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Phylogenetic Study of *Alternaria* Potato and Tomato Pathogens in Russia

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Abstract: Early blight (EB) is a destructive disease affecting potato and tomato plants in Russia, caused by a heterogeneous group of plant pathogenic *Alternaria* fungi. The current species delimitation in *Alternaria* sect. Porri with medium to large conidia and a long (filamentous) beak is based on molecular data. In this study, the ITS, GAPDH, RPB2, TEF1, and Alt a 1 gene regions were analyzed in 41 large-spored *Alternaria* isolates obtained from diseased potato and tomato plants collected from 13 regions in Russia. Our data revealed five pathogenic species (*A. alternariacida*, *A. grandis*, *A. linariae*, *A. protenta*, and *A. solani*). Two species (*A. solani* and *A. linariae*) were found to be associated with early blight of tomato. *Alternaria linariae* and *A. protenta* were confirmed as the major causal agents of tomato and potato early blight, respectively. There were no phylogenetic groupings among tested Russian *Alternaria* isolates associated with their locality.

Keywords: *Alternaria solani*; early blight; multi-gene phylogeny; *Alternaria linariae*

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1. Introduction

In Russia, early blight (EB) is considered to be one of the most destructive diseases of potato (*Solanum tuberosum* L.) and tomato (*S. lycopersicum* L.) plants, the leading vegetable crops in the country. The volume of potato production in Russia is about 30 million tons and financial losses from the development of potato diseases can be very significant. A wide variety of plant pathogenic *Alternaria* fungi cause early blight. It is characterized by necrotic lesions in the aerial parts of plants. Species delimitation among *Alternaria* spp. pathogens was always challenging, and all large-spored species were generally considered to be *Alternaria solani* Sorauer. Based on conidial morphology, Simmons described 21 species occurring on Solanaceae plants [1]. The main EB-inducing agents for potato were *A. solani* and *A. grandis* E.G. Simmons. *A. tomatophila*, *A. cretica*, and *A. subcylindrica* were identified to cause EB on tomatoes [2]. In 2014, a large-scale phylogenetic reconstruction of large-spored *Alternaria* species was performed [3]. Multilocus analyses using concatenated phylogeny of internal transcribed spacers 1 and 2 and 5.8S gene (ITS), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), DNA-dependent RNA polymerase II (RPB2) gene, transcription elongation factor 1α (TEF1) and Alternaria major allergen gene (Alt a 1) have separated *Alternaria* species into different groups according to their original hosts [3]. At present, this is the most extensive phylogenetic study of large-spored *Alternaria*. There were only *A. solani*, *A. protenta*, and *A. grandis* strains isolated from potato plants in the study; *A. alternariacida* and *A. linaria* from tomato plants; and *A. nitrimali* and *A. solani-nigri* from other Solanaceae plants.

There have only been a few studies conducted on species affecting potato and tomato plants according to the revised Porri classification proposed by Woudenberg et al. [3]. As far as we know, such work has not been conducted in Russia, the former Soviet Union, or Eastern Europe. Thus, our work aimed to revise large-spored *Alternaria* strains that infect

potato and tomato plants by DNA sequencing species-specific regions in accordance with the research of Woudenberg et al. [3].

2. Materials and Methods

2.1. Isolates

Alternaria isolates were collected from commercial potato and tomato fields and small private gardens in different regions of Russia (Figure 1, Table 1): Voronezh (site 9), Leningrad (7), Astrakhan (5,6), Krasnodar (10,11,13), Moscow (12), Primorsky (3,4), Khabarovsk (2) regions, and Tatarstan republic (1) as well as in Belarus (8). For direct isolation, plant material was incubated for 24 h in moist chambers. Under a binocular microscope (MBS 10, Russia), conidia were transferred with a preparation needle to potato dextrose agar (PDA) medium with an antibiotic solution (1000 U/mL benzylpenicillin sodium). After that, the hyphal tips were transferred under a binocular microscope onto another Petri dish with PDA medium.

