The Potential use of Bacterial Diversity Analysis of Soft Rotted Potato Tubers and Their Geocaulospheres

Sanja Marković
University of Belgrade Institute for Multidisciplinary Research: Univerzitet u Beogradu Institut za multidisciplinarna istrazivanja

Tatjana Popović (tanjaizbis@gmail.com)
Institut za zastitu Bilja I Zivotnu Sredinu https://orcid.org/0000-0003-0125-5905

Tanja Berić
Univerzitet u Beogradu Bioloski Fakultet

Ivica Dimkić
Univerzitet u Beogradu Bioloski Fakultet

Aleksandra Jelušić
University of Belgrade Institute for Multidisciplinary Research: Univerzitet u Beogradu Institut za multidisciplinarna istrazivanja

Renata Iličić
Univerzitet u Novom Sadu Poljoprivredni fakultet

Slaviša Stanković
Univerzitet u Beogradu Bioloski Fakultet

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Abstract

The soft rot caused by *Pectobacterium* and *Dickeya* spp. is among the most important potato disease, responsible for outbreaks worldwide. In 2018, potato tubers (cultivar Lady Claire) with and without visible soft rot symptom, together with their geocaulospheres were sampled from the field in Bačka region (Serbia). The 16S rRNA Next Generation Sequencing (NGS) of tubers with and without soft rot symptom and their corresponding soils was performed to detect differences in microbial diversity and, using this data to predict causal agent(s) of disease and which samples are potentially best for isolating biocontrol strains. The ubiquitous soil bacteria from the phyla Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes were dominant in all samples. The sequences identified as *Pectobacterium aroidearum*, *P. carotovorum*, and *P. polaris* were present in all tested samples and it can be hypothesized that they caused soft rot. The K3 sample showed the presence of genera with potential antimicrobial activity (*Pseudomonas, Arthrobacter, Chryseobacterium, Bacillus*, and *Exiguobacterium*). This result shows that diversity analysis could be used for checking for the presence of potential antagonistic bacteria at infected sites. Also, following the presence or absence of particular taxa could point out a capacity of soil to endure a growth season without an outbreak of soft or to maintain it without significant losses. This is a new approach to interpreting the results of the diversity of bacterial communities of tubers and can be useful for screening the health status of the soil.

Introduction

Most plant pathogenic bacteria are culturable and usually, they are identified based on visible symptoms of the disease on the host, biochemically, molecular-genetically, and through their pathogenicity (Riesenfeld et al. 2004; Neelakanta and Sultana 2013). On the other hand, analysis of microbiomes, through the development of Next Generation Sequencing (NGS) technologies provides a taxonomic characterization of up to 90% cultivation-independent microbial taxa (which are not or are poorly described), including the pathogens and antagonistic microorganisms, as well (Faure and Joly 2016; Bakshi et al. 2020). The metagenomic approach towards revealing bacterial communities' diversity of healthy plants, diseased plant tissue, and the soil is a useful tool for providing information about the distribution of different taxa across ecosystems and their relative abundance (Doonan et al. 2017).

The soft rot *Enterobacteriaceae* (SRE) with the *Pectobacterium* and *Dickeya* as representing genera are considered to be the most important phytopathogenic bacteria which affect a range of plant species worldwide (Mansfield et al. 2012; Pritchard et al. 2016) including potato (*Solanum tuberosum* L.) (Toth et al. 2011; Charkowski 2018; Charkowski et al. 2020). The SRE causes symptoms of the blackleg in the field on potato plants, and the soft rot symptom on the tubers in the field and/or in storage (Czajkowski et al. 2015). The *Pectobacterium* and *Dickeya* species can colonize the plant intercellular spaces, and they can cause a latent infection without any visible symptoms (Pérombelon 2002; Lebeau et al. 2008; Czajkowski et al. 2011).

During the last decade, a trend of increasing the blackleg and the soft rot potato diseases has been observed worldwide (Czajkowski et al. 2011; Toth et al. 2011; Ngadze et al. 2012; Nabhan et al. 2013; Moretti et al. 2016; Motyka et al. 2017; Waleron et al. 2019; Charkowski et al. 2020; van der Wolf et al. 2020). As causal agents, the species that are most commonly found on potatoes include *Pectobacterium atrosepticum*, *P. brasiliense*, *P. carotovorum*, *P. odoriferum*, *P. parmentieri*, *P. peruviense*, *P. polaris*, and *P. punjabense*; as well as *Dickeya dianthicola* and *D. solani* (Charkowski et al. 2020). In Serbia, in Bačka region - the largest potato growing area, blackleg, and soft rot diseases start to occur frequently in recent years (Bijelić, personal communication).

This study provides insights into bacterial communities of tubers with or without visible soft rot symptom and their surrounding soil (geocaulosphere) taken from the blackleg and soft rot outbreak in a commercial potato field in Serbia during 2018. The differences in overall diversity among samples, determined by amplicon sequencing-based on 16S

rRNA, were explored for use in the detection of potential plant pathogen(s) and antagonists and in the pinpointing of taxa that indicate soil health status.

Material And Methods

Sample collection

During September 2018, potato tubers (cv. Lady Claire) with and without visible soft rot symptom, together with their geocaulospheres were sampled from the field in the Bačka region in Serbia (GPS coordinates: 45°21'05.0"N; 19°22'47.8"E). Two potato samples (K1 and K2), both consisted of 3 tubers, with the visible soft rot symptom and their surrounding soils (Z1 and Z2), were taken from opposed field sites where the highest number of diseased plants were noticed. Additionally, K3 and Z3 were samples of 3 symptomless tubers and their surrounding soils collected from the infield place with the lowest number of diseased plants. For each potato and surrounding soil sample, a total of three replicates were collected.

