AGRICULTURAL BIOLOGY,
2019, V. 54, № 5, pp. 990-1001
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 635.21:632.4:577.21

doi: 10.15389/agrobiology.2019.5.990eng
doi: 10.15389/agrobiology.2019.5.990rus

ANALYSIS OF MYCOBIOME IN DAMAGED POTATO (*Solanum tuberosum* L.) LEAVES BY USING METAGENOMIC APPROACHES

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The authors declare no conflict of interests
Acknowledgements:
Supported financially by Russian Science Foundation, grant No. 18-16-00073. Sample collecting and description were financially supported by Ministry of Education and Science of the Russian Federation (Agreement No. 14.607.21.0178, RFMEFI60717X0178).
Received March 21, 2019

Abstract

The problem of potato diseases caused by fungi and fungi-like organisms is relevant for all regions of the world cultivating this crop, since it is mycoses that cause the most significant damage to plants (A. Bernreiter, 2017). The traditional approaches for identification of potato pathogens are aimed at identifying a specific pathogen and do not take into account neither other, often unknown pathogens, nor the other most important component — the beneficial microbiota of the phyllosphere community whose alterations can also become one of the causes of diseases. The novelty of this work lies in the fact that the high-throughput sequencing methods we used here is free of this disadvantages and makes it possible to identify virtually all plant microorganisms, including the phyllosphere and endosphere. The purpose of this study was to use metagenomic approaches to analyze the total fungal and fungi-like community in potato leaves that have morphological markers of damage by pathogens of the genus *Alternaria* and *Phytophthora* which are the causative agents of early blight and late blight. For fungal and fungi-like communities analysis, DNA samples was extracted from leaves of potato (*Solanum tuberosum* L.) cultivar Nikulinsky, affected by "alternaria" and "phytophthora" diseases types, which were later used to create amplicon libraries of ITS1 and ITS2 fragments and high-throughput sequencing on the Illumina MiSeq platform (Illumina, Inc., USA). During the bioinformatic data processing with the Illumina software and the PIPITS software package (H.S. Gweon et al, 2015), 187 OTU, 113 phylotypes for the ITS1 library and 249 OTU, 127 phylotypes for ITS2 were identified. Subsequent annotation of OTU and taxonomic analysis of the resulting communities were carried out with the QIIME program (J.G. Caporaso et al., 2010), the diversity coefficients within the community were calculated using the PAST software package (Ø. Hammer et al., 2001). Comparison of the fungal communities obtained for both types of lesion using different universal primers for the ITS1 regions (M. Usyk et al., 2017) and ITS2 (T.J. White et al., 1990) showed that only the first pair is suitable for the detection of phytophthora, and in general gives a more even community structure. The tools of automatic annotation turned out to be insufficient for objective identification of alternaria in samples, as a result we had to use the methods of manual search with the BLASTn program (S.F. Altschul et al., 1990). Since the primer pair ITS2 does not allow identification of *Phytophthora* in the samples, the further comparative analysis of the fungal communities of the two types of lesion was carried out using data only from the ITS1 library. The data of taxonomic analysis showed that in the affected areas for both types of mycoses a rich fungal community is formed, and, in the case of "late blight", the fraction of the pathogen is about 30 % in the community, and in the variant with "early blight", only 2.07 % with a significant part (about 15 %) accounted for by *Phytophthora*, which does not exclude the case of secondary lesion. Thus, it was shown that in the fungal and fungi-like communities formed in the areas affected by disease, the proportion of pathogens is no more than 30 %, which indicates a pronounced dynamics of the taxonomic composition of fungi in the affected area. It is obvious that high-throughput sequencing methods have a very high po-

tential in fundamental and applied research on plant diseases of a microbiological nature.

Keywords: fungi, fungi-like organisms, pathogens, potato, *Phytophthora infestans*, *Alternaria* sp., high throughput sequencing

Potato is an essential agricultural crop actively cultivated in many regions of the world; therefore, the problem of losing potato crops due to various diseases is relevantly high both in Russia and abroad. Plant diseases can be caused by bacteria, viruses, fungi and fungus-like organisms. Mycoses and lesions are characterized by the most severe course of the disease where representatives of fungus-like microbial flora, and oomycetes in particular [1], act as pathogens. The pathologies caused by them consist in manifestation of mildew, spotting and molds of fruit and seeds [2].

