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Talaromyces minioluteus: New Postharvest Fungal Pathogen in Serbia

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Abstract

Talaromyces minioluteus is one of the important species of genus *Talaromyces*, which has cosmopolitan distribution and is encountered on a wide range of different habitats. This species has not been considered as an important plant pathogen, even though it has been isolated from various plant hosts. Fruits and vegetables with *Penicillium*-like mold symptoms were collected from 2015 to 2017 from markets in Serbia. Isolates originating from quince, tomato, and orange fruits, onion bulbs, and potato tubers were identified and characterized on a morphological, physiological, and molecular level. Morphological and physiological examination included observing micromorphology, testing growth on six different media and at five different temperatures, and production of three enzymes. Molecular identification and characterization were performed using four molecular markers: internal transcribed spacer, β-tubulin, calmodulin, and DNA-dependent RNA polymerase II second largest subunit. The results of morphological and molecular analyses were in agreement, and they proved that the obtained isolates are *T. minioluteus*. In the pathogenicity assay, *T. minioluteus* was confirmed as a pathogen of all species tested with the exception of potato tubers. This is the first report of *T. minioluteus* as a postharvest plant pathogen on quince, tomato, and orange fruit and onion bulbs. Also, this is the first record of *T. minioluteus* in Serbia.

Keywords: *Talaromyces minioluteus*, postharvest pathogen, identification, characterization, fruits and vegetables

The genus *Talaromyces* contains polyphagous fungal species with a worldwide distribution. Species of this genus are important as contaminants in food industry, as mycotoxin producers, and in medicine as human pathogens. They are also used in biotechnology for the production of enzymes and soluble pigments and in plant protection as biological control agents (Yilmaz et al. 2014).

Benjamin (1955) introduced the genus *Talaromyces* to group sexual *Penicillium* species that produce "soft, usually yellow ascocarps with wall composed of loosely to tightly interwoven hyphae" and have ovate or globose asci and spiny ascospores. However, the genus has recently been redefined and now includes both sexual and asexual species (Samson et al. 2011). In the monograph by Yilmaz et al. (2014), 88 species have been determined in *Talaromyces*. New species have been described continually since then (Chen et al. 2016; Jiang et al. 2018; Peterson and Jurjević 2017; Visagie et al. 2014a), so now this genus contains 143 species (Rajeshkumar et al. 2019).

One of the important species of this genus, *Talaromyces minioluteus*, was first described by Dierckx (1901) as *Penicillium minioluteum*. Later, Raper and Thom (1949) placed this species in synonymy with *P. funiculosum*, which was a decision contrary to the Botanical Code. Pitt (1979) reintroduced *P. minioluteum* and based its description on isolate FRR 1714. Due to an error in the neotypification of this species it was described later in scientific literature with other two names: *P. gaditanum* (Ramírez and Martínez 1981) and *P. samsonii* (Quintanilla 1985). *P. minioluteum* was subsequently retypified and redefined by Van Reenen-Hoekstra et al. (1990) and Frisvad et al. (1990). Samson et al. (2011) analyzed the phenotypic data, extrolite patterns, and multiple-gene phylogeny and transferred all the taxons from the subgenus *Biverticillium* of the genus *Penicillium* to *Talaromyces* to follow the new nomenclatural rules. These authors are the first that renamed *P. minioluteum* to *T. minioluteus*, and nowadays all abovementioned names are considered synonyms of the name *T. minioluteus* (Yilmaz et al. 2014).

T. minioluteus is placed in section *Trachyspermi*, whose members have restricted growth on Czapek yeast autolysate agar (CYA), yeast extract sucrose agar (YES), and dichloran 18% glycerol agar (DG18), slightly faster on malt extract agar (MEA), and poor growth on creatine sucrose agar (CREA). They usually have biverticillate conidiophores, and some species of this section produce red pigments. Some of the distinctive features of *T. minioluteus* are moderate growth on general media (CYA, MEA), weak acid production on CREA, and the inability to grow at 37°C (Yilmaz et al. 2014).

T. minioluteus has been isolated from the following plant hosts: apple fruits (Quintanilla 1985; Viñas et al. 1993), corn (Camiletti et al. 2014), grape berries (Behr et al. 2013; Khodaei et al. 2016; Sage et al. 2004), pomegranate (Labuda et al. 2004; Palou et al. 2010, 2013), and *Tulipa* sp. (Van Reenen-Hoekstra et al. 1990). This fungus has also been collected from numerous other substrates and environments: air (Alananbeh et al. 2017; Ramírez and Martínez 1981), declining trees (Kubátová 2000), house dust (Visagie et al. 2014a), pickled onions (Williams 1990), poultry feed (Magnoli et al. 1998), raisins (Khodaei et al. 2016), soil (Hujslová et al. 2010; Lee et al. 2002; Nesci et al. 2006; Okada et al. 1998), tree bark (McGee et al. 2006), jute sugar bags (Van Reenen-Hoekstra et al. 1990), wood (Seifert and Frisvad 2000), and even marine sponge (Ngokpol et al. 2015) and Antarctic mosses (Tosi et al. 2002).