DNA extraction, library preparation, and NGS sequencing

The extraction of ultra-pure DNA from three potato tuber samples K1, K2, and K3 with the approximate weight of about 200 mg was performed using the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research). The PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, USA) was used for the extraction of total DNA from three soil samples Z1, Z2, and Z3. The DNA was isolated and purified using the protocol provided by the manufacturers. The DNA concentration of all six analyzed samples was determined using Qubit Fluorometric Quantitation (Qubit 4 Fluorometer, Invitrogen™, USA). Each pooled DNA sample was composed of out of three individual replicates.

The obtained DNA was amplified according to the Metagenomic Sequencing Library Preparation Illumina protocol (Cod. 15044223 Rev. A), using the gene-specific 16S rRNA primers (For-5'-CCTACGGGNGGCWGCAG-3'; Rev-5'-GACTACHVGGGTATCTAATCC-3') targeting V3 and V4 regions (Klindworth et al. 2013). Illumina adapter overhang nucleotide sequences are added to the gene-specific primers. The protocol was initiated with a microbial genomic pooled DNA (5 ng μL^{-1} in 10 mM Tris pH 8.5). After 16S rRNA gene amplification, the multiplexing step was performed using the Nextera XT Index Kit, (FC-131-1096). A total of 1 µL from each PCR product was analyzed on a Bioanalyzer DNA 1000 chip to verify the expected size of 450 bp. The libraries were then sequenced using a 2 × 300 bp paired-end run [MiSeq Reagent kit v3 (MS-102-3001)] on a MiSeq Sequencer according to manufacturer's instructions (Illumina, USA).

Sequence data process and taxa annotation

Quality data control was performed using the *prinseq-lite* program (Schmieder et al. 2011). The parameters were set as follows: min_length (50); trim_qual_right (30); trim_qual_type (mean), and trim_qual_window (10). R1 and R2 reads from Illumina sequencing were merged using fastq-join within the ea-utils suite (Aronesty 2011). Data have been obtained using an *ad-hoc* pipeline written in R Statistics environment (R Core Team 2012). Denoising, paired-ends joining, and chimera depletion were performed starting from paired ends data using the DADA2 pipeline (Callahan et al. 2016).

Taxonomic assignments have been determined using the RDP classifier from the Ribosomal Database Project (Cole et al. 2009). Taxonomic assignation tables were summarized using the Krona tool for interactive metagenomic visualization of bacterial taxa distributions within samples, allowing more informed interpretation and easy browsing of the results (Ondov et al. 2011). Main data reported in contingency and proportion tables, or the Krona diversity representations, have been obtained by considering the whole dataset, from whom are excluded the taxa represented by less than 3 sequences. However, all operational taxonomic units (OTUs) including all singletons (a total of 294.374

unique sequences), were subjected to homology search on GenBank via BLAST of the National Centre for Biotechnology Information (NCBI) database to reveal and secure taxonomic relevance of aggregated top hit species including *Pectobacterium* and *Dickeya* genera.

Bioinformatic analysis

All computations and statistics have been carried out within R statistics environment with reported packages: knitr, knitcitations, markdown: report and reference environment (Allaire et al. 2014; Boettiger 2014; Xie 2014); bioconductor packages for genomics data (Biostrings); community ecology package – Vegan (Oksanen et al. 2017).

Alpha diversity, a bacterial diversity within communities, was determined by sampling-based analysis of OTUs and shown through estimators Shannon, Simpson, invSimpson, and FisherAlpha indices. Observed and estimated richness was determined with different estimators (number of observations -OBS, Chao1, Chao1standard error, ACE, and ACE standard error). Rarefaction analysis was performed to permit estimating the overall diversity covered by the obtained sequences.

Beta diversity (diversity shared across sample communities) was determined using Principal Coordinates Analysis (PCoA) which allows visualization of similarities or dissimilarities in a data set containing individuals/observations described by multiple inter-correlated quantitative variables (Lê et al. 2008), as well as through clustering analyses and Canonical Correspondence Analysis (CCA) explaining taxonomical data through environmental factors. The functions are based on the Legendre algorithm (Legendre and Anderson 1999): in Chi-square transformed data matrix is subjected to weighted linear regression on constraining variables, and the fitted values are submitted to correspondence analysis performed via singular value decomposition. All clustering and CCA figures have been obtained using distance matrices for phyla, families, and genera. One thousand bootstraps have been applied to obtain statistics. The alpha and beta diversities of the bacterial communities were determined by comparing differences at the phylum, family, and genus levels.

Results

Diversity of bacterial communities associated with tubers and their geocaulospheres

The phylogenetic composition of bacterial communities associated with symptomless (K3) and potato tubers with the symptom of soft rot (K1 and K2) as well as corresponding geocaulospheres (Z1, Z2, and Z3) was analyzed using six DNA samples isolated from commercial potato field by amplifying and sequencing the V3 and V4 regions of the 16S rRNA gene. The mean length of the obtained sequences ranged between 455 and 465 bp. After trimming and quality filtering, classifiable paired-end 537.192 sequence reads were retained. Following the OTU-clustering and chimera-checking steps, total numbers of 257.843 and 279.349 OTUs were obtained in tubers and surrounding soil, respectively.