Plant leaf is an ecological niche populated by a community of microorganisms, including fungi and fungus-like organisms of phylloplane, endophytes and phytopathogens. Any imbalance in the community due to changing weather conditions, chemicals in the soil or for other reasons can result in pathogen domination and disease development [3, 4]. The useful inhabitants of phyllosphere which increase resistance to harmful representatives of microbial flora are diazotrophs, antagonists and bacteria ensuring plant growth [3]. The pathogenic organisms inhibiting the phylloplane represent potential threat for the host and can belong both to biotrophs and necrotrophic pathogens [3, 4]. The saprophytes affecting weakened plants, e.g. Fusarium fungi, can develop as the secondary infection masking the primary pathogen.

Phytophthora rot is one of the most dangerous potato diseases in the regions with humid and moderate climate, which is caused by *Phytophthora infestans* (Mont.) de Bary oomycete [5]. This pathogen affects the leaves reducing the assimilatory activity of the plant during tuber formation and provokes their further decay during storage [6]. The spread of phytophthora rot at the end of 1840s in Europe, especially in Ireland, resulted in loss of potato crops, which caused the Great Irish famine (Irish potato famine) [7]. In Russia and Europe the damage caused by phytophthora rot (depending on edaphoclimatic conditions of the region) can be from 10-12% to 50% of the entire potato crop yield [8, 9]. *Alternaria* imperfect fungi cause potato blight, which poses a serious threat for the regions with more arid climate characterized by presence of short-term precipitations and abundant night dew [4, 5]. Alternaria also affects leaf surface reducing assimilatory activity of the plant and resulting in crop yield loss of up to 40%. Furthermore, alternaria causes reduction of potato starch content, increases the share of non-saleable tubers [5, 6, 10] and causes accumulation of mycotoxins and allergens [2].

Saprophytic fungi of *Fusarium* genus cause fusarial potato wilt. Smirnov et al. [11] showed that pathogenic *Fusarium-Alternaria* complex becomes one of the reasons behind potato lodging in different regions of Russia and can emerge as primary and secondary lesion after rhizoctonia solani, phytophthora rot or bacteriosis. Earlier fusarial rot and early blight were widespread in southern regions, but now these diseases became typical for the European part of the country [4, 5, 11].

Early blight and phytophthora rot are widespread in all potato farming regions, and scientists in Russia and abroad analyze how they can be countered. In particular, the studies of phylloplane [3, 12], endophytic [13, 14] and edaphic fungal communities are known [15, 16], as well as studies of plant sections directly affected by mycosis [17, 18]. As a result, the pathogens of phytophthora rot, early blight and other mycological diseases have been identified. The effect of various agricultural methods [15] and fungicides [8, 10, 19], which can be

used in combination with the other means [20], e.g. with culture liquid of *Klebsiella planticola* [21], on pathogens is actively researched. The resistance of a number of potato varieties to pathogens of early blight and phytophthora rot has been studied [9, 22].

It is critical to properly identify pathogens in order to effectively combat them [23]. It is known that early blight pathogens are a complex of alternaria fungi where fungal forms with small and large spores [10] are distinguished by ecological properties, host specificity, pathogenicity, toxicogenity, sensitivity to fungicides, and geographic distribution [2, 4]. For identification, cytological methods are traditionally used, along with microscopy and isolation of pure cultures [1, 24], as well as specific symptoms on affected plants [1, 25], moist chamber method to stimulate formation of infected structures, and checking pathogenicity on susceptible plants. Furthermore, spectroscopy [25-27], mass spectroscopy [1], biosensor diagnostics [25], molecular methods based on polymerase chain reaction [28-30], cloning followed by Sanger sequencing with universal primers [17, 31] and enzyme-linked immunosorbent assay [32] are modern methods.

However, as has been noted above, it is important to analyze the community in general, including imbalance detection in phylloplane communities. This task is beyond the scope of methods focused on identification of a specific pathogen and not taking into account the other pathogenic organisms and the condition of normal nonpathogenic microbial flora, whose plant protection function is often underestimated. High throughput sequencing [3, 16, 33] provides an opportunity to evaluate not only known and unknown pathogens, for which no diagnosticums have been created to date, but also the normal flora. We selected two pairs of universal primers to ITS1 and ITS2 regions [35, 36] among primers developed for the creation of fungal amplicon libraries [23, 33, 34], which enable taxonomic identification of most fungal groups and fungus-like organisms to genus and even species [16, 37, 38].

