition of the bacterial and fungal microorganism communities (Fig. 3). A certain distinction was observed between both currants based on the structure of the bacterial population (Fig. 3A). This was likely influenced by several bacterial genera present exclusively on one type of the berries (*e.g. Mucilaginibacter* - on red currant berries, *Bacteroides* - on white currant). The clustering of red and white currant bacterial community into the separate sections of PCoA plot from sea buckthorn and black chokeberry can be explained by the increased number of several less abundant genera. The fungal microbiota of red and white currants clustered together, while the communities of the fungal microorganisms on sea buckthorn and black chokeberry were clearly separated from each other and both currants (Fig. 3B).

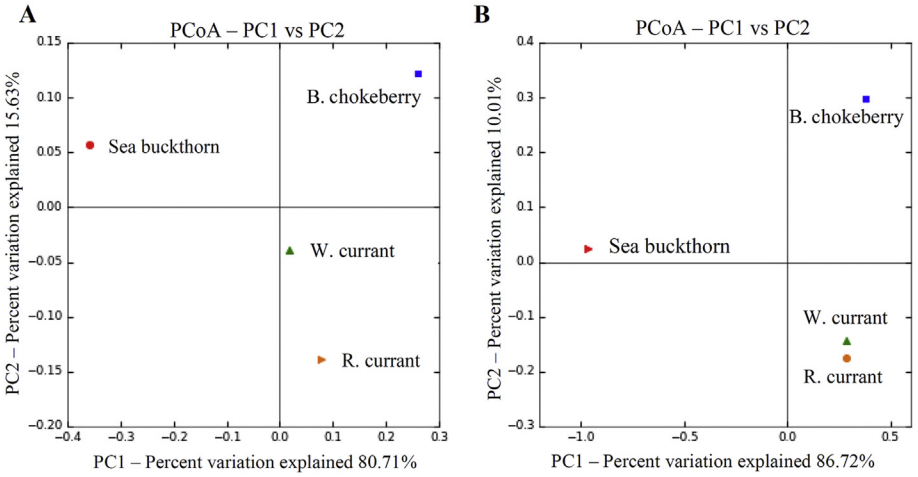


Fig. 3. Principal coordinates analysis (PCoA) of the relative abundance of bacterial (A) and fungal microorganism (B) OTUs.

The heatmap diagram illustrated distribution of the most abundant fungal and bacterial OTUs on various fruit surfaces (Fig. 4). Among bacteria community, OTUs of *Methylobacterium* (OUT12, OTU23 and OTU30*)*, *Pedobacter* (OTU36*)* and *Frondihabitans* (OTU14, OTU32) inhabited mainly red currant, while those belonging to *Flavobacterium* (OTU33), *Duganella* (OTU24) and *Bacteroides* (OTU61) were more abundant on white currant. Higher abundance of OTUs matching in GenBank to *Okibacterium* (OTU11) and *Pantoea* (OTU4) was documented on sea buckthorn as compared to both currants and black chokeberry. OTUs from the *Acinetobacter* (OTU22), *Bacillus* (OTU89), *Kineasporia* (OTU42) and *Variovorax* (OTU20) bacteria were more abundant on black chokeberry as compared to other berries tested (Fig. 4A). Among *Pseudomonas* genera, different distribution of closely-related microorganisms is observed, e. g. *Pseudomonas* OTU5 is present mainly on sea buckthorn surface, while OTU8 distributed similarly on sea buckthorn and red currant. Differently developed OTUs from *Hymenobacter* genera also possess various distribution: OTU25 and OUT123 is inhabiting mainly black chokeberry, OTU105 is related to white currant and OTU148 inhabiting red currants.

Among fungal microorganism community, OTUs assigned to *Hanseniaspora* (OTU0) and *Cystofilobasidiales* (OTU55) were more prevalent on sea buckthorn samples comparing to other fruits tested. Conversely, we found a significantly higher proportion of OTUs belonging to *Taphrina* (OTU14), *Lewia* (OTU66), *Rhodotorula* (OTU16, OTU18) and *Mrakiella* (OTU61) on black chokeberry compared to sea buckthorn and both currants. OTUs from *Phoma* (OTU13), Tremellales (OTU10, OTU28) and *Articulospora* (OTU60) were more distributed on both currants as compared to other berries tested, while *Knufia* (OTU36*)*, *Septoria* (OTU19) and *Colletotrichum* (OTU12) were more prevalent on red currant (Fig. 4B). Some OTUs of the same genera developed differently and vary on inhabitation pattern, e. g. *Cryptococcus* OTU17 is present mainly on black chokeberry, OTU4 inhabits both currants, OTU11 is distributed on sea buckthorn and OTU23 is more prevalent on black chokeberry and white currants.