The bacterial richness and diversity indices for each sample at the phylum, family, and genus levels are presented in Table 1. Bacterial communities were rich and diverse for each sample, at all taxonomic levels. The highest alpha diversity (presented through the FisherAlpha index) was detected in samples of symptomless tubers and their geocaulospheres (K3 and Z3). Also, the same samples had the highest values for all estimators except for Shannon and Simpson indices which failed to detect a significant difference. For all samples OBS values, as a richness estimator, were highest at the genus level. The differences between observed and estimated richness were in positive correlation according to Chao1 and ACE indices at all taxonomic levels.

Table 1
Biodiversity measures of obtained taxa trough microbial richness and alpha diversity indices

	Phylum/*Family*/Genus								
	Shannon	**Simpson**	**invSimpson**	**FisherAlpha**	**OBS***	**CHAO1**	**CHAO1.SE**	**ACE**	**ACE.SE**
K1	1.07	0.63	2.69	1.17	13	19.00	7.19	21.08	2.09
	2.62	*0.90*	*9.88*	*10.25*	*92*	*116.43*	*15.17*	*110.46*	*5.28*
	3.09	**0.92**	**12.35**	**23.71**	**193**	**234.00**	**16.77**	**233.37**	**7.55**
K2	1.05	0.62	2.65	1.15	13	13.00	0.12	13.89	1.89
	2.26	*0.84*	*6.10*	*10.61*	*96*	*117.11*	*12.61*	*117.86*	*5.52*
	2.71	**0.86**	**7.34**	**23.1**	**191**	**234.75**	**16.31**	**250.78**	**8.07**
K3	0.65	0.35	1.54	1.66	18	23.00	6.00	25.30	1.97
	1.86	*0.75*	*4.07*	*15.94*	*137*	*200.07*	*26.09*	*197.9*	*7.27*
	2.55	**0.87**	**7.62**	**32.46**	**256**	**347.00**	**27.65**	**343.15**	**9.48**
	Phylum/*Family*/**Genus**								
	Shannon	**Simpson**	**invSimpson**	**FisherAlpha**	**OBS***	**CHAO1**	**CHAO1.SE**	**ACE**	**ACE.SE**
1.73	0.73	3.73	2.04	22	22.00	0.24	22.53	1.66	
3.69	*0.95*	*18.61*	*27.81*	*227*	*236.10*	*6.49*	*234.54*	*6.62*	
4.20	**0.96**	**23.65**	**68.11**	**495**	**551.45**	**18.28**	**549.07**	**11.35**	
1.44	0.67	3.02	2.05	22	22.50	1.29	23.89	1.89	
3.21	*0.91*	*11.55*	*27.58*	*224*	*240.87*	*9.29*	*238.39*	*7.24*	
3.77	**0.93**	**13.95**	**63.68**	**464**	**514.73**	**16.52**	**509.17**	**11.01**	
Z3	2.05	0.82	5.58	2.80	29	31.00	2.88	32.29	2.42
	4.13	*0.97*	*30.04*	*33.06*	*261*	*294.07*	*15.56*	*289.37*	*7.24*
	4.54	**0.97**	**34.69**	**86.55**	**600**	**685.85**	**22.33**	**693.23**	**12.83**

*OBS – observed species richness

The beta diversity estimated through the PCoA analysis revealed a close association between all tuber samples (Fig. 1a). Bacterial community composition of K1, K2, and K3 samples was more similar to each other than to samples of the geocaulosphere, which can be seen from very high values for the first dimension (Dim1 in the range from 59–75%) at phylum, family, and genus levels. According to the main axis of disjunction, the bacterial communities of Z1 and Z2 soil samples were grouped and more similar compared to the Z3. The PCoA analysis clearly showed the separation of the Z3 sample from all other samples (Fig. 1a). According to the second dimension, all samples of potato tubers were more like the soil sample Z2. However, according to hierarchical clustering analysis based on the abundances of specific taxa, the most a like samples were K1 and K2, and their surrounding soil samples Z1 and Z2, and these findings were also confirmed through CCA (Fig. 1b).

Bacterial community composition

Bacterial diversity at the phylum and family levels for all samples are summarized in Fig. 2. The relative abundance of different phyla indicated a higher diversity for geocaulosphere samples (Z1, Z2, and Z3) when compared to potato tubers samples (K1, K2, and K3). The Proteobacteria were present in all samples in range from 32–78%. The Bacteroidetes and Firmicutes were also present in all six samples, but in a higher proportion ratio in tuber samples with visible soft rot symptom. The Bacteroidetes was the least represented phylum in symptomless samples (K3 and Z3). The Actinobacteria and Acidobacteria were presented with a low number of taxa or at the limit of detection in tuber samples, but more abundant in symptomless samples and the most abundant in soil samples. Besides Acidobacteria, Planctomycetes, Verrucomicrobia, Chloroflexi, Gemmatimonadetes, Nitrospirae, and Candidatus Saccharibacteria were the most abundant in the Z3 geocaulosphere sample.