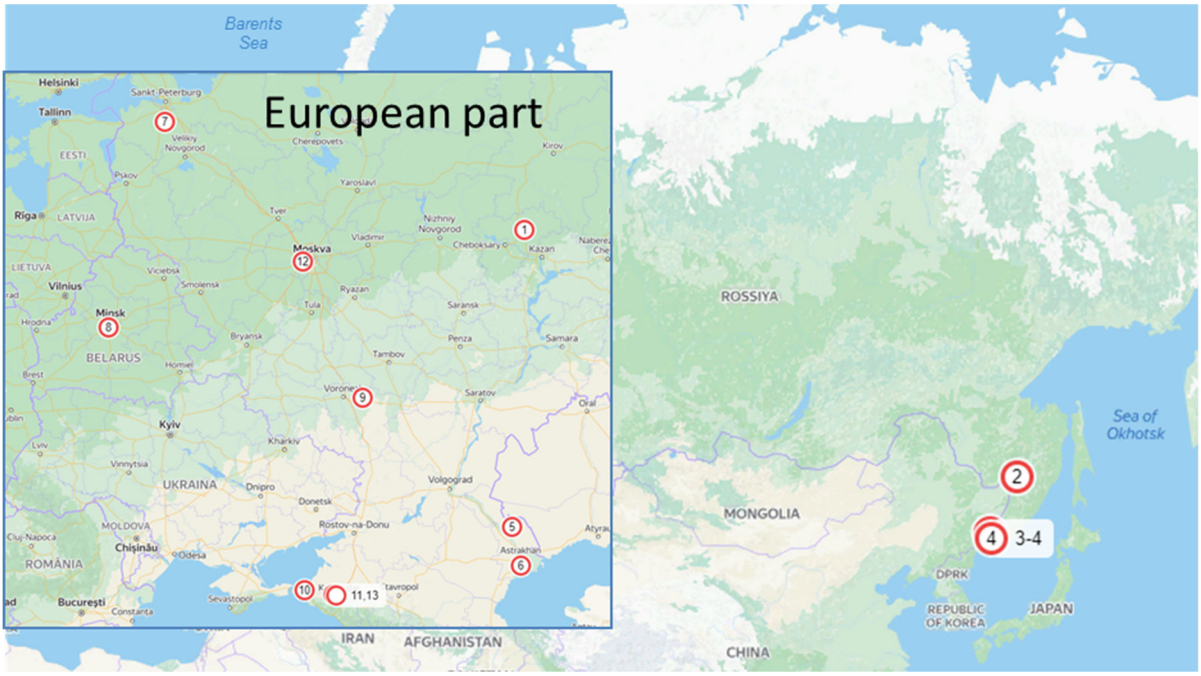

Figure 1. Collection sites of *Alternaria* isolates. 1—Mari El republic; 2—Khabarovsky krai; 3—Primorsky krai, Ussurijsk district; 4—Primorsky krai, Vladivostok district, 5—Astrakhan region, Habarlin district; 6—Astrakhan region, Kamyzyak district; 7—Leningrad region; 8—Republic of Belarus; 9—Voronezh region; 10—Krasnodar region, Strelka village; 11—Krasnodar region, Temryuk district; 12—Moscow region; 13—Krasnodar region, Anapa district.

Table 1. Isolates used in this study.

Name	Strain Number	Year of Isolation	Host/Substrate	Locality of Collection Site (See Figure 1)	GenBank Accession Numbers				
					ITS	TEF	RPB2	GADPH	Alt a 1
Alternaria grandis	A16UsPL21	2016	S. t., leaf	3	OM640142	MN580515	MN580526	MN544407	MN562255
	A16PrPL22	2016	S. t., leaf	3	OM640143	ON098332	ON098335	ON098326	ON098329
	A17VlPL41a	2017	S. t., leaf	4	OM640144	ON098333	ON098336	ON098327	ON098330
	A16KhPL41	2016	S. t., leaf	2	OM640145	ON098334	ON098337	ON098328	ON098331
Alternaria solani	A17SpbPL10	2017	S. t., leaf	7	OM640146	MN580516	MN580527	MN544406	MN562256
	A21KrTL8	2021	S.l., leaf	11	OM640147	ON098290	ON098292	ON098286	ON098288
	A21MTSt3	2021	S.l., stem	12	OM640148	ON098291	ON098293	ON098287	ON098289
Alternaria alternariacida	A16PrPL21	2016	S. t., leaf	3	OM348531	MN580518	MN580529	MN544404	MN562258
Alternaria protenta	A16PrPL11	2016	S. t., leaf	3	OM640149	MN580517	MN580528	MN544405	MN562257
	A17VlPL41	2017	S. t., leaf	4	OM640150	ON098306	ON098312	ON098294	ON098300
	A17KhPL51	2017	S. t., leaf	2	OM640151	ON098307	ON098313	ON098295	ON098301
	A16PrPL45	2016	S. t., leaf	3	OM640152	MN580523	MN580534	MN593316	MN593309
	A17VlPL31	2017	S. t., leaf	4	OM640153	MN580524	MN580535	MN593317	MN593310
	A16UsPL31	2016	S. t., leaf	3	OM640154	ON098308	ON098314	ON098296	ON098302
	A17VlPL51	2017	S. t., leaf	9	OM640155	ON098309	ON098315	ON098297	ON098303
	A16KhPL11	2016	S. t., leaf	2	OM640156	ON098310	ON098316	ON098298	ON098304
	A17VlPL51a	2017	S. t., leaf	9	OM640157	ON098311	ON098317	ON098299	ON098305
Alternaria linariae	7AHTF 11a	2017	S.l., fruit	5	KY496637	MN580520	MN580531	MN593313	MN562260
	A17AHTL 14e/2	2017	S.l., leaf	5	OM640158	ON135533	ON135537	ON135525	ON135529
	A18MYKTL7	2018	S.l., leaf	1	OM640159	ON135534	ON135538	ON135526	ON135530
	A18MYKTL18/1	2018	S.l., leaf	1	OM640160	ON135535	ON135539	ON135527	ON135531
	A17AHTL3a*	2017	S.l., leaf	5	OM640161	ON135536	ON135540	ON135528	ON135532
	A17VlPL31a	2017	S. t., leaf	4	OM640162	MN580519	MN580530	MN593312	MN562259
	A17MYKTL10/1	2017	S.l., leaf	1	OM640163	MN580521	MN580532	MN593314	MN562261
	A18MYKTL25/2(1)	2018	S.l., leaf	1	OM640164	ON098322	ON098324	ON098318	ON098320
	A17MYKTL11/2	2017	S.l., leaf	1	OM640165	ON098323	ON098325	ON098319	ON098321
	A18BlTF1	2018	S.l., fruit	8	OM640166	MN580522	MN580533	MN593315	MN562262
	A18VTL10/2	2018	S.l., leaf	9	OM640167	ON149482	ON149496	ON149454	ON149468
	A16KhTL21	2016	S.l., leaf	2	OM640168	ON149483	ON149497	ON149455	ON149469