T. minioluteus has also been exploited in chemistry, biochemistry, biotechnology, and other disciplines of science. De Souza et al. (2012) used this species to biotransform clovane derivatives, which have the potential to act as fungistatic agents. Tang et al. (2015) produced novel furanones, miniolins A to C, using epigenetic manipulation on the culture of *T. minioluteus*. Two spiro-orthoesters, (±)-peniorthoesters A and B, were obtained from *T. minioluteus* and were the first spiro-orthoesters of fungal origin (Liu et al. 2018). This species is a producer of a cell cycle inhibitor (HY 558) that can help in cancer treatment (Lee et al. 2002). Frisvad et al. (2013) noted that *T. minioluteus* produces red pigments (monascorubrin, rubropunctatin, and other azaphilone pigments), and Sudha et al. (2017) explored the potential and application of the extracted dyes for coloring materials in the textile industry.

T. minioluteus has been considered a postharvest plant pathogen of small economic importance and has limited attention in

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phytopathology. Palou et al. (2010, 2013) investigated postharvest diseases on pomegranate in commercial packinghouses in Spain and among other fungi detected *T. minioluteus*. Characteristic *Penicillium*-like blue mold symptoms were noticed on the fruits, and the isolated fungus has been identified using morphological characteristics and gene sequencing. In pathogenicity trials, *T. minioluteus* did not produce disease symptoms on pomegranate (Palou et al. 2010, 2013). The only confirmation of *T. minioluteus* as a plant pathogen was in the study of Viñas et al. (1993). They explored imazalil resistance of the *Penicillium* isolates sampled from apple fruits, package boxes, and the air of the storage rooms. Isolates of *T. minioluteus* (referred as *P. minioluteum*) produced decay on the apple fruits in the pathogenicity tests and were among the most resistant to imazalil (Viñas et al. 1993). Furthermore, it has been determined that *T. minioluteus* produces secalonic acid D and F, mycotoxins that can compromise human health (Frisvad et al. 2006; Yilmaz et al. 2014).

According to the available literature, *T. minioluteus* has not been reported in Serbia. Only two *Talaromyces* species were detected in Serbia, *T. funiculosus* and *T. rugulosus* (reported as *Penicillium funiculosum* Thom and *P. rugulosum* Thom, respectively), on ready-to-use fresh mixed salads (Kocić-Tanackov et al. 2010). The objectives of this study were (i) to examine the etiology of *Penicillium*-like symptoms on fruits of quince (*Cydonia oblonga* Mill.), tomato (*Solanum lycopersicum* L.), and orange (*Citrus sinensis* [L.] Osbeck), bulbs of onion (*Allium cepa* L.), and tubers of potato (*Solanum tuberosum* L.); (ii) to identify and characterize isolates of *T. minioluteus* by using morphological, physiological, and molecular methods; (iii) to investigate the relationship of Serbian isolates of *T. minioluteus* and their placement within the section *Trachyspermi* of genus *Talaromyces* by sequencing four genetic loci of ribosomal DNA and protein coding regions (internal transcribed spacer [ITS], β-tubulin [*BenA*], calmodulin [*CaM*], and DNA-dependent RNA polymerase II second largest subunit [*RPB2*]); and (iv) to evaluate pathogenicity of the isolates on the originating hosts.

Materials and Methods

Sampling and isolation. Various samples of fruits and vegetables with *Penicillium*-like mold symptoms—brown, watery spots or characteristic blue/green sporulation on their surface—were randomly collected from markets in 2015 to 2017. Three hundred twenty-one samples were recovered, and all originated from Serbia, except the citrus fruits (Table 1).

Three small pieces on the border of necrotic and healthy tissue of each sample were excised with a sterilized scalpel, sterilized in 1% aqueous solution of NaOCl for 2 min, rinsed three times with sterile distilled water, placed on MEA, and incubated in dark conditions for 7 days at 25°C. Single-spore isolates were obtained, transferred onto potato dextrose agar slants, and kept at 4°C. Eight isolates were chosen for comprehensive investigation (Table 1). The number of selected isolates per host corresponded to the relative frequency of the isolates determined on the originating plant hosts.

Cultivation conditions. Isolates included in the analysis were three-point inoculated by placing 1 µl of conidial suspension from semisolid agar (0.2% agar and 0.05% Tween 80) in 90-mm Petri dishes on CYA, MEA, CREA, YES, DG18, and oatmeal agar (OA) and incubated for 7 days in the dark at 25°C (Visagie et al. 2014b; Yilmaz et al. 2014). The impact of temperature on fungal growth was examined by incubating cultures for 7 days at 5, 15, 30, and 37°C on CYA. After the incubation, all plates were photographed (Olympus digital camera, model FE-220/X-785, Olympus Corporation, Japan), two perpendicular diameters were measured for each colony, and the average was calculated. Phenotypic characteristics such as culture appearance and mycelium color (obverse, reverse), colony texture, sporulation, presence and color of exudates, diffusible color, and change of the medium color were also recorded.

Microscopic investigation. Preparation of microscopic slides was done from 10-day-old cultures grown on MEA. The conidia were suspended in 1 ml of 60% lactic acid (Yilmaz et al. 2014). Phenotypic characteristics of conidia (shape, ornamentation of the cell wall) and conidiophores (number of branching points between stipe and phialides, shape and texture of phialides) were recorded. Microscopic examinations were done using an Olympus phase contrast microscope (model BX51) and photographed using an Olympus camera (model E620). Two diameters (length and width) were measured for 100 conidia of each isolate in the Quick Photo Camera software program (PROMICRA, Czech Republic).