At a family level, the relative abundance of different families indicated a higher diversity for geocaulosphere samples comparing to tuber samples. In samples consisted of tubers with visible soft rot symptom and their geocaulospheres, families such as Moraxellaceae, Bacteroidaceae, Flavobacteriaceae, Porphyromonadaceae, Xanthomonadaceae, Lachnospiraceae were the most abundant. Less represented were Enterococcaceae, Neisseriaceae, Comamonadaceae, Acidaminococcaceae, Alcaligenaceae, Campylobacteraceae, Leuconostocaceae, Sphingobacteriaceae, and Veillonellaceae. In symptomless potato tubers Enterobacteriaceae and Pseudomonadaceae were prevalent; Planococcaceae, Micrococcaceae, Incertae Sedis XII, and not defined Bacillales were more abundant than the family of Bacillaceae 1. Other families such as Gp6 (Acidobacteria phylum), Planctomycetaceae, Sinobacteriaceae, Gaiellaceae, and Chitinophagaceae were the most abundant families in the Z3 sample.

The sequences were classified at the genus level using the RDP classifier (Fig. 3). Again, higher diversity was observed in soil samples compared to tuber samples and different genera dominated the communities of these samples. The most dominant genus was *Acinetobacter* in K1 and K2 (with a relative abundance of more than 20%), and also in Z1 and Z2 samples (15 and 21%, respectively). Genera such as *Bacteroides, Dysgonomonas, Empedobacter, Myroides, Morganella*, and *Propionispira* were almost exclusively present in tuber samples with visible soft rot symptom and their geocaulosphere (K1, K2, Z1, and Z2). Additionally, *Wohlfahrtiimonas*, *Clostridium* XlVa, *Providencia*, *Proteus, Arcobacter, Kerstersia*, and *Weissella* dominated only in communities of tubers with the symptom (K1 and K2). On the other hand, *Sphingobacterium, Stenotrophomonas, Comamonas, Achromobacter*, and *Corynebacterium* were abundant within Z1 and Z2. Genera *Enterobacter, Pseudomonas, Raoultella, Kurthia, Arthrobacter*, and *Exiguobacterium* were most abundant in communities of the K3 sample. The most abundant genera in the Z3 sample were *Sphingobium, Sphingomonas, Lysobacter, Gaiella*, and *Gemmatimonas*. For all tuber samples *Vagococcus, Lactococcus, Enterococcus, Stenotrophomonas*, *Flavobacterium*, and *Pectobacterium* were ubiquitous and almost equally distributed.

The diversity and abundance of genera within the Enterobacteriaceae family in all samples were presented in Fig. 4. The *Pectobacterium* spp. were most abundant in K3 (18%) and geocaulosphere samples Z1, Z2, and Z3 (19–21%). According to the whole dataset with singletons excluded, the *Dickeya* genus was not found, although bacteria belonging to the *D. dianthicola* were isolated and identified from the same field (Marković et al. 2020).

However, separate manual BLAST analysis of top hit species, out of a total dataset including singletons, was performed to obtained information of hidden hints related to the *Pectobacterium* and *Dickeya*, spp. and the results are showed in Table 2. The sequences identified as the *Dickeya* were at the limit of detection in all samples. Same approach showed that the sequences of *Pectobacterium* species were also presented in all samples, in up to 7% (K3 sample). Among determined species the most important were *P. aroidearum*, *P. carotovorum*, and *P. polaris*. The *P. carotovorum* was the most abundant in K3, Z1, and Z2 samples.

Table 2
A total percentage of top hit BLAST aggregated species including *Pectobacterium* and *Dickeya* singletons

Species name	Sample name					
	K1	K2	K3	Z1	Z2	Z3
Dickeya aquatica	–	–	0.001	–	–	–
Dickeya chrysanthemi	0.001	–	0.008	–	0.001	0.001
Dickeya dadantii	–	–	–	0.001	–	0.002
Dickeya fangzhongdai	–	–	0.002	–	–	–
Dickeya zeae	–	–	–	0.001	–	–
Σ Dickeya total	**0.001**	**–**	**0.012**	**0.002**	**0.001**	**0.003**
Pectobacterium aroidearum	0.337	1.000	6.029	0.065	0.550	0.008
Pectobacterium atrosepticum	0.009	0.002	0.015	–	0.002	–
Pectobacterium betavasculorum	0.005	–	0.006	–	0.001	0.001
Pectobacterium cacticida	0.001	–	–	–	–	0.003
Pectobacterium carotovorum	0.138	0.119	0.739	1.714	0.938	0.062
Pectobacterium polaris	0.020	0.012	0.306	0.016	0.015	–
Σ Pectobacterium total	**0.509**	**1.133**	**7.094**	**1.795**	**1.506**	**0.074**
Σ Other total	**99.490**	**98.867**	**92.894**	**98.203**	**98.493**	**99.922**

Discussion

The metabarcoding analysis of communities from our samples showed a higher diversity of samples from the soil than from tubers, at all examined taxonomic levels, which was expected, but also the diversity of bacterial communities of geocaulosphere of symptomless tubers was considerably higher than the ones surrounding rotten tubers. And while sequences of the Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria were present in all samples, nine more phyla had representatives almost exclusively in soil samples (Fig. 2). Similar findings have been reported in other studies of the microbial diversity of potato tubers and surrounding soil (Janssen 2006; Delmont et al. 2011; Kõiv et al. 2015; Roquigny et al. 2018). Amongst these ubiquitous phyla, it was indicative that the Bacteroidetes were considerably lower represented in symptomless tubers and their geocaulosphere. Since they play a fundamental role as decomposers in the rhizosphere environment (Wu et al. 2018) that could be taken as a health indicator and can be monitored relatively easy only by checking the presence/absence of taxa of the genus *Bacteroidetes* (whose presence was not detected at all in samples K3 and Z3).