Table 1. *Cont.*

Name	Strain Number	Year of Isolation	Host/Substrate	Locality of Collection Site (See Figure 1)	GenBank Accession Numbers				
					ITS	TEF	RPB2	GADPH	Alt a 1
Alternaria linariae	A18AKTL117/7	2018	S.l., leaf	6	OM640169	ON149484	ON149498	ON149456	ON149470
	A20KrTL14	2020	S.l., leaf	10	OM640170	ON149485	ON149499	ON149457	ON149471
	A20KrTL16	2020	S.l., leaf	10	OM640171	ON149486	ON149500	ON149458	ON149472
	A21KrTS1.2	2021	S.l., seed	10	OM640172	ON149487	ON149501	ON149459	ON149473
	A21KrTL3	2021	S.l., leaf	11	OM640173	ON149488	ON149502	ON149460	ON149474
	A21KrTL5	2021	S.l., leaf	11	OM640174	ON149489	ON149503	ON149461	ON149475
	A21KrTL6	2021	S.l., leaf	11	OM640175	ON149490	ON149504	ON149462	ON149476
	A21KrTL10	2021	S.l., leaf	11	OM640176	ON149491	ON149505	ON149463	ON149477
	A21KrTS22	2021	S.l., seed	10	OM640177	ON149492	ON149506	ON149464	ON149478
	A21MTSt2	2021	S.l., stem	12	OM640178	ON149493	ON149507	ON149465	ON149479
	A21MTSt6	2021	S.l., stem	13	OM640179	ON149494	ON149508	ON149466	ON149480
	A21MTSt7	2021	S.l., stem	13	OM640180	ON149495	ON149509	ON149467	ON149481

2.2. PCR and Sequencing

In order to isolate the DNA, the mycelium of fungi was grown on liquid pea medium [4]. DNA was extracted according to the standard CTAB protocol [5]. The internal transcribed spacer 1 (ITS1) and ITS2 regions and the 5.8S ribosomal DNA (rDNA) region of the fungi were amplified with ITS1 and ITS4 primers [6], parts of the GAPDH gene—with gpd1 and gpd2 [7], the RPB2 gene—with RPB2-5F2 [8] and fRPB2-7cR [9], and the TEF1 gene—with the primers EF1-728F and EF1-986R [10]. PCR was performed with GenPak® PCR Core kit (Isogene Lab., Moscow, Russia). The PCR program consisted of an initial denaturing step at 94 °C for 5 min, 35 amplification cycles, and an additional extending step at 72 °C for 3 min. For the primer pairs ITS1/ITS4, RPB2-5F2/fRPB2-7cR, EF1-728F/EF1-986R, and Alt-for/Alt-rev, the amplification cycles were 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s. For the primer pair gpd1/gpd2, the amplification cycles were 94 °C for 30 s, 62 °C for 30 s, and 72 °C for 1 min. After the reaction, the length and purity of the PCR products were monitored by electrophoresis in 1% agarose gel. Ethidium bromide was used to visualize the PCR product. A piece of gel containing the amplicon of the desired size was cut with a sterile scalpel and placed in a microtube. Then, the instructions specified in the description of the CleanUp Standard kit for DNA isolation from the gel (Evrogen Co Ltd., Moscow, Russia) were followed. DNA sequencing was carried out according to the Sanger method at the Evrogen company with both forward and reverse primers. The raw sequence reads were assembled into the consensus sequence in Geneious v. 7.13 (Biomatters Limited, Auckland, New Zealand) using default settings.