Lipase, esterase, and amylase production test. All isolates were assayed for lipase, esterase, and amylase production. Lipase production was tested using spirit blue agar and lipase reagent (Difco, BD, Franklin Lakes, NJ) (Pianzzola et al. 2004). Esterase production was examined using Tween 80 medium. Amylase production was assessed using a medium with starch (Paterson and Bridge 1994). Each isolate was inoculated by placing 25 µl of conidial suspension from semisolid agar in the center of 90-mm Petri dishes containing the abovementioned media and incubated in the dark at 25°C. Reactions were recorded after 7 days with the lipase production test and after 10 days for esterase and amylase production. In the lipase production test, a positive reaction was the manifestation of a halo effect around the culture. In the esterase production test, a positive reaction was the change of medium color from green to blue-purple. In the amylase production test, a positive reaction was growth on this medium and manifestation of a brown ring around the culture appearing 5 to 10 min after flooding them with 4 ml of Lugol's solution.

DNA extraction. The isolates were cultivated for 7 days at 25°C in the dark on MEA for DNA extraction. Approximately 100 mg of mycelium was scraped from the surface of the plate using a sterile blade, transferred to a 1.5-ml sample tube, frozen in liquid nitrogen, and ground using a sterile micropestle. DNA extractions were performed using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, and the extracts were kept at –20°C.

Polymerase chain reaction (PCR). PCR was performed for four molecular markers: ITS, *BenA*, *CaM*, and *RPB2* (Table 2). Total volume of reactions was 40 µl for each individual marker. PCR reactions for ITS and partial *BenA* amplification contained 20 µl of 2× PCR Master mix (TaqNova-RED: 4 mM $MgCl_2$; 1.6 mM dNTPs mix [0.4 mM of each dNTP]; 0.04 U/µl TaqNova DNA polymerase; DNA Gdansk, Poland), 4 µl of each primer (Microsynth AG, Switzerland, for ITS; Metabion International AG, Germany, for *BenA*), 10.4 µl of sterile nuclease-free water (Thermo Fisher Scientific, U.S.A.), and 1.6 µl of DNA. The compounds for *CaM* gene amplification were 20 µl of 2× PCR Master mix (TaqNova-RED, DNA Gdansk), 8 µl of each primer (Microsynth), 2.4 µl of sterile nuclease-free water, and 1.6 µl of DNA. In *RPB2* gene amplification, reagents in PCR reaction were 4 µl of PCR buffer (without $MgCl_2$), 28.9 µl of sterile nuclease-free water, 1.6 of $MgCl_2$ (50 mM) (Invitrogen, Thermo Fisher Scientific), 1.6 µl of dNTPs (1 mM) (DNA

Table 1. Metadata associated with the *Talaromyces* spp. isolates included in this study

Year	Sample type	No. of collected samples/no. of *Talaromyces* spp. isolates	Isolates included in the analysis
2015–2017	Apple fruit	54/–	–
2015–2016	Quince fruit	31/4	DnjP/2
2015–2016	Pear fruit	43/–	–
2015–2016	Onion bulb	48/13	CLP/3
			CLP/4
			CLP/5
			CLP/7
2016–2017	Tomato fruit	25/3	ParP/2
2016	Potato tuber	6/2	KroP/1
2015–2016	Lemon fruit	39/–	–
2016–2017	Orange fruit	35/4	PP/14
2016–2017	Mandarin fruit	40/–	–
Total		321/26	8

Gdansk), 0.8 μl of each primer (Invitrogen, Thermo Fisher Scientific), 0.3 μl of Taq polymerase (KAPATaq, KAPA Biosystems, Roche Holding AG, Switzerland), and 2 μl of DNA. Amplifications were conducted in an Eppendorf thermal cycler Mastercycler nexus GSX1 (Eppendorf, Germany). PCR cycling conditions are presented in Table 2. Electrophoretic mobility of the amplified DNA products (10 μl) was tested in 1% agarose gel at 110 V for 45 min (stained with Midori green dye, Bulldog Bio, U.S.A.) and visualized with a UV transilluminator (Vilber Lourmat UV transilluminator, Germany).

Sequencing and sequence analysis. The PCR products of all genomic regions were purified and sequenced in both directions in an automated sequencer (ABI 3730XL Automatic Sequencer, Macrogen, South Korea), using the same primers as for the amplification. Consensus sequences were computed using the ClustalW algorithm (Thompson et al. 1994), integrated in MEGA7 software (Kumar et al. 2016), and deposited in GenBank (Table 3). All generated sequences were compared with each other by calculating nucleotide identities, as well as with previously deposited *T. minioluteus* isolates available in the GenBank, using the similarity search tool, BLAST.

Phylogenetic analysis. Newly generated ITS, *BenA*, *CaM*, and *RPB2* sequences were analyzed with reference sequences of *Talaromyces* section *Trachyspermi* available in NCBI and previously listed type-derived sequences of *Talaromyces* spp. (Rajeshkumar et al. 2019). The analyses of individual gene alignments for the purpose of identification of Serbian isolates and the analyses of concatenated four gene alignments for the purpose of characterization were performed using MEGA7 software (Kumar et al. 2016).

Phylogenetic trees were inferred using the maximum likelihood implemented in MEGA version 7.0 software (Kumar et al. 2016) on individual as well as combined datasets of ITS, *BenA*, *CaM*, and *RPB2* gene sequences. The sequence of *T. pinophilus* (CBS 631.66) was used as an outgroup. The gamma-distributed Tamura–Nei model (G+I) determined by Modeltest implemented in MEGA7 was used as the best fitting model of nucleotide substitution. The reliability of the obtained trees was evaluated using 1,000 bootstrap replicates, and bootstrap confidence values <70% were omitted. Phylogenetic trees were visually prepared and edited in Adobe Photoshop CS6 (Adobe, U.S.A.).