Dickeya and *Pectobacterium* spp. are responsible for economically significant losses, mostly in the potato production area. Within the Euphresco project risk assessment of these pathogens and their propagation was analyzed. In Norway, *P. carotovorum* and *P. atrosepticum* were dominant in infected plants, while in Switzerland *P. brasiliense* was prevalent in blackleg symptomatic plants, followed by *P. carotovorum, P. parmentieri, D. dianthicola*, and *D. solani* (van der Wolf et al. 2020). These studies were based on isolation methods. Likewise, in Poland, *P. parmentieri* was dominant in diseased plants, but also *P. atrosepticum*, *P. c. carotovorum*, *P. c. odoriferum*, *P. c. brasiliense*, *D. solani*, and *D. dianthicola* have

been isolated from symptomatic plants as well (Motyka et al. 2017). *P. aroidearum*, as a new soft rot pathogen with a preference for monocotyledonous plants, was described by Nabhan et al. (2013), while the study of Moretti et al. (2016) revealed for the first time *P. aroidearum* as a causal agent of soft rot on potato in a mixed infection with *P. carotovorum* subsp. *carotovorum*. On the other hand, more recently *P. polaris* was reported as a newly reclassified species and shortly thereafter was described as a casual soft rot agent of potato in Poland (Waleron et al. 2019).

The results of our 16S rRNA amplicon sequencing research pointed to combined infection also with several *Pectobacterium* species (*P. aroidearum*, *P. carotovorum*, and *P. polaris*) as probable causative agents of soft rot disease. Using traditional isolation and molecular characterization in the same field in Northern Serbia during 2018, combined infection of *P. carotovorum* subsp. *brasiliensis* and *D. dianthicola* was determined (Marković et al. 2020) while the *Dickeya* spp. was found at the limit of detection in metabarcoding samples. This suggests the possibility that *Dickeya* spp. is a secondary pathogen that participates in, or even just succeeds infection of the primary pathogen (*Pectobacterium*) rather than inducing it, as shown by Kõiv et al. (2015) for some endophytes.

In our study, symptomless potato samples had a higher relative abundance of *Pectobacterium* than samples with a visible symptom. This finding could be explained by the life strategy of these pathogens considering that they are necrotrophic and actively destroying host tissue whereupon saprotrophic microorganisms competitively colonize and outgrown rotten tubers (Kõiv et al. 2015). Although symptomless tubers were sampled from the field with a low number of infected plants, the *Pectobacterium* finding could be explained as a consequence of latent or initial phase of infection, since it has been unequivocally shown that species of the genus *Pectobacterium* are present in a non-negligible percentage in the soil around the tubers with and without the symptom of rot. The reduced pathogenicity of the strains may also be the reason why *Pectobacterium* spp. were detected but the symptoms of the disease were not manifested in the K3 sample. This may be due to genetic mutations in the genome of the pathogen itself (Moleleki et al. 2017), but it may be caused by the presence of antagonistic bacteria whose mechanism of biocontrol action is to reduce the pathogenicity of the pathogen (Dong et al. 2004).

In addition to the obvious culprits for the appearance of soft rot, in the samples of tubers with the symptom, the genera such as *Wohlfahrtiimonas, Clostridium, Providencia, Proteus, Arcobacter, Kerstersia*, and *Weissella* were abundant. Many of them have representatives of opportunistic and pathogenic species, whose presence could be interpreted as a rotting indicator (Kõiv et al. 2015). Also, it was interesting that *Bacteroides*, as well as some genera with opportunistic pathogens of humans (*Dysgonomonas, Empedobacter, Myroides, Morganella*, and *Propionispira*), were exclusively present in samples with soft rot symptom. This study also indicated a higher abundance of *Sphingobacterium, Stenotrophomonas, Comamonas*, and *Achromobacter* in geocaulosphere samples of potato tubers with visible soft rot symptom, all of which have been associated with the biocontrol of various plant pathogens (Berg et al. 2001; Dhaouadi et al. 2019). These findings confirm that community diversity is shaped so that there is a balance between pathogens and antagonists in soil, but the balance was disturbed in tubers in favor of pathogens. However, what could be considered valuable data is that when our goal is to isolate bacteria with biocontrol potential, the unavoidable sampling point should be the soil around plants with disease symptoms.

A recent study was shown that the *Flavobacterium, Acinetobacter, Dickeya, Sphingobacterium*, and *Myroides* were prevalent in the rhizosphere microbiota of soft rot potatoes, where *Dickeya* was found as the causal pathogen (Mao et al. 2019). Our results correspond to these findings for samples with visible soft rot symptom and their geocaulospheres with exception of *Flavobacterium* which was equally distributed in all our samples. Using a qPCR Jiao et al. (2018) determined antibiotics-resistant bacteria from accumulating places in *S. tuberosum* (potato peel and potato tuberous root), among which *Acinetobacter, Achromobacter, Stenotrophomonas*, and *Corynebacterium* were found in higher content. Our results indicated a higher presence of *Acinetobacter* in tubers with soft rot symptom and their geocaulosphere, while *Stenotrophomonas* and *Corynebacterium* were prevalent in the geocaulosphere samples.

Results presented in this work showed differences at the genus level for the symptomless sample K3 in terms of the higher presence of *Pseudomonas, Arthrobacter, Chryseobacterium, Bacillus*, and *Exiguobacterium*. All of them possess antimicrobial activities (Foldes et al. 2000; Dardanelli et al. 2010; Vacheron et al. 2013; Chauhan et al. 2015; Mashiane et al. 2017; Puri et al. 2019; Yadav 2020) and perhaps their higher abundance could be the reason for decay symptom absence. The *Pseudomonas* species are important members of the rhizosphere and their colonization of the root surface prevents colonization of plant pathogens (Vacheron et al. 2013). *Bacillus* strains produced natural biocontrol metabolites and can be used against rot causal microorganisms (Foldes et al. 2000).