2.3. Phylogenetic Analysis

The sequences of each gene were aligned and cut at the ends. Specifically, for the ALT-A1 gene, a fragment of about 475 nucleotides (nt) was considered; for the ITS—538 nt, for the GAPDH gene—580 nt, for the RPB2—772 nt, and for the TEF1—355 nt. Multiple sequence alignments were generated with MAFTT algorithm plugin in Geneious ver. 7.13 (Biomatters Ltd., Auckland, New Zealand). Sequences of fragments of ITS-5,8S-ITS2, GAPDH, RPB2, TEF1, and Alt a1 genes were used for phylogenetic study (Table 2). Additionally, gene sequences, including the outgroup, were retrieved from Woudenberg et al. [3] (Table 2).

Table 2. Reference isolates from Solanaceus plants from Woudenberg et al., 2014 [3].

Current Species Name	Old Species Name	Strain	Host/Substrate	Locality	GenBank Accession Numbers				
					ITS	GAPDH	Alt a1	TEF1	RPB2
A. alternariacida	*A. solani*	CBS 105.51	*S. lyc.*, fruit	UK	KJ718105	KJ717959	KJ718625	KJ718454	KJ718279
A. grandis		CBS 109158	*S. tuber.*, leaf	USA	KJ718239	JQ646341	JQ646425	EU130547	KJ718414
		CBS 116695	*S. tuber.*, leaf	USA	KJ718241	KJ718070	KJ718748	KJ718587	KJ718416
A. linariae	*A. solani*	CBS 108.53	–	–	KJ718181	KJ718025	KJ718693	KJ718529	KJ718354
	A. solani	CBS 107.61	–	Belgium	KJ718182	KJ718026	KJ718694	KJ718530	KJ718355
	A. tomatop-hila	CBS 109156	*S. lyc.*, leaf	USA	KJ718183	JQ646347	GQ180101	KJ718531	KJ718356
	A. subcylin-drica	CBS 109161	*S. lyc.*, leaf	USA	KJ718184	JQ646345	JQ646429	KJ718532	KJ718357
	A. cretica	CBS 109164	*S. lyc.*, leaf	Greece	KJ718185	JQ646342	JQ646426	EU130545	KJ718358
	A. tomatophila	CBS 116704	*S. lyc.*, leaf	USA	KJ718188	KJ718029	KJ718697	KJ718535	KJ718361
		CPC 21620	*S. lyc.*, leaf	Thailand	KJ718189	KJ718030	KJ718698	KJ718536	KJ718362
A. nitrimali		CBS 109163	*S. viarum* leaf	Puerto Rico	KJ718201	JQ646358	KJ718710	KJ718547	KJ71837
A. protenta	*A. solani*	CBS 347.79	*S. lyc.*, fruit	New Zealand	KJ718219	KJ718054	KJ718728	KJ718565	KJ718392
	A. solani	CBS 116651	*S. tuber.*, tuber	USA	KC584217	KC584139	GQ180097	KC584688	KC584430
	A. solani	CBS 135189	*S. tuber.*,	New Zealand	KJ718224	GQ180082	GQ180098	KJ718570	KJ718397
A. solani		CBS 106.21	–	–	KJ718236	KJ718066	KJ718743	KJ718582	KJ718410
		CBS 111.41	*S. aviculare*, leaf	–	KJ718237	KJ718067	KJ718744	KJ718583	KJ718411
	A. danida	CBS 109157	*S. tuber.*, leaf	USA	KJ718238	GQ180080	KJ718746	KJ718585	KJ718413
A. solaninigri	*A. cyphoman-drae*	CBS 113403	*S. nigrum*, leaf	New Zealand	KJ718243	KJ718071	KJ718749	KJ718589	KJ718418