Pathogenicity test. Pathogenicity of our isolates was tested on intact, symptomless quince, tomato, and orange fruits, onion bulbs, and potato tubers. The assay was conducted only on hosts from which isolates were obtained. The fruits, tubers, and bulbs were surface sterilized with 70% ethanol, air dried, wounded with a sterile needle, and inoculated with 50 μl of spore suspension. Conidial suspensions for each isolate were prepared in 1 ml of sterile distilled water from the 14-day-old cultures grown on MEA. The spore concentration was determined with a hemocytometer (Neubauer chamber) and adjusted to 1×10^6 conidia/ml. Each host was inoculated in three replicates with the corresponding isolate. Control fruits, tubers, and bulbs were inoculated with 50 μl of sterile distilled water. All inoculated hosts were placed in a plastic container and incubated at 25°C and 95% relative air humidity. Symptoms were assessed 7 days after inoculation by measuring the horizontal and vertical (stem-calyx axis vertical) diameters of lesions. To confirm that symptoms were caused by *T. minioluteus*, reisolations were done from 100% of the inoculated fruits, tubers, and bulbs. Reisolation was performed as described above (see "Sampling and isolation"). Colony and spore morphology of the developed MEA cultures was checked for the fulfillment of Koch's postulates. Inoculated fruits, bulbs, and tubers were monitored after the expiration of the specified incubation period to observe further symptom development. The experiment was performed twice, and it was arranged according to a completely randomized design.

Statistical analysis. Data were analyzed in IBM SPSS Statistics, version 23 (IBM Corporation, U.S.A.). Data from independent experiments were pooled if the individual trials proved not to be significantly different. Basic descriptive statistics (minimal, maximal, and average diameters, standard deviation) were calculated for colony growth of all tested isolates on the above-named six media and five temperatures. Mean diameters for colony growth and lesions in the pathogenicity assay were analyzed by one-way ANOVA ($P \leq 0.05$) and then compared and separated using Tukey's honest significant difference test. Levene's test for homogeneity of variance was used. In cases in which the condition of the equality of variances was not fulfilled, we applied more robust Welch and Brown–Forsythe tests to compare the growth diameters. Minimal, maximal, and average length and width were calculated for conidia.

Results

Symptoms and fungal isolation. *Penicillium*-like mold symptoms were observed on all collected types of fruit and vegetables. Discoloration of the tissue, brown, circular, slightly sunken spots, presence of white to yellow mycelia, and in some cases sparse to intensive sporulation with dark green spores on the surface of samples were recorded. The decayed area was soft and watery.

After isolation on MEA, 26 isolates were obtained with significantly different morphological characteristics (colony color and texture, very slow growth and micromorphology) in comparison with the other obtained single-conidial isolates of *Penicillium* spp. (unpublished data). For detailed morphological and molecular identification and characterization, eight isolates originating from quince, tomato, and orange fruits, onion bulbs, and potato tubers were chosen (Table 1).

Growth on different media. Each isolate was inoculated on six media (CYA, MEA, CREA, DG18, OA, and YES) and incubated for 7 days at 25°C. The results of the radial growth diameters and the macromorphological characteristics are presented in Figures 1A and 2A to K, respectively. All tested isolates had plane, compact colonies on CYA, DG18, OA, and YES, plane-filamentous on CREA, and slightly umbonate on MEA. Textures were velutinous on CYA, DG18, and CREA, strongly funiculose on MEA and YES, and loosely funiculose on OA. Mycelia were visible on the edges of the cultures, white on DG18, whiteish on CYA, white to yellowish on MEA and OA, and transparent to white on CREA. Sporulation was abundant on all media, except on CREA, for which it was moderate. Conidia were dark green on CYA, YES, CREA, DG18, and

Table 2. List of primers and PCR conditions used in molecular identification of Serbian *Talaromyces* spp. isolates

Locus	Primer set	Direction	Primer sequence (5′-3′)	Reference	Cycling conditions
Internal transcribed spacer (ITS)	V9G	Forward	TTACGTCCCTGCCCTTTGTA	de Hoog and van den Ende (1998)	94°C, 5 min; 94°C, 45 s, 55°C, 45 s, 72°C, 1 min (35 cycles); 72°C for 7 min
	LS266	Reverse	GCATTCCCAAACAACTCGACTC	Masclaux et al. (1995)	
Calmodulin (*CaM*)	CMD5	Forward	CCGAGTACAAGGARGCCTTC	Hong et al. (2006)	
	CMD6	Reverse	CCGATRGAGGTCATRACGTGG		
β-tubulin (*BenA*)	Bt2a	Forward	GGTAACCAAATCGGTGCTGCTTTC	Glass and Donaldson (1995)	94°C, 5 min; 94°C, 30 s, 55°C, 45 s, 72°C, 1 min (35 cycles); 72°C, 7 min
	Bt2b	Reverse	ACCCTCAGTGTAGTGACCCTTGGC		
DNA-dependent RNA polymerase II second largest subunit (*RPB2*)	5F	Forward	GAYGAYMGWGATCAYTTYGG	Liu et al. (1999)	94°C, 5 min; 94°C, 45 s, 60°C, 45 s, 72°C, 1 min (35 cycles); 72°C, 7 min
	7CR	Reverse	CCCATRGCTTGYTTRCCCAT		

OA and bluish-green on MEA. Margins were low, entire, and up to 2 mm wide on all media, except on CREA, for which they were filiform. Acid production on CREA was absent or very weak (weak change of the medium color from purple to yellow). Exudates and soluble pigments were absent on all media. On the reverse side of the plates, cultures were light yellowish green on CYA, light cream yellow on MEA and OA, pale green on DG18, orange on YES, and light dull green on CREA.