In this study, for the first time we analyzed pathogenic and nonpathogenic fungal and fungus-like microbial flora using metagenomic approach on potato plants affected by diseases of early blight type and phytophthora rot type. We have assessed the presence and absence of the main pathogen and taxonomic structure of the entire community in the affected leaf area. It was shown for the first time that the actual pattern of the community is of complex and not completely definitive nature (e.g. the primary pathogen can be a minor component of the entire community). Taxonomic analysis of communities with both primers revealed pathogenic *Alternaria* fungus in a sample with signs of early blight type. Primers to ITS1 region helped identify oomycete *Phytophthora* for both lesion types.

Our subjective was to fully identify species composition of fungi and fungus-like organisms involved in potato leaf phytopathogenesis by high throughput sequencing and two universal primers.

Techniques. Leaf samples of potato (*Solanum tuberosum* L.) Nikulinsky cultivar with visible symptoms were collected in experimental field of Vavilov Russian National Plant Genetics Resources Institute (St.Petersburg—Pushkin, 59°42′37,78″C; 30°25′41,26″B) in 2017. Two types of lesions were identified based on morphological characters.

DNA was extracted directly from the affected leaf section using AxyPrep Multisource Genomic DNA Miniprep Kit (Axygen, USA) according to the manufacturer's instruction.

Taxonomic composition of fungal community and fungus-like organisms was identified in each sample with amplicon libraries of intergenic transcribed

spacers of ribosomal operons (ITS1 and ITS2). The diagnostic fragment was amplified in PCR (T100 Thermal Cycler, BIO-RAD Laboratories, Inc., USA) using following primer pairs: ITS1_30F-GTCCCTGCCCTTTGTACACA/ITS1_217R-TTTCGCTGCGTTCTTCATCG for ITS1 [35], ITS3-GCATCGATGAAGAACGCAGC/ITS4-TCCTCCGCTTATTGATATGC for ITS2 [36] with addition of service sequences as per the protocol of Illumina, Inc. (USA) containing linkers and barcodes. Phusion Hot Start II High-Fidelity polymerase (Thermo Fisher Scientific, USA) was used in PCR according to the manufacturer's protocol.

PCR products were purified with AM Pure XP (Beckman Coulter, USA) as per Illumina, Inc. recommendation. The libraries were further prepared in compliance with the manufacturer's instructions (MiSeq® Reagent Kit Preparation Guide, USA). The libraries were sequenced using Illumina MiSeq and MiSeq® Reagent Kit v3 (600-cycles) (Illumina, Inc., USA) with bilateral reading (2×300 nt).

The identified sequences were processed with Illumina software (Illumina, Inc.), QIIME software [39] and PIPITS software [40].

Data of sequencing were used for taxonomic profiling and comparing variants for ITS1 and ITS2 primers. During the analysis the representation of different taxa as well as abundance of communities were analyzed. Parameter reflecting the number of taxa (richness, i.e. the expected number of phylotypes), Simpson index (evenness, i.e. even distribution by phylotypes) and Shannon index were calculated using PAST software [41].

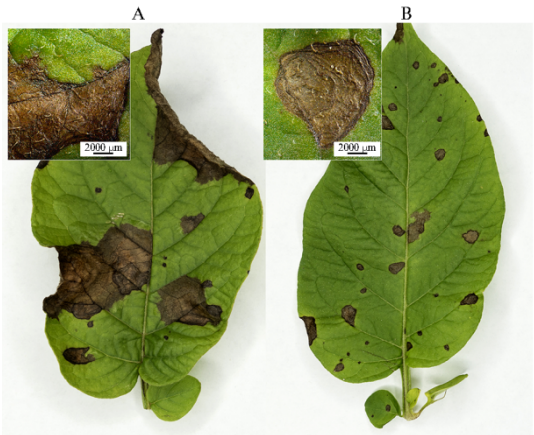

Fig. 1. Potato leaves (*Solanum tuberosum* L. cultivar Nikulinsky) with lesions of phytophthora rot (A) **and early blight** (B) **types** (experimental fields of Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg—Pushkin, 59°42′37,78″N; 30°25′41,26″E, 2017). A microscope Stemi 508 and light microscopy camera AxioCam ERc 5s (ZEISS, Germany).