2.4. Bioinformatic Methods

Sequences were aligned with the MAFFT version 7 web tool (http://mafft.cbrc.jp/alignment/server/ accessed on 1 December 2021) with subsequent manual processing. Phylogenetic reconstructions were performed with maximum likelihood (ML) and Bayesian (BI) analyses. Nucleotide substitution models for BI were chosen with TOPALI v. 2.5 (The

Apache Software Foundation, Maryland, CA, USA) based on the Bayesian information criterion (BIC). Bayesian analyses were performed with Geneious v. 7.13. In these analyses, three parallel runs with four chains each and other default parameters were run for one million generations. A burn-in of 25% was used in the final analyses, ensuring the average standard deviation of split frequencies had reached <0.01 for all data sets. Support at nodes was indicated when posterior probabilities were ≥0.6. For ML analyses, the best-fit substitution model for the alignment was estimated based on the Akaike information criterion (AIC) using the IQ-TREE Web Service (http://iqtree.cibiv.univie.ac.at/ accessed 12 December 2021). The Tamura–Nei (TN) [11] model plus empirical base frequencies allowing for a proportion of invariable sites was chosen for the "potato" dataset. For the "tomato" dataset, TN plus empirical base frequencies and a freeRate model with 2 categories were used. The RAxML program ver. 7.0.3 (The Exelixis Lab, Heidelberg, Germany) was used for the heuristic search.

3. Results

3.1. Bioinformatic Analysis

The aligned sequences of ITS, GAPDH, RPB2, TEF1, and Alt a1 regions had a total length of 2690 characters for the alignments of both potato- and tomato-related strains, with 2, 6, 28, 6, and 8 unique site patterns, respectively. The aligned "potato" data set for seven species included 2572 constant sites and 54 parsimony informative sites. Alignment of the "tomato" data set sequences resulted in 2574 constant sites and 47 parsimony informative sites. The phylogenetic analyses based upon Maximum Likelihood inference of ITS, GAPDH, RPB2, TEF1, and Alt a1 regions of 41 *Alternaria* isolates are shown in Figures 2 and 3. Bayesian Inference and ML returned similar topologies and relevant support values. Two species, *A. solani-nigri* (R. Dubey, S.K. Singh and Kamal) and *A. nitrimali* (E.G. Simmons and M.E. Palm), which also occur on Solanaceae plants, were used in tree *A. nitrimali* and found to be a proper out-group as indicated by its clear segregation from the other strains used in the study.

3.2. Phylogeny

Isolates from affected potato leaves included species of *Alternaria alternariacida*, *A. grandis*, *A. linariae*, *A. protenta*, *A. solani* (Figure 2, Table 1). Most of the potato strains (9) were grouped with *A. protenta*. Among them are isolates from the eastern and western parts of Russia. Seven strains were completely identical to the reference isolate CBS 116651; the sequence of the GADPH gene of strain A17VoPL51a differed from the reference only by one nucleotide. A16PrPL45 strain from the Far East belongs to the *A. protenta* clade as well but differed from CBS 116651 by one nucleotide in each of four gene regions (ITS, GADPH, TEF1, Alt). Among the strains isolated from the Far East, four were *A. grandis.* Their DNA sequences were completely identical to that of the *A. grandis* CBS 109158 and CBS 116695 reference strains. From all tested potato isolates, the only strain isolated in northern Europe in 2017 belonged to *A. solani* and was identical to CBS 109157. Although *A. solani* clustered with *A. grandis* and differs by only one nt in its GAPDH sequence and one in the ITS sequence from *A. grandis*, although they were retained as a distinct species [3]. The A16PrPL21 strain from the Far East clustered with *A. alternariacida* CBS 105.51, although it differed from the reference strain by one nucleotide deletion in the ITS region. Among the strains studied was one that belonged to the clade *A. linariae*. This isolate from the Far East, A17VlPL31a, was closest to the first subclade of *A. linariae*.

The tomato strains studied belong to the *A. linariae* and *A. solani* (Figure 3) species. Twenty-three out of twenty-five tomato strains studied belong to the *A. linariae* species. The isolates came from leaves, fruits, and stems of tomato plants cultivated in different Russian regions and in different years. According to the reference strains CPC 21620, CBS 109164, and CBS 108.53, *A. linariae* strains were grouped into three subclades. There were two strains found in the European part of Russia in 2021 that were identified as *A. solani* and were analogous to CBS reference strains.