Significant differences ($P \leq 0.05$) were determined among isolates grown on the same medium on CYA, YES, OA, and DG18 (Fig. 1A). Growth diameters were not significantly different on MEA and CREA. All isolates had the fastest growth on YES (from 22.67 to 28 mm, isolates CLP/4 and DnjP/2, respectively) and the slowest on CREA (6.33 to 8.42 mm, isolates ParP/2 and CLP/4, respectively). The isolate from orange fruit (PP/14) had the slowest mycelial growth on three of the six tested media (CYA, MEA, and OA). The isolate from tomato (ParP/2) had the fastest growth on CYA and OA, and the isolate from quince (DnjP/2) was the fastest growing on YES and CREA.

Growth on CYA at different temperatures. At 5 and 37°C none of the tested isolates manifested growth (Fig. 1B). Significant differences ($P \leq 0.05$) were detected in growth at the remaining three temperatures. Also, colony morphology was different at these temperatures: cultures had one appearance at 15°C and another at 25 and 30°C. Colonies of all isolates at 15°C were raised, with very slow growth, white or somewhat yellowish mycelia, floccose texture, absent or very weak sporulation, and no exudates. Margins were white and entire. Reverse, cultures were orange-brown. At 25 and 30°C, colonies had similar appearance to cultures on CYA described in the previous section. The only difference was that at 30°C soluble pigments (light yellow color) were visible in PP/14 (isolate from orange) and in CLP/3 (isolate from onion).

The fastest growth of all isolates was recorded at 30°C, and the slowest was at 15°C (Fig. 1B). The mean radial growth diameters at 15°C ranged from 2.75 mm (isolate PP/14) to 4.71 mm (ParP/2) (Fig. 1B). At 25°C mycelial growth was in the range from 15.33 mm (isolate PP/14) to 19.08 mm (ParP/2). The average growth diameters at 30°C were in the range from 18.67 mm (isolate CLP/3) to 21.25 mm (ParP/2). Isolate PP/14 from orange had the least rapid growth at two (15 and 25°C) out of three temperatures, and isolate ParP/2 from tomato had the fastest growth at all three temperatures for which growth was registered.

Microscopic investigation. All analyzed isolates originating from various hosts formed biverticillate conidiophores, with smooth stipes and acerose phialides. Conidia were smooth-walled and ellipsoidal to subglobose (Fig. 2L). Conidia sizes ranged from 2.49 × 2.35 μm

Table 3. Accession numbers for *Talaromyces* spp. isolates used in the phylogenetic analysis; isolates from this study are in bold[a]

Species	Strain/isolate	Substrate and origin	GenBank accessions			
			ITS	*BenA*	*CaM*	*RPB2*
T. aerius	CBS 140611^T	Indoor air, China	KU866647	KU866835	KU866731	KU866991
T. albobiverticillius	CBS 133440^T	Decaying leaves of a broad-leaved tree, Taiwan	HQ605705	KF114778	KJ885258	KM023310
	CBS 140498	Air from HVAC system, China	KR855658	KR855648	KR855653	KR855663
T. amyrossmaniae	NFCCI 1919^T	Fallen decaying fruits of *Terminalia bellirica* (Combretaceae), Maharashtra, India	MH909062	MH909064	MH909068	MH909066
	NFCCI 2351	Fallen decaying fruits of *Terminalia bellirica* (Combretaceae), Maharashtra, India	MH909063	MH909065	MH909069	MH909067
T. assiutensis	CBS 147.78^T	Soil, Egypt	JN899323	KJ865720	KJ885260	KM023305
	CBS 645.80	*Gossypium*, India	JN899334	KF114802	*	*
T. atroroseus	CBS 133442^T	House dust, South Africa	KF114747	KF114789	KJ775418	KM023288
	CBS 133449	Mouse dung, Denmark	KF114744	KF114788	*	*
T. austrocalifornicus	CBS 644.95^T	Soil, U.S.A.	JN899357	KJ865732	KJ885261	*
T. brasiliensis	CBS 142493^T	Honey of *Melipona scutellaris*, Recife, Pernambuco, Brazil	MF278323	LT855560	LT855563	LT855566
T. convolutus	CBS 100537^T	Soil, Nepal	JN899330	KF114773	*	JN121414
T. diversus	CBS 320.48^T	Leather, U.S.A.	KJ865740	KJ865723	KJ885268	KM023285
	DTO 244-E6	House dust, New Zealand	KJ775712	KJ775205	*	*
T. erythromellis	CBS 644.80^T	Soil from creek bank, New South Wales, Australia	JN899383	HQ156945	KJ885270	KM023290
T. heiheensis	HMAS 248789^T	Rotten wood, China	KX447526	KX447525	KX447532	KX447529
T. minioluteus	CBS 642.68^T	Unknown	JN899346	KF114799	KJ885273	JF417443
	CBS 270.35	*Zea mays*, U.S.A.	KM066172	KM066129	*	*
	CBS 137.84	Fruit damaged by insect, Spain	KM066171	KF114798	*	*
	CLP/3	**Onion, Serbia**	**MN311444**	**MN306500**	**MN306508**	**MN306516**
	CLP/4	**Onion, Serbia**	**MN311445**	**MN306501**	**MN306509**	**MN306517**
	CLP/5	**Onion, Serbia**	**MN311446**	**MN306502**	**MN306510**	**MN306518**
	CLP/7	**Onion, Serbia**	**MN311447**	**MN306503**	**MN306511**	**MN306519**
	DnjP/2	**Quince, Serbia**	**MN311448**	**MN306504**	**MN306512**	**MN306520**
	KroP/1	**Potato, Serbia**	**MN311449**	**MN306505**	**MN306513**	**MN306521**
	ParP/2	**Tomato, Serbia**	**MN311450**	**MN306506**	**MN306514**	**MN306522**
	PP/14	**Orange, Serbia**	**MN311451**	**MN306507**	**MN306515**	**MN306523**
T. minnesotensis	CBS 142381^T	Human ear, U.S.A.	LT558966	LT559083	LT795604	LT795605
T. solicola	DAOM 241015^T	Soil, South Africa	FJ160264	GU385731	KJ885279	KM023295
	CBS 133446	Soil, South Africa	KF114730	KF114775	*	*
T. systylus	BAFCcult3419^T	Soil, Argentina	KP026917	KR233838	KR233837	*
T. trachyspermus	CBS 373.48^T	Unknown, U.S.A.	JN899354	KF114803	KJ885281	JF417432
	CBS 118437	Soil, Morocco	KM066169	KM066127	*	*
T. ucrainicus	CBS 162.67^T	Unknown	JN899394	KF114771	KJ885282	KM023289
	CBS 127.64	Soil treated with cyanimide, Germany (ex-type of *T. ohiensis*)	KM066173	KF114772	*	*
T. udagawae	CBS 579.72^T	Soil, Japan	JN899350	KF114796	KX961260	*