The results of this study showed that diversity analysis could be used for checking the presence of potential antagonistic bacteria at an infected site and consequently, suitable enrichment media, which would allow the isolation of the strains of interest, could be prepared, and used. Another conclusion that emerges from our results is that it is possible that most infections that cause soft rot are probably mixed infections, with multiple pathogens acting simultaneously and consequently succeeded by saprotrophs. Therefore, caution should be exercised in naming only one pathogen as the causative agent of a disease and additional efforts should be made to identify and verify the involvement of potentially several different pathogens that cause the observed symptoms. Moreover, diversity analysis in terms of the presence or absence of particular taxa could point out a capacity of soil to endure a potato growth season without an outbreak of soft rot or to maintain it without significant losses.

Declarations

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Figures

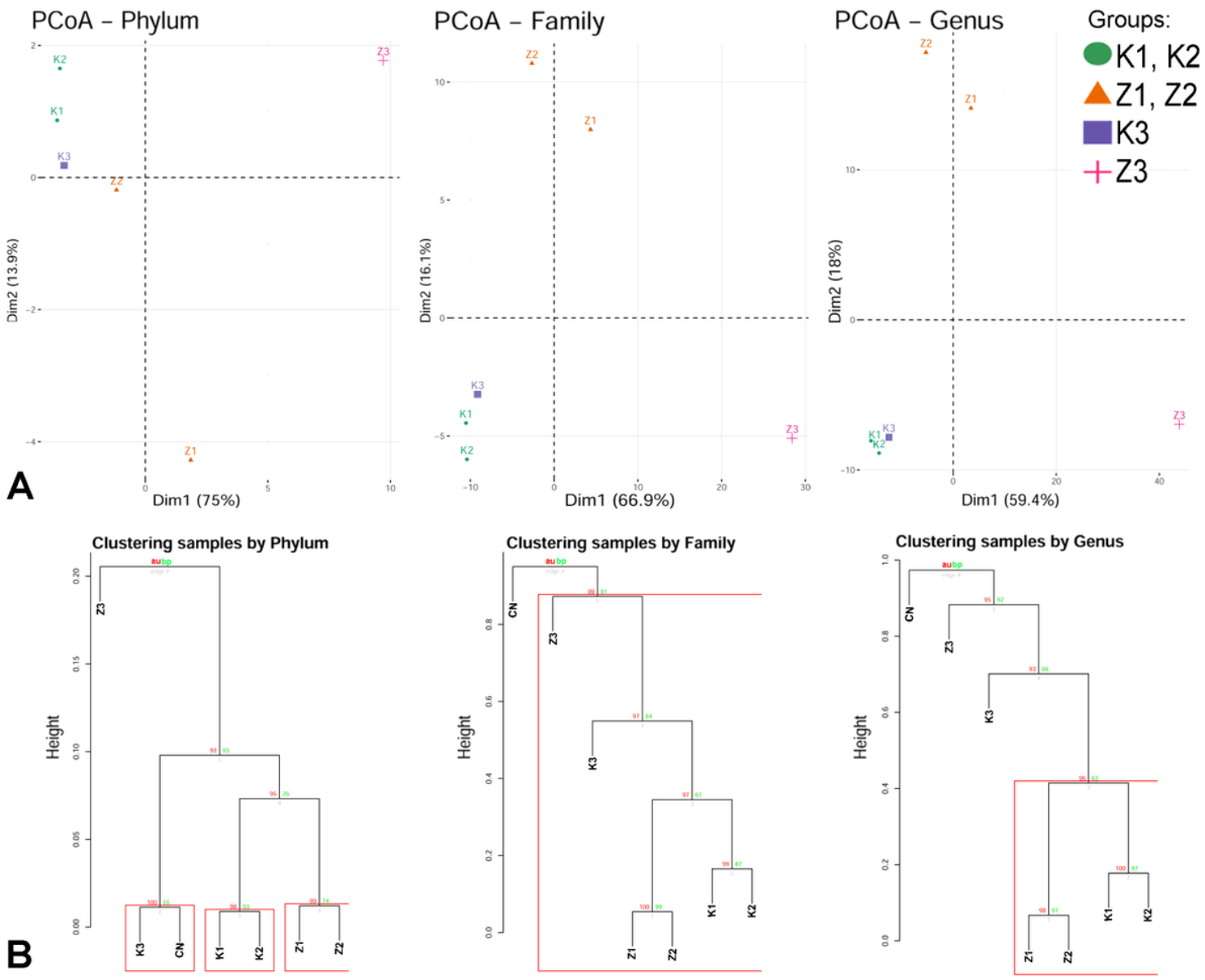

Figure 1

The Principal Coordinate Analysis (PCoA) of bacterial compositions in six different samples at phylum, family, and genus taxonomic levels. The individual samples are color coded to indicate differences between them (a). Cluster analysis of bacterial compositions for six different samples at phylum, family, and genus taxonomic levels (b). Dendrograms reports AU (Approximately Unbiased) values and BP (Bootstrap Probability) values scores (p-values). AU p-values (marked with red) were calculated by multiscale bootstrap resampling. Clusters (edges) with high AU values (≥ 95%) were strongly supported by the data. Red frames report approximately unbiased p-values higher than 95%. BP values (marked with green) were less accurate than the AU value