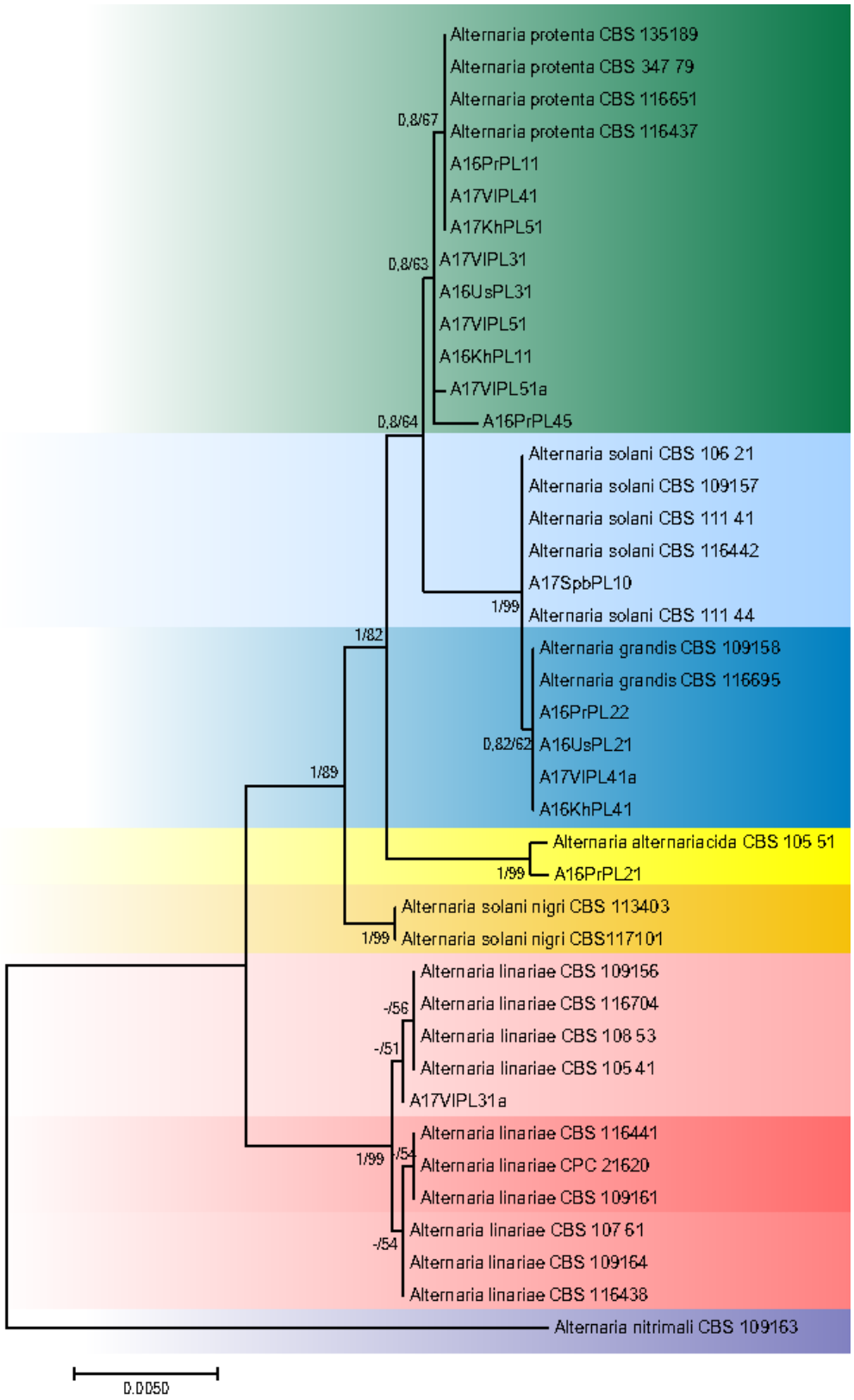

Figure 2. Phylogenetic tree based on the combined gene sequences of ITS, GAPDH, Alt a1, TEF1, and RPB2 of strains isolated from *S. tuberosum* plants. Bayesian posterior probabilities followed by ML bootstrap values are shown at nodes.