Results. Based on the symptoms on potato leaves lesions were classified as diseases of early blight or phytophthora rot types (Fig. 1).

Common primers to ITS2 region [36] and wide-range primers to ITS1 region [35] were used (Fig. 2) in high throughput sequencing to identify pathogen species in the affected zone. Both lesion types were evaluated for each region in terms of composition and fungal and fungus-like community.

The number of reads (sequences) for samples obtained with a pair of primers for ITS1 with early blight and phytophthora rot signs amounted to 55000 and 54000 respectively, for ITS2 — 36000 and 21000. Processing of ITS1 and ITS2 libraries was performed separately, because OUT (operational taxonomic units) isolation can be performed only for homolog libraries. Bioinformatic processing for ITS1 resulted in 187 OUT and 113 phylotypes, for ITS2 — in 249 OUT and 127 phylotypes.

After automatic OTU annotation (UNITE databank, https://unite.ut.ee/, PIPITS software) a large group of OTU was taxonomically attributed only at kingdom level. Furthermore, a significant amount of *Alternaria* fungus (0.01 and 0.08% for ITS1, and 0.07 and 0.04% for ITS2 in variants with early blight and phytophthora rot signs, respectively) were not found in libraries for both primer pairs; moreover, phytophthora was not detected using ITS2 library. For this reason,

unannotated OTU were checked manually for taxonomic affiliation with BLASTn program [42]. The issue of incorrect OTU taxonomic diagnosis is mentioned by Halwachs et al. [43] who further recommend to manually adjust data with BLAST-based algorithm to clarify the results of automatic annotation. Some of the reasons why proper OTE are unannotated may be insufficient number of reference sequences in databases or different taxonomic resolution by ITS for different groups of fungi at genus and species levels [37].

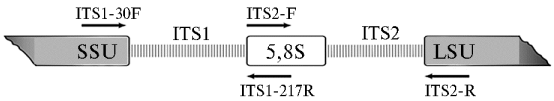

Fig. 2. Localization of ITS1 and ITS2 primers [35, 36] used to derive amplicon libraries and description of microbial communities in affected potato leaves (*Solanum tuberosum* L. cultivar Nikulinsky): SSU — small subunit, LSU — large subunit (rDNA regions encoding small and large ribosomal subunits, respectively).

The communities of fungi and fungus-like organisms were generally evaluated based on automatic taxonomic diagnosis. When comparing the results obtained for two regions we observed differences in taxonomic composition both among communities characteristic of two lesion types and among libraries obtained using primers to ITS1 and ITS2 (Table 1). For further comparison we used only the taxa, the share of which at least in one of the libraries amounted to more than 1% (see Table 1).

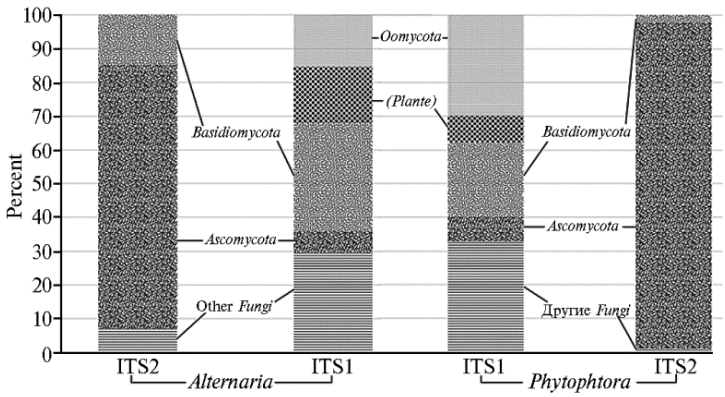

Fig. 3. Taxonomic composition of fungal and fungus-like communities in potato leaves (*Solanum tuberosum* L. cultivar Nikulinsky) with lesions of phytophthora rot and early blight types when constructed using primers to ITS1 and ITS2 regions (experimental fields of Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg—Pushkin, 59°42′37,78″N; 30°25′41,26″E, 2017)**.**

The differences in specificity of the primers we chose were observed as early as at the large taxon level (Fig. 3). For instance, broadly specific primers to ITS1 captured representatives of *Oomycota* phylum, which includes phytophthora rot; however, a significant amount of reads corresponded to plant organisms. This coincides with features of ITS1 primers described by Xu [16]. In turn, the primers to ITS2 that we selected captured only representatives of the fungal kingdom without identifying plant homologs. However, they demonstrated a more narrow specificity as compared to the ITS1 pair, resulting in lack of oomycetes in libraries, which makes this primer pair not suitable for phytophthora rot identification in affected samples.