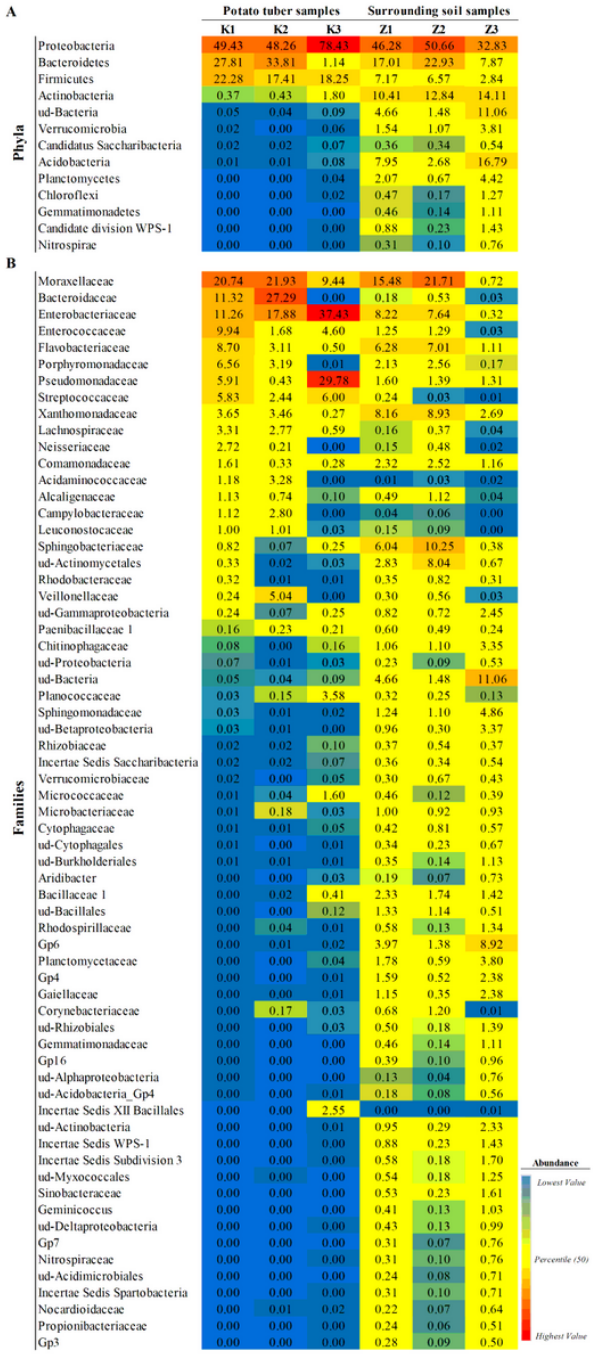

Figure 2

The relative abundance of bacterial taxa as assessed by 16S rRNA gene sequences on phylum (a) and family (b) levels. Only taxa with total percentage abundance above 0.5% for all samples were included