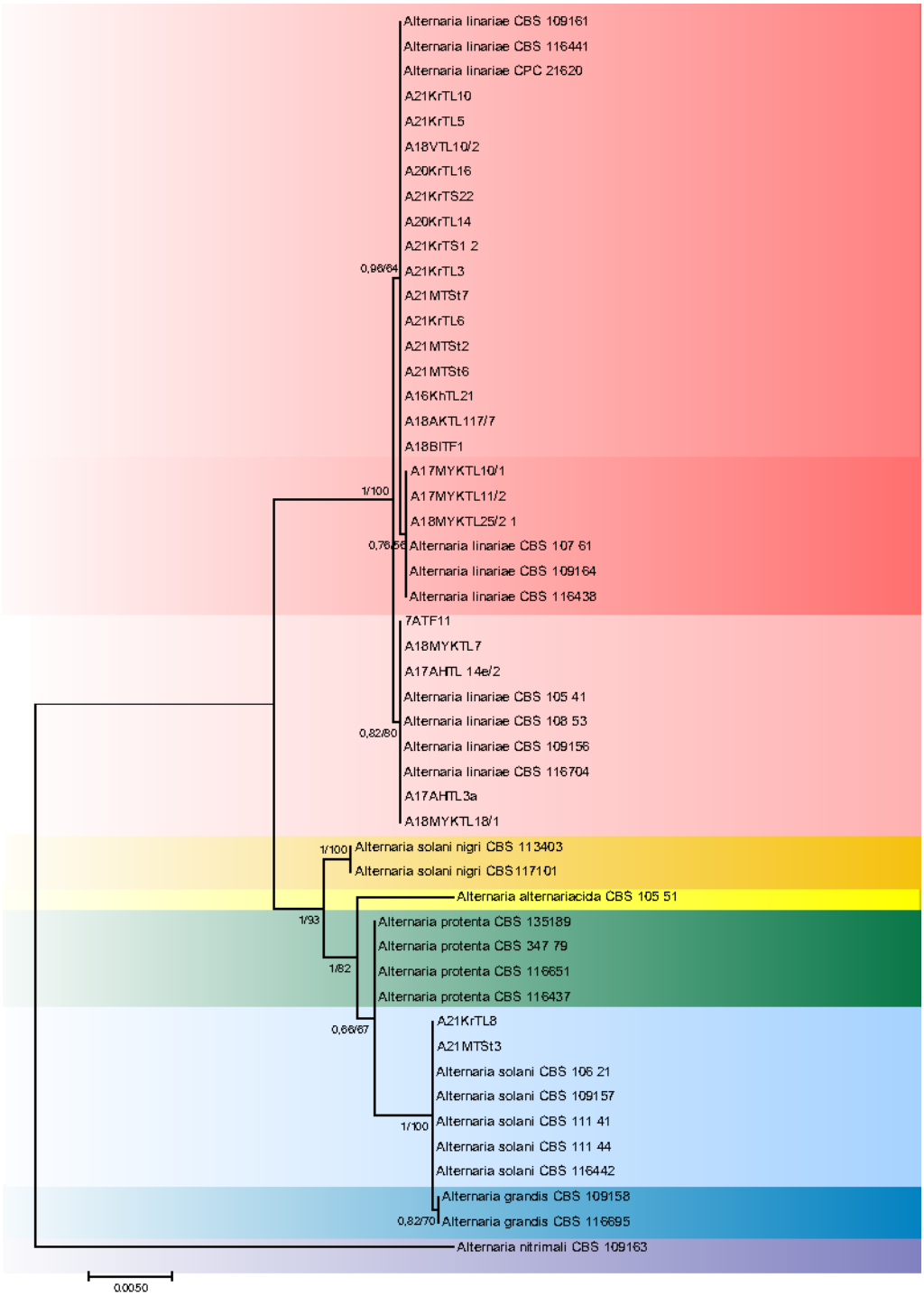

Figure 3. Combined phylogenetic ITS, GAPDH, Alt a1, TEF1 and RPB2 topology from maximum likelihood analysis of strains isolated from *S. lycopersicum* plants. Bayesian posterior probabilities followed by ML bootstrap values are shown at nodes.

4. Discussion

In our study, five species of large-spore *Alternaria* were identified on potato leaves: *A. grandis*, *A. solani*, *A. alternariacida*, *A. protenta*, *A. linariae*. The *A. alternariacida* description (Woudenberg) is based on the strain isolated from the fruit of *Solanum lycopersicum*. In the present work, we first discovered a strain of this species on potato. We confirmed disease caused by *A. alternaricida* on potato plants [12]. Moreover, two species, *A. solani* and *A. linariae*, were found to be associated with tomato. There have been similar observations