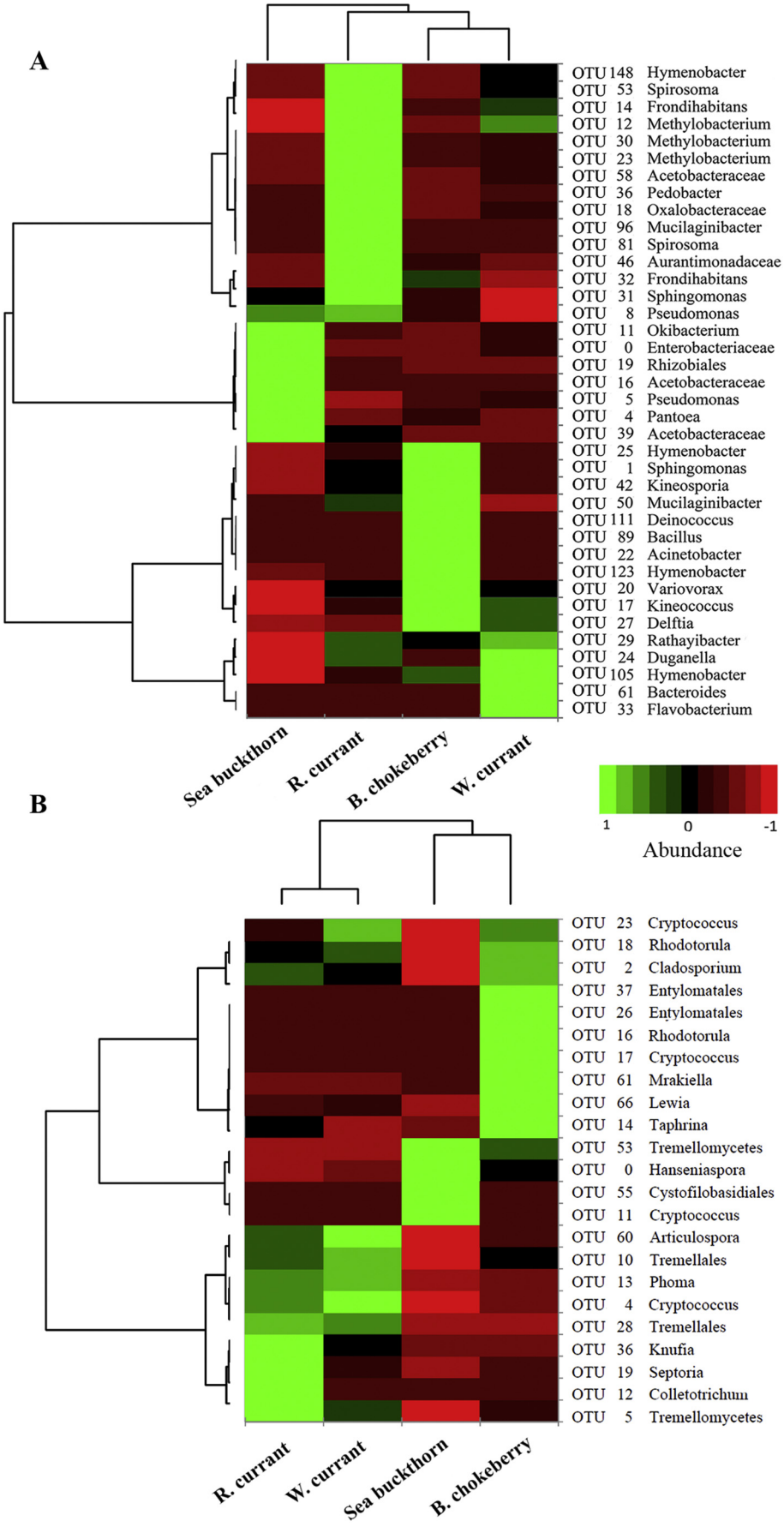
4. Discussion

The current study for the first time uncovered the structure of microbial populations associated with sea buckthorn, black chokeberry, red and white currant berries. For a comprehensive and culture-free analysis, we have applied Next Generation Sequencing and observed the wide diversity of prokaryotic and eukaryotic microorganisms on all berries tested.