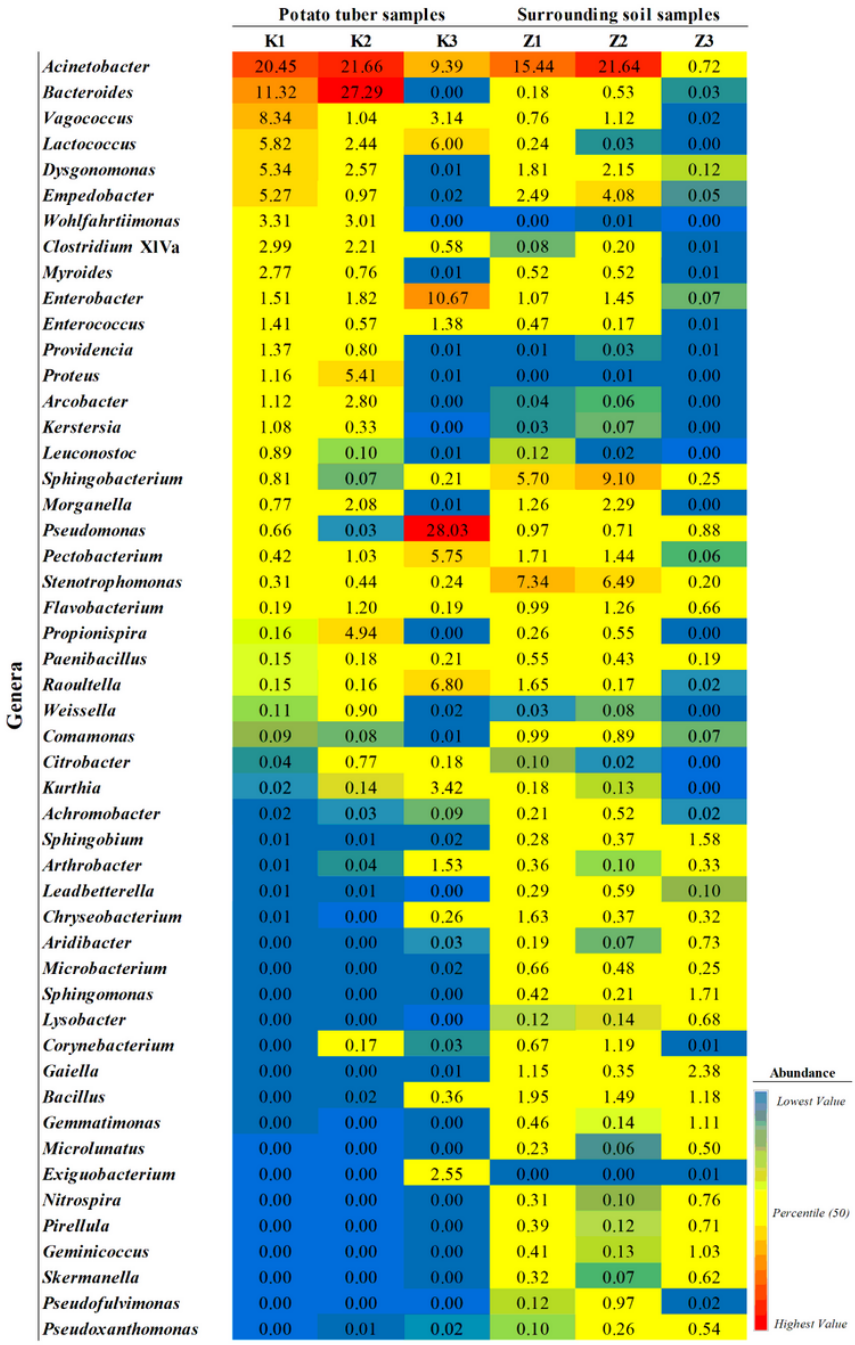

Figure 3

The relative abundance of bacterial taxa as assessed by 16S rRNA gene sequences on the genus level. Only taxa with total percentage abundance above 0.5% for all samples were included

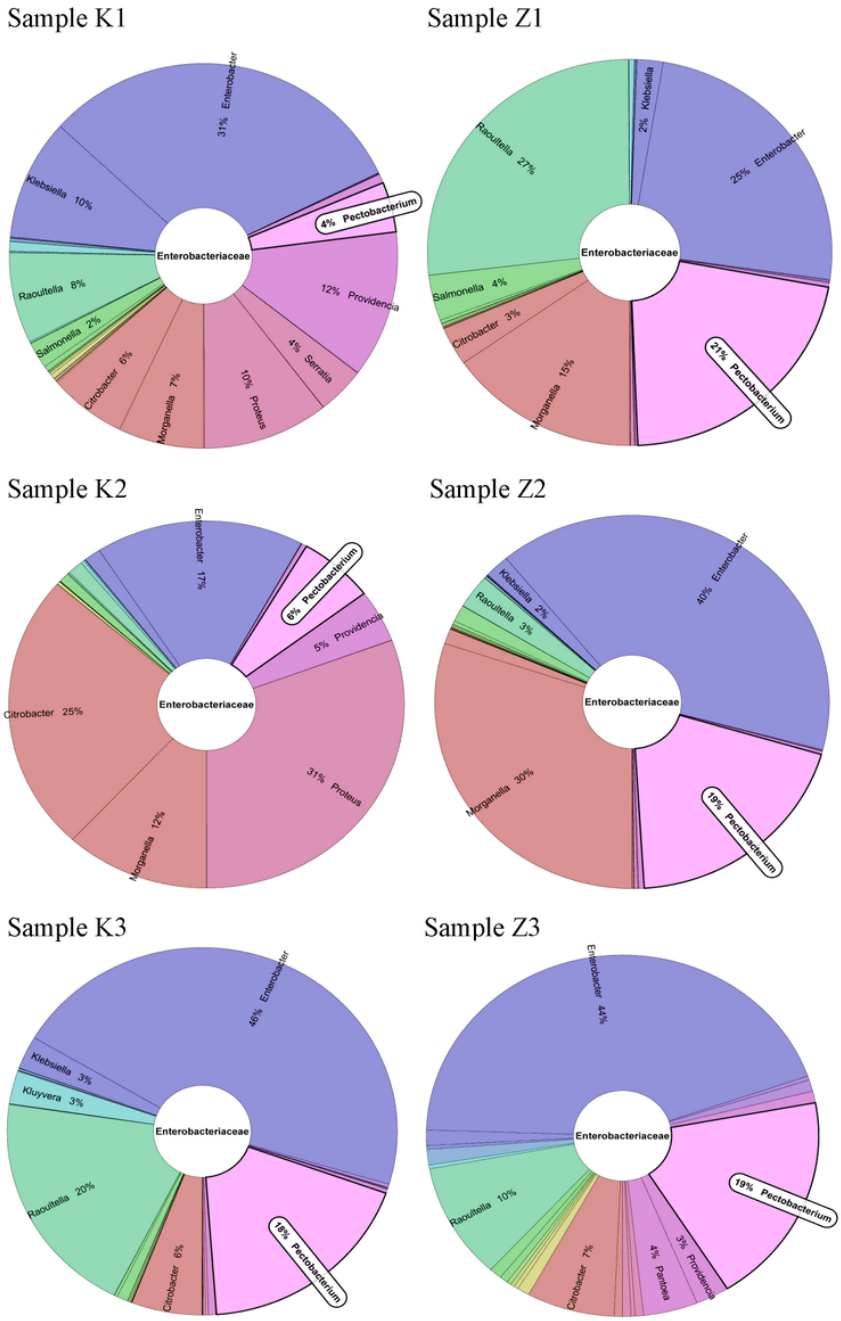

Figure 4

The relative abundance of genera within the Enterobacteriaceae family, for all samples, presented through the Krona interactive viewer