To evaluate the percent of pathogens in the resulting communities, the results of automatic annotation for UNITE database and sequence check in BLASTn program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) were summarized (Table 2). The table was used to evaluate the efficiency of selected primers to identify the pathogens in question.

1. Taxonomic diagnosis of main operational taxonomic units (OTU) of fungal and fungus-like organisms in potato leaves (*Solanum tuberosum* L. cultivar Nikulinsky) with lesions of phytophthora rot and early blight types (experimental fields of Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg—Pushkin, 59°42′37,78″N; 30°25′41,26″E, 2017)

Taxonomic assignment	OUT percent in the community			
	Altern_Its1	Phyth_Its1	Altern_Its2	Phyth_Its2
k__Fungi; Other; Other; Other; Other; Other	29.45	32.85	7.15	1.08
k__Fungi; p__Ascomycota; c__Dothideomycetes; o__Capnodiales; f__Cladosporiaceae; g__Cladosporium	0.03	0.79	0.69	2.48
k__Fungi; p__Ascomycota; c__Dothideomycetes; o__Capnodiales; Other; Other	0.02	0.00	63.38	71.10
k__Fungi; p__Ascomycota; c__Dothideomycetes; o__Pleosporales; f__Didymellaceae	1.02	0.77	6.03	21.57
k__Fungi; p__Ascomycota; c__Dothideomycetes; o__Pleosporales; f__Phaeosphaeriaceae	1.59	0.23	2.29	0.13
k__Fungi; p__Ascomycota; c__Dothideomycetes; o__Pleosporales; Other; Other	0.04	0.10	1.85	0.46
k__Fungi; p__Ascomycota; c__Sordariomycetes; o__Hypocreales; f__unidentified; g__unidentified	0.00	3.23	0.00	0.00
k__Fungi; p__Ascomycota; Other; Other; Other; Other	1.26	0.43	0.00	0.00
k__Fungi; p__Basidiomycota; c__Microbotryomycetes; o__Sporidiobolales; f__Sporidiobolaceae; g__Sporobolomyces	0.82	2.64	0.00	0.00
k__Fungi; p__Basidiomycota; c__Tremellomycetes; o__Cystofilobasidiales; f__Cystofilobasidiaceae; g__Cystofilobasidium	2.70	1.46	1.15	0.00
k__Fungi; p__Basidiomycota; c__Tremellomycetes; o__Cystofilobasidiales; f__Mrakiaceae	0.00	0.00	6.35	0.11
k__Fungi; p__Basidiomycota; c__Tremellomycetes; o__Cystofilobasidiales; f__Mrakiaceae; g__Itersonilia	18.40	8.86	0.00	0.00
k__Fungi; p__Basidiomycota; c__Tremellomycetes; o__Cystofilobasidiales; f__Mrakiaceae; g__Udeniomyces	0.20	1.59	0.00	0.00
k__Fungi; p__Basidiomycota; c__Tremellomycetes; o__Filobasidiales; f__Filobasidiaceae; g__Filobasidium	0.22	1.39	0.00	0.00
k__Fungi; p__Basidiomycota; c__Tremellomycetes; o__Tremellales; f__Bulleraceae	0.03	0.00	3.44	0.79
k__Fungi; p__Basidiomycota; c__Tremellomycetes; o__Tremellales; f__Bulleraceae; g__Bullera	4.41	2.01	0.00	0.00
k__Fungi; p__Basidiomycota; c__Tremellomycetes; o__Tremellales; f__Bulleribasidiaceae	0.10	0.51	3.05	1.10
k__Fungi; p__Basidiomycota; c__Tremellomycetes; o__Tremellales; f__Bulleribasidiaceae; g__Dioszegia	1.18	0.83	0.00	0.00
k__Fungi; p__Basidiomycota; c__Tremellomycetes; Other; Other; Other	1.51	0.60	0.00	0.00
k__Plantae; p__unidentified; c__unidentified; o__unidentified; f__unidentified; g__unidentified	16.68	8.00	0.00	0.00
k__Stramenopila; p__Oomycota; c__Oomycetes; o__Peronosporales; f__Peronosporales_fam_Incertae_sedis; g__Phytophthora	15.47	29.85	0.00	0.00

N o t e. The data of high throughput sequencing with MiSeq platform (Illumina, Inc., USA) and an automatic annotation using UNITE database (https://unite.ut.ee/); k — kingdom, p — phylum, c — class, o — order, f — family, g — genus. Altern and Phyt, respectively, are samples with symptoms of early blight and phytophthora rot types; Its1 and Its2 are primers we used to create amplicon libraries.