According to our data, sea buckthorn, black chokeberry and both currants were dominated by fungal microorganisms prevalent on intact fruits due to the early harvesting time. In grapes, it was previously demonstrated that the intact berries were dominated by basidiomycetous yeasts, such as *Cryptococcus*, *Rhodotorula*, *Sporobolomyces* and the yeast-like fungus *Aureobasidium pullulans* (Barata, Malfeito-Ferreira, & Loureiro, 2012). During the ripening, the oxidative or weakly fermentative ascomycetous populations, such as of *Candida*, *Hanseniaspora*, *Metschnikowia*, and *Pichia* increased in frequency (Barata et al., 2012). *Cladosporium* and *Cryptococcus* were the most abundant genera observed in our study on black chokeberry, red and white currant berries. This is not surprising since *Cladosporium* are considered as ubiquitous fungi, with some species causing plant or human diseases (Sandoval-Denis et al., 2016; Wit et al., 2012), while others providing numerous antifungal agents (Wang et al., 2013). The abundant presence of *Cryptococcus* may be relevant, considering that species of this genus have been used as biocontrol agents against many pathogens (Chand-Goyal & Spotts, 1996; Hashem, Alamri, Hesham, Al-Qahtani, & Kilany, 2014). The genus *Cryptococcus* also encloses species capable of biofilm formation, thus reducing fungal cell susceptibility to heat, cold and UV irradiation (Martinez & Casadevall, 2007). Of note, certain species of *Cryptococcus* can cause infections in humans and animals, e. g., nervous system mycoses or pulmonary diseases (Bernal-Martinez et al., 2010).

It is generally accepted that more than 98% of microorganisms present in nature cannot be isolated (Lucero, Unc, Cooke, Dowd, & Sun, 2011). Therefore, the most informative estimates of the fungal diversity in plants are obtained from the metagenomic sequence data (Handelsman & Alvarez-Cohen, 2007). When fresh-cut fruits such as apple, plum, pear, or orange were analyzed by culture-dependent techniques, only microbial populations of limited diversity, mainly consisting of yeasts and molds, were isolated (Graca et al., 2015; Janisiewicz, Jurick, Peter, Kurtzman, & Buyer, 2014; Vadkertiova, Molnarova, Vranova, & Slavikova, 2012; Volschenk et al., 2016). Employment of the high-throughput sequencing techniques allowed detection of not only prevalent microorganisms, but also species present at low abundance or unculturable, thus overcoming the limitations of the culture-dependent approaches (Clooney et al., 2016; Pinto et al., 2015; Vepstaite-Monstavice et al., 2018). According to our data, the majority of fungal microorganisms present on sea buckthorn were represented by uncultured *Aureobasidium*. On the other berries, uncultured Ascomycota (such as uncultured Metschnikowiaceae and uncultured *Taphrina*) were detected. Recently, it was demonstrated that some *Aureobasidium* species demonstrated biological control activity against leaf pathogens, especially against mold such as *Botrytis* and *Bacillus* bacteria (Grube, Schmid, & Berg, 2011; Parsa et al., 2016). *Metschnikowia* also includes species commonly found on the fruit surface and acting as biocontrol agents against different pathogens (Parafati, Vitale, Restuccia, & Cirvilleri, 2015).