elsewhere in the world: in Algeria, *A. protenta*, *A. linariae*, *A. solani* and *A. grandis* have been found on potato leaves [13–16]; *A. solani* was found on a potato tuber in Egypt [17]. We have also found a large-spore *Alternaria* strain on potatoes grown in Uganda. The DNA sequence analysis indicated that it was similar to the reference *A. linariae* strain, differing by one nucleotide in the GADPH gene and two nucleotides in the Alt a 1 gene (OL450058and OL450057). In Wisconsin, USA, [18] strains isolated from potato leaves were found to be *A. protenta* or *A. solani*. Researchers did not analyze the sequence of the rpb2 gene that differentiates the two species, so a more precise identification was not possible. Einspanier et al.'s [19] genome-wide study involved 43 large-spore *Alternaria* isolates collected from potato plants in Europe and the United States. By analyzing the sequences of species-specific markers, it was observed that eight of the isolates studied were identical to strain CBS 116651, which belongs, according to Woudenberg et al. [3], to the *A. protenta* species. Whole-genome analysis of strains revealed that large-spore species have high levels of single nucleotide substitution rates. This corresponds well with our results. Only one-point substitutions separated the strains that had no complete similarity with the reference strains. This may be caused by the absence of sexual reproduction in large-spore *Alternaria* populations.

Most of the isolates from the leaves, stems, and fruits of affected tomato plants belonged to the *A. linariae* species. This is not surprising, since the revised *A. linariae* species include *A. tomatophila*, *A. cretica* and *A. subcylindrica* [3], which were previously considered the main species of *Alternaria* infecting tomato [2]. Additionally, a potato A17VPL31a isolate was included in the *A. linariae* clade. In the Moscow and Krasnodar regions (European part of Russia), two strains of *A. solani* were isolated from affected leaves and stems of tomato plants. Tomato plants in Algeria have also been found affected by both *A. linariae* and *A. solani* [15].

There are relatively few molecular genetic studies of the species structure of large-spore *Alternaria* in the world. As a result, it is difficult to compare the species and intraspecific composition of *Alternaria* that infect different Solanaceae plants. Yet, if we look at our and literature-based data, we can find some patterns. Our study found that almost all tomato isolates belonged to the *A. linariae* species, and only two isolates belonged to the *A. solani* species. Furthermore, of the tested potato isolates, only one was *A. linariae* and one was *A. solani*, and the rest were distributed among the species *A. grandis*, *A. alternariacida*, and *A. protenta*. The CBS *A. protenta* reference strains were isolated from both *S. lycopersicum* and *S. tuberosum* plants. An isolate of *A. solani* CBS 111.41 was isolated from *S. aviculare*. It was identical to the *A. solani* strains we observed causing early blight on potatoes and tomatoes. The *A. linariae* strains that cause early blight on tomatoes and potatoes were also similar. This indicates that there is no evidence to support the assumption of species-specificity. This corresponds well with the results of studies in North Carolina and Wisconsin involving the *Alternaria* species from tomato and potato plants [20]. However, the hypothesis of a lack of host specialization needs to be confirmed by cross-inoculation of *Alternaria* isolates on tomato and potato plants. Our previous studies of the virulence of *Alternaria alternata* detected intraspecific differences in the virulence and aggressiveness of strains towards potato and tomato cultivars. Some isolates successfully infected cultivars that were highly resistant to other isolates, suggesting that potato and tomato cultivars have genes of specific resistance to *A. alternata* [21].

The phylogenies of the single-gene trees were not congruent with the consensus tree. Only RPB2 gene trees had the same topology as the consensus tree. We found that the sequences of the ITS region, Alt A1 and GAPDH genes alone could not resolve the phylogeny of closely related *Alternaria* pathogens of Solanaceae. These results agree with Lourencßo et al. [22] and Peixoto et al. [23] which also found a relatively low number of polymorphisms in the Alt a1 gene sequence among EB-inducing isolates from potato and tomato plants. Therefore, the RPB2 gene is the most relevant for this species complex.

We hypothesized that the genetic diversity of species can vary between different locations, at least in the European region and the Far East. Despite this, we found no rela-

tionship between the variable characters and the geographical spread between species. The low number of differences corresponds well with whole-genome results [19], suggesting the existence of true clones that have been transported by seed tubers. In North Carolina and Wisconsin, Adhakiri et al. [20] analyzed field populations of three *Alternaria* species, finding that *A. solani* had much lower diversity than *A. alternata* and *A. linariae*. Indeed, we found three different haplotypes in *A. linariae* species. However, it has been shown that *A. solani* in China has relatively high levels of genetic variation, suggesting parasexual reproduction [24].

Thus, we found five pathogenic Alternaria species on potato plants and two species on tomato plants in Russia. These findings allow us to study the host range and possible options for disease control. We found no phylogenetic groupings among Russian *Alternaria* isolates associated with their locality. Yet, the sister relationship between the potato and tomato plants makes these species excellent subjects for studying the model of genetic divergence and speciation. A better understanding of their virulence and fungicide resistance can help in the elaboration of the most effective methods of plant protection.

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