2. Percentage of pathogens in fungal and fungus-like communities of potato leaves (*Solanum tuberosum* L. cultivar Nikulinsky) with lesions of phytophthora rot and early blight types as resulted from high throughput sequencing libraries of ITS1 и ITS2 fragments (experimental fields of Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg—Pushkin, 59°42′37,78″N; 30°25′41,26″E, 2017)

Community		Percent in the community	
by primer type	by lesion type	*Alternaria*	*Phytophthora*
ITS1	early blight	2.07	15.47
	phytophthora rot	0.11	29.85
ITS2	early blight	4.57	0
	phytophthora rot	0.006	0

N o t e. The results of automatic annotation for UNITE (https://unite.ut.ee/) database are adjusted by additional search in BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

3. Diversity in fungal and fungus-like communities of potato leaves (*Solanum tuberosum* L. cultivar Nikulinsky) with lesions of phytophthora rot and early blight types (experimental fields of Vavilov Russian National Plant Genetics Resources Institute, St. Petersburg—Pushkin, 59°42′37,78″N; 30°25′41,26″E, 2017)

Parameter, index	Altern_Its1	Phyth_Its1	Altern_Its2	Phyth_Its2
Number of taxa	19	18	10	9
Simpson index	0.81	0.77	0.54	0.43
Shannon index	1.90	1.87	1.31	0.84

Note. Altern and Phyt areleaves with lesion of early blight and phytophthora rot types, respectively; Its1 and Its2 are primers used to create libraries.

It must be pointed out that major irregularity was observed in ITS2 libraries in presenting taxa, for instance, there was a noticeable bias in the direction of representatives of *Capnodiales* (*Ascomycota*) order (see Table 1), which were predominant in two liaison variants resulting in significant reduction of variety parameter of the resulting community. This can be clearly seen when comparing the Simpson index characterizing the eveness of taxon distribution (Table 3). The same ratios were identified during analysis of the number of taxa or species richness and Shannon index. It is interesting that in reports of other authors [37] who studied the same community using ITS1 and ITS2, the libraries showed rather identical results during annotation of resulting OTE. The degree of result reproducibility was higher for basidiomycetes and lower for ascomycetes, particularly, a large number of clusters were allocated for them in a variant with ITS1. On the contrary, our data show oversaturation of ITS2 libraries with representatives of *Ascomycota* phylum due to *Capnodiales* order members.

When comparing communities at family level, the representatives of *Cladosporiaceae*, *Didymellaceae*, *Phaeosphaeriaceae*, *Cystofilobasidiaceae*, *Mrakiaceae*, *Bulleraceae* and *Bulleribasidiaceae* turned out common for both primer variants. The representatives of *Sporidiobolaceae*, *Filobasidiaceae* and *Peronosporales*, to which *Phytophthora infestans* belongs (see Table 1), were typical only for the ITS1variant.

Both primer pairs were suitable for identification of alternaria; however, for no explicable reason the automatic identification system did not attribute the fungi of this type and attributed them to the group of unidentified organisms. Nevertheless, by using manual adjustment based on BLASTn data the *Alternaria* genus fungi were identified and, apparently, they account for a noticeable part of the community.

Whereas comparison of ITS1 and ITS2 libraries, and more precisely, of the primers used, demonstrated apparent predominance of the former, we subsequently worked with ITS1 library only.