We have identified beneficial fungal microorganisms, such as



(caption on next page)

Fig. 4. Heatmap of bacterial and fungal microorganisms OTU abundance on sea buckthorn, black chokeberry, red and white currant. The colour intensity is proportional to the relative abundance of bacterial and fungal microorganism OTUs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Hanseniaspora, *Rhodotorula* and *Dioszegia*, distributed at a low frequency. *Rhodotorula* spp. were previously found on grapes during all ripening stages and shown to be capable of producing biofilms on surfaces of berries, thus protecting them from pathogens (Lederer, Nielsen, Toldam-Andersen, Herrmann, & Arneborg, 2013). *Hanseniaspora* spp. displays antagonistic properties against the development of molds responsible for fruit spoilage (Liu et al., 2010). *Dioszegia* spp. is referred as a beneficial microorganism associated with arbuscular mycorrhizal fungi and possessing antagonistic activities (Renker, Blanke, Borstler, Heinrichs, & Buscot, 2004). From the potential pathogens for plants and/or humans, *Phoma*, *Lewia*, *Colletotrichum*, *Septoria*, *Taphrina* were identified (Aveskamp, de Gruyter, & Crous, 2008; Cisse et al., 2013; Eyal, 1999; Phalip, Hatsch, Laugel, & Jeltsch, 2006; Wang et al., 2013).

The presence of fungal microorganisms can potentially alter the composition of bacterial communities and *vice versa* (Grube et al., 2011). Among the prokaryotic consortia on all berries tested, we have identified microorganisms either potentially beneficial or pathogenic. *Pantoea* and *Pseudomonas* were abundant on sea buckthorn. It was reported previously that both genera contain species possessing antibacterial and antifungal activity (Ligon et al., 2000; Trotel-Aziz, Couderchet, Biagianti, & Aziz, 2008). However, certain *Pantoea* or *Pseudomonas* species, distributed in diverse ecological niches, including water, soil, fruits, vegetables and foodstuffs, are recognized plant, animal or human pathogens (Coutinho & Venter, 2009; Cruz, Cazacu, & Allen, 2007; Higgins et al., 2018; Pukatzki, Kessin, & Mekalanos, 2002). Bacteria from the genera *Sphingomonas* and *Hymenobacter* residing on black chokeberry, red and white currants could be advantageous for plants due to their ability to induce plant resistance and promote growth, as well as by exhibiting antagonism against food-spoilage bacteria (Kim et al., 1998; Mageswari, Subramanian, Srinivasan, Karthikeyan, & Gothandam, 2015; Sukweenadhi et al., 2015). Similar beneficial traits may be possessed by other bacteria documented in our study, such as *Massilia* spp. (Ofek, Hadar, & Minz, 2012), *Acinetobacter* spp. (Suzuki, Sugawara, Miwa, & Morikawa, 2014), *Flavobacterium* spp. (Sang & Kim, 2012), *Methylobacterium* spp. (Ryu et al., 2006). Some species of abovementioned genera may induce metabolic processes that cause food to be unsuitable for human consumption (Ercolini, Russo, Nasi, Ferranti, & Villani, 2009; Rawat, 2015). Since microorganisms cohabiting sea buckthorn, black chokeberry and both currants at the species level were undescribed or assigned to the uncultured bacterium, it is plausible to suggest that some species undesirable for plants and humans are also present at a low frequency.

Co-existence of the different microbial population on the plants is driven by the multiple forces. It is dependent on produced enzymatic or bioactive compounds and generates competition for the nutrients (Pinto et al., 2014). The distribution of fungal and bacterial microorganisms on sea buckthorn, black chokeberry and both currants was showed to be essential to understand the existing balance of pathogenic and beneficial microorganisms. This is highly relevant for the development of strategies for plant cultivation and disease management. Furthermore, an increasing demand of natural food, minimally processed and prepared without chemical preservatives, requires comprehensive analysis of microbiota residents of the plants. The technology of production of natural food therefore should be bounded with the understanding of the balance among beneficial and pathogenic microorganisms, ubiquitously present even under good farming practices. The limited processing underscores the significance of quality of raw material for preparation of food truly valuable for human health.

5. Conclusion

In this study, we analyzed bacterial and fungal microorganism populations associated with the black chokeberry, see buckthorn, red and white currant berries harvested in Lithuania by applying NGS techniques. Differences in diversity, composition and overall prevalence of eukaryotic and prokaryotic microorganism were dependent on the host plant. Among prokaryotic and eukaryotic consortia, we have identified potentially beneficial and pathogenic microorganisms. Obtained data substantiate understanding of the interactions between resident microflora and plant. The study uncovers the importance of microbiota in berries-based food production and deepens knowledge of their ecological and medical potential.

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Author contributions

Investigation: IVM, JL.
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.

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