[a] Superscript T indicates an ex-type strain. Asterisks indicate no corresponding entry exists. ITS = internal transcribed spacer; *CaM* = calmodulin; *BenA* = β-tubulin; and *RPB2* = DNA-dependent RNA polymerase II second largest subunit.

(CLP/7) to 3.73 × 3.34 μm (KroP/1) (Table 4). Ascomata were not observed.

Micromorphological features, colony morphology, and growth diameters on tested media and temperatures are in agreement with the description of species *T. minioluteus* (Visagie 2012; Yilmaz et al. 2014).

Production of lipase, esterase, and amylase. In enzyme production tests all tested isolates manifested similar reactions, with minor differences (Table 4). In the lipase production test, all isolates had a negative result except the isolate ParP/2 from tomato fruit, for which very small brightness of the medium color around the culture was noticed. Tested isolates expressed a negative reaction in the esterase production assay. In the amylase production test, all isolates had weak growth on Tween 80 medium, and the margins of the cultures were brown, which was considered a weak positive reaction. Isolates DnjP/2 (from quince fruit) and KroP/1 (from potato tuber) had

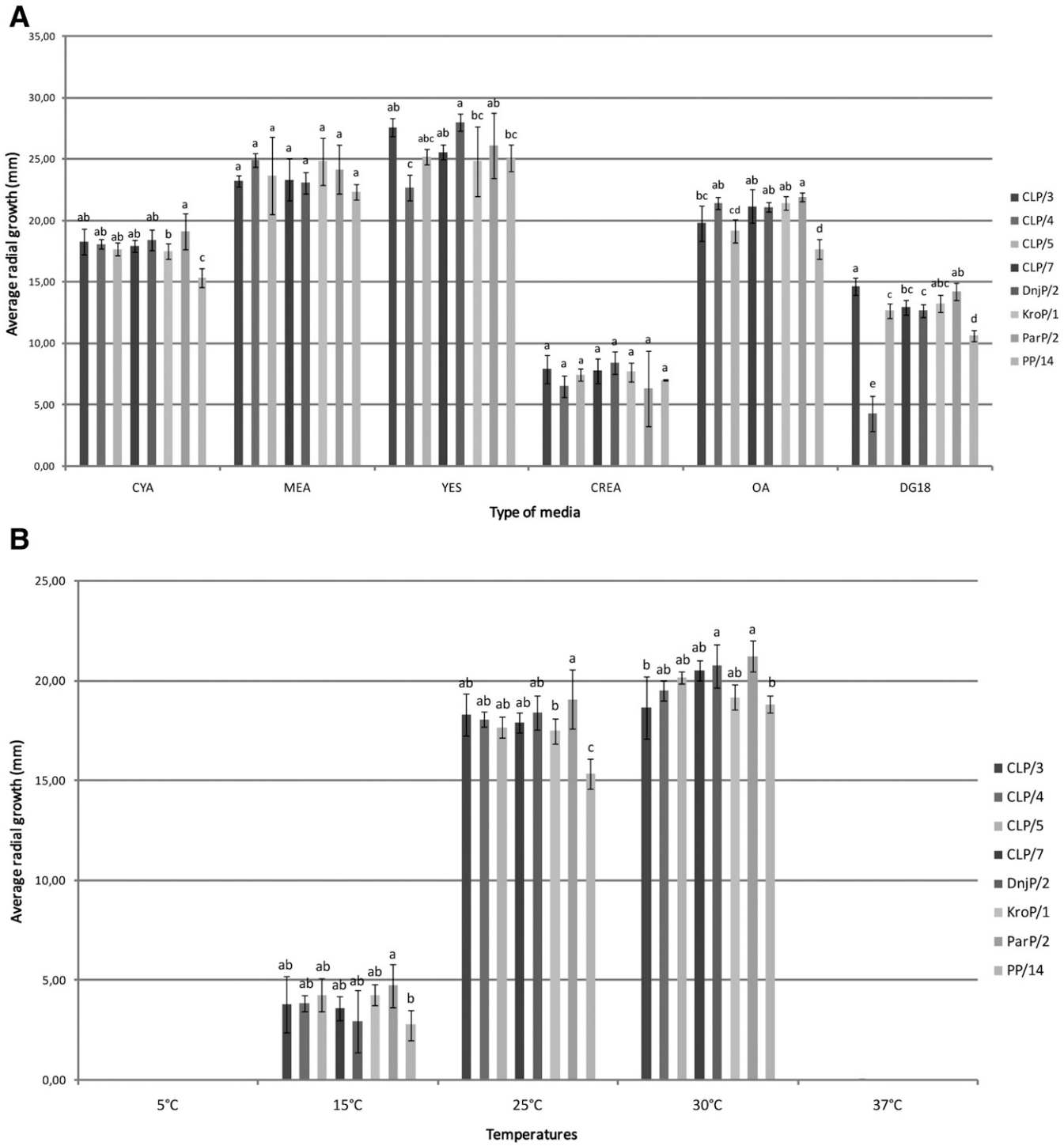

Fig. 1. The average colony growth of *Talaromyces minioluteus* isolates. **A,** Growth on six media after 7 days of incubation at 25°C. Numbers followed by the same letters in the column for Czapek yeast autolysate agar (CYA) represent values that are not significantly different according to Tukey's HSD test ($P \leq 0.05$); numbers followed by the same letters in the column for malt extract agar (MEA) represent values that are not significantly different according to the Brown–Forsythe test ($P \leq 0.05$); numbers followed by the same letters in the columns for yeast extract sucrose agar (YES), creatine sucrose agar (CREA), oatmeal agar (OA), and dichloran 18% glycerol agar (DG18) represent values that are not significantly different according to the Welch test ($P \leq 0.05$). Vertical error bars on all media type indicate standard deviation of the mean (SD). **B,** Growth on CYA at five different temperatures. Numbers with the same letters represent values that are not significantly different according to Tukey's HSD test ($P \leq 0.05$). Vertical error bars indicate SD.

slightly different reactions than the others: DnjP/2 had a narrow brown zone around the culture (weaker reaction), and KroP/1 had a clear positive reaction.

Molecular identification and sequence analysis. All eight ITS sequences from Serbian isolates were similar (99.9 to 100%; 1 bp difference) to each other. BLAST analysis revealed the highest nucleotide identities of 99.2% with one sequence of *T. minioluteus* from Spain (MH861709). The *BenA* sequences of our *T. minioluteus* isolates shared a similarity of 99.8 to 100% (1 bp differences), and BLAST results revealed a nucleotide identity of 99.8 to 100% with five *T. minioluteus* isolates from Iran (KU516400 to 404). The *CaM* sequences of all eight Serbian *T. minioluteus* isolates proved to be 100% identical at the nucleotide level, and BLAST analysis confirmed the highest nucleotide homology of 99.6% with one *T. minioluteus* from Iran (KU711896). The *RPB2* sequences of Serbian isolates showed nucleotide identities of 99.9 to 100% (1 bp differences), and BLAST analysis confirmed the highest nucleotide homology of 99% with one *T. minioluteus* isolate from Brazil (KX650058). BLAST analyses of all four targeted genome regions (ITS, *BenA*, *CaM*, and *RPB2*) confirmed the conventional identification based on morpho-physiological characteristics of selected Serbian *T. minioluteus* isolates.

Molecular characterization and phylogeny. Maximum likelihood analyses of the ITS, *BenA*, *CaM*, and *RPB2* sequences alignment of 475, 370, 487, and 517 nucleotides each, including the outgroup taxa, resulted in phylogenetic trees with established resolution and topology for species of section *Trachyspermi* (Fig. 3). In the ITS phylogeny, the Serbian isolates were placed in a single distant branch with high bootstrap support (98%) from the main clade with the rest of *T. minioluteus* isolates, showing a similarity of 99.1, 98.9, and 98.7%, respectively, to CBS 137.84, CBS 270.35, and CBS 642.68. Our isolates were positioned in the *BenA* phylogeny in a single distant branch with high bootstrap support (99%) from the main clade with the rest of *T. minioluteus* isolates, showing a similarity of 95.3, 94.7, and 94%, respectively, to CBS 642.68, CBS 270.35, and CBS 137.84. In the *CaM* and *RPB2* phylogeny our isolates had 95.8 and 97.8% sequence similarity with the sequence of CBS 642.68 with high bootstrap support (100%). Also, our isolates were clustered with *T. minnesotensis* and *T. udagawae* in the ITS, *BenA*, *CaM*, and *RPB2* analyses.

Multilocus analysis, based on the Tamura–Nei model assuming gamma distribution (Kumar et al. 2016), resulted in a maximum likelihood tree for the four concatenated loci (Fig. 4). The ITS, *BenA*, *CaM*, and *RPB2* sequence alignments consisted of 475, 370, 487, and 517 nucleotides, respectively (1,849 nucleotides in total), from 37 different isolates, including the outgroup taxa. At the *Trachyspermi* section level, the phylogenetic tree with the combined data set shared a similar topology with the ITS, *BenA*, *CaM*, and *RPB2* single-locus trees obtained in this study. Within the clade, *T. minioluteus* isolates were, with great confidence, separated in two subgroups (bootstrap support of 100%). The first branch included the Serbian isolates, whereas the remaining *T. minioluteus* isolates from the United States, Spain, and one unknown location were grouped in the second branch. These isolates were included in a highly supported clade with *T. minnesotensis* and *T. udagawae*.

Pathogenicity test. Tested *T. minioluteus* isolates manifested rot symptoms on quince, tomato, and orange fruits and onion bulbs 7 days after inoculation (Fig. 5A to H). Our assay showed that *T. minioluteus* (isolate KroP/1) was not pathogenic on potato tubers (Fig. 5I and J). Control fruits remained without symptoms. Significant

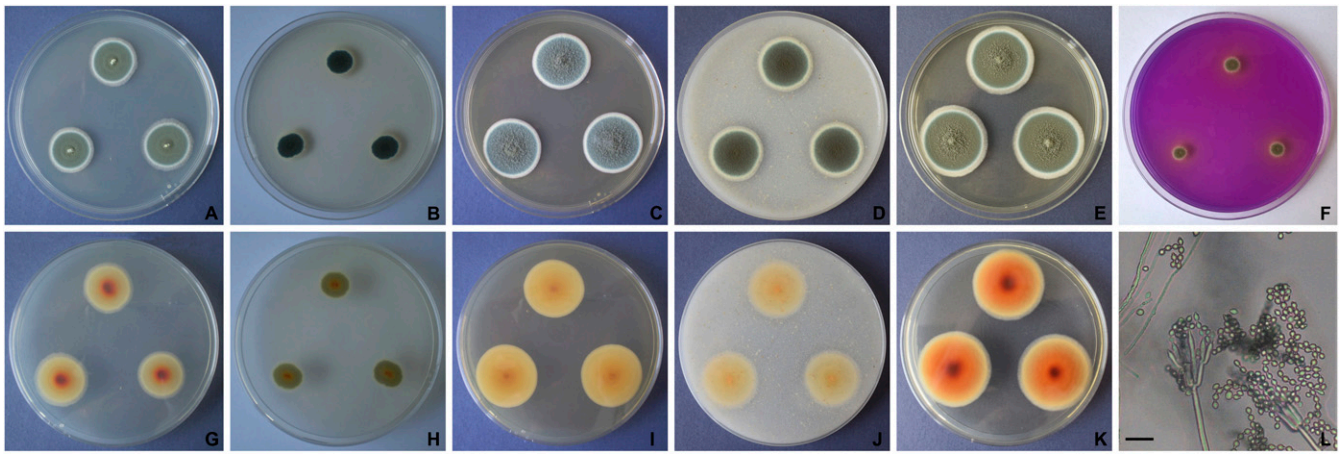

Fig. 2. Morphological characteristics of *Talaromyces minioluteus* isolate DnjP/2. Obverse: **A,** Czapek yeast autolysate agar (CYA); **B,** dichloran 18% glycerol agar (DG18); **C,** malt extract agar (MEA); **D,** oatmeal agar (OA); **E,** yeast extract sucrose agar (YES); and **F,** creatine sucrose agar (CREA). Reverse: **G,** CYA; **H,** DG18; **I,** MEA; **J,** OA; and **K,** YES. **L,** Conidiophores and conidia, bar = 10 μm.

Table 4. Dimensions of spores and enzyme production of *Talaromyces minioluteus* isolates

Isolate	Conidial dimensions (μm), minimum-(average)-maximum	Enzyme production		
		Lipase test[a]	Esterase test[b]	Amylase test[c]
CLP/3	3.00–(3.08)–4.00 × 2.00–(2.65)–3.00	–	–	+–
CLP/4	3.00–(3.04)–4.00 × 2.00–(2.72)–3.00	–	–	+–
CLP/5	2.00–(2.51)–2.75 × 2.25–(2.46)–2.75	–	–	+–
CLP/7	2.25–(2.54)–3.00 × 2.25–(2.54)–3.00	–	–	+–
DnjP/2	2.25–(2.61)–3.75 × 2.00–(2.50)–3.00	–	–	+
KroP/1	2.00–(2.49)–3.00 × 2.00–(2.35)–2.75	–	–	+
ParP/2	3.00–(3.11)–4.00 × 2.00–(2.52)–3.00	+–	–	+–
PP/14	2.50–(3.73)–5.00 × 2.50–(3.34)–5.00	–	–	+–

[a] – = no halo effect; + = strong halo effect; and +– = weak halo effect.
[b] – = medium color green; and + = medium color purple-blue.
[c] – = blue zone around the culture; + = brown zone around the culture; and +– = margin of the culture is brown.

differences ($P \leq 0.05$) were determined in lesions caused by isolates originating from different hosts (Fig. 6). The most severe decay was observed on quince fruits (30.83 mm), whereas the smallest lesions were measured on onion bulbs (10.17 and 11.00 mm, isolates CLP/7 and CLP/4, respectively). Isolates CLP/3 and CLP/5 were not significantly different from each other, but they were distinguished from isolates CLP/4 and CLP/7, also originating from onion bulbs.

The symptoms reproduced in the pathogenicity assay were quite similar to those observed on the original hosts. Tissue around the wound became discolored, soft, watery, with a clear distinction between diseased and healthy flesh, on all inoculated fruits except orange. The intensity of developed symptoms on the surface of orange fruit was different than on the other inoculated hosts. The lesions observed on orange fruit were small, and the pericarp of the fruit was not soft and watery (Fig. 5C). Five days after