The data of taxonomic analysis for the sample affected with early blight type showed that unidentified representatives of *Fungi* kingdom were predominant (27.38 %). The representatives of *Itersonilia* (18.4 %) and *Phytophthora* (15.47%) genera were rather numerous. The plant ITS accounted for 16.68%. Fungi of *Bullera* (4.41%), *Cystofilobasidium* (2.7%) and *Alternaria* (2.07 %) genera (see Table 3) along with non-attributed representatives of *Tremellomycetes* order (1.51%) and ascomycetes (1.26%), as well as *Dioszegia* (1.18%) genus fungi were observed at family level. Representatives of *Bulleraceae* and *Bulleribasidiaceae* families, as well as *Udeniomyces* and *Filobasidium* genera (see Table 1) were observed in insignificant quantities (below 1% of the community). In the event of phytophthora rot type, two groups of organisms were predominant, i.e. unidentified representatives of *Fungi* kingdom (32.85 %) and oomycetes of *Phytophthora* genus (29.85%). The representatives of *Itersonilia* genus and plant ITS in the community accounted for 8.86 and 8.00%, respectively. They were followed, in

descending order, by representatives of *Hypocreales* order (3.23%), *Sporobolomyces* (2.64%), *Bullera* (2.01%), *Udeniomyces* (1.59%), *Cystofilobasidium* (1.46%) and *Filobasidium* (1.40%) genera. Also, a small number of taxa were observed in the community, whose share did not exceed 1% (see Table 1).

The ITS1 data showed presence of phytophthora rot pathogen in both samples, 15.47% and 29.85% of community, respectively, for early blight and phytophthora rot damage types. The representatives of *Alternaria* genus were also found, their share was 2.1 and 0.1%, respectively, for early blight and phytophthora rot types (see Table 3).

Regardless of lesion type, significant number of reads fell upon unidentified fungal organisms. The structure of the community obtained for sample with early blight showed presence of two subdominant taxa, *Itersonilia* and *Phytophthora* genera. In the phytophthora rot variant the *Phytophthora* genus acted as the second dominant. Also, both communities showed taxa either not identified in the other community or identified in insignificant quantities. For early blight damage type these were unidentified representatives of *Tremellomycetes* class and *Phaeosphaeriaceae* family fungi; for phytophthora rot these were unidentified representatives of *Hypocreales* order and *Sporobolomyces*, *Udeniomyces* and *Filobasidium* genera.

In studies on pathogens in fungal communities of phyllospheres and potato tubers, the *Alternaria* genus fungi are observed on affected plants in significant quantities; however, it has to be pointed out that the authors mostly use isolates grown on special nutrient media [12, 17, 18]. The studies of endophytic mycobiota of a healthy plant based on this approach also confirm significant presence of *Alternaria* genus fungus [13]. At the same time, detailed analysis of high throughput sequencing shows a small share of pathogen in the community (0.35%) [14], and in weakened plant phyllosphere affected by *Podosphaera* fungus it shows a more significant (0.35-4.6 %) portion. These data demonstrate the advantages of metagenomic approach in similar studies.

Therefore, we have identified serious differences in taxonic specificity of two primer pairs, ITS1 and ITS2. The ITS1 primers help identify not only more fungal taxa and fungus-like organisms, but also demonstrate higher levels of eveness in distributing the sequences by taxa. However, due to broader specificity, primers to ITS1 capture plant sequences. The automatic taxonomic database diagnosis not always reveal several taxa in the community. This can strongly distort the results of analysis, specifically in cases when certain process or absence of a specific pathogenic organism should be diagnosed. For this reason, combination of several tools to diagnose a target organism can have a positive impact on the results of the research. The community of affected potato leafs of Nikulinsky cultivar turned out quite rich. Interestingly, in case of phytophthora rot the share of pathogen did not exceed 29.9%, which is indicative of the dynamics of taxonomic composition of affected tissues. Apparently, during the first stage of infection there is almost always a major pathogen, and subsequently damaged zones are populated by opportunist microbial flora. Apparently, this explains low numbers of alternaria in the sample corresponding to early blight. There is a good chance that in the described case the presence of phytophthora rot can also be the secondary lesion.

To summarize, the results of taxonomic analysis showed a rather rich community of fungi and fungus-like organisms for lesions of both types, whereas a share of primary pathogen in the community when affected by phytophthora rot type accounts for about 30%, and only 27% when affected by early blight type whereas a significant part (about 15%) can be attributed to phytophthora rot (possibly, due to secondary damage). It is certain that high throughput sequencing

methods show a lot of promise in identifying plant pathogens; however, these methods require significant methodic work both at choosing primers and during analysis of libraries, when certain important taxa can be lost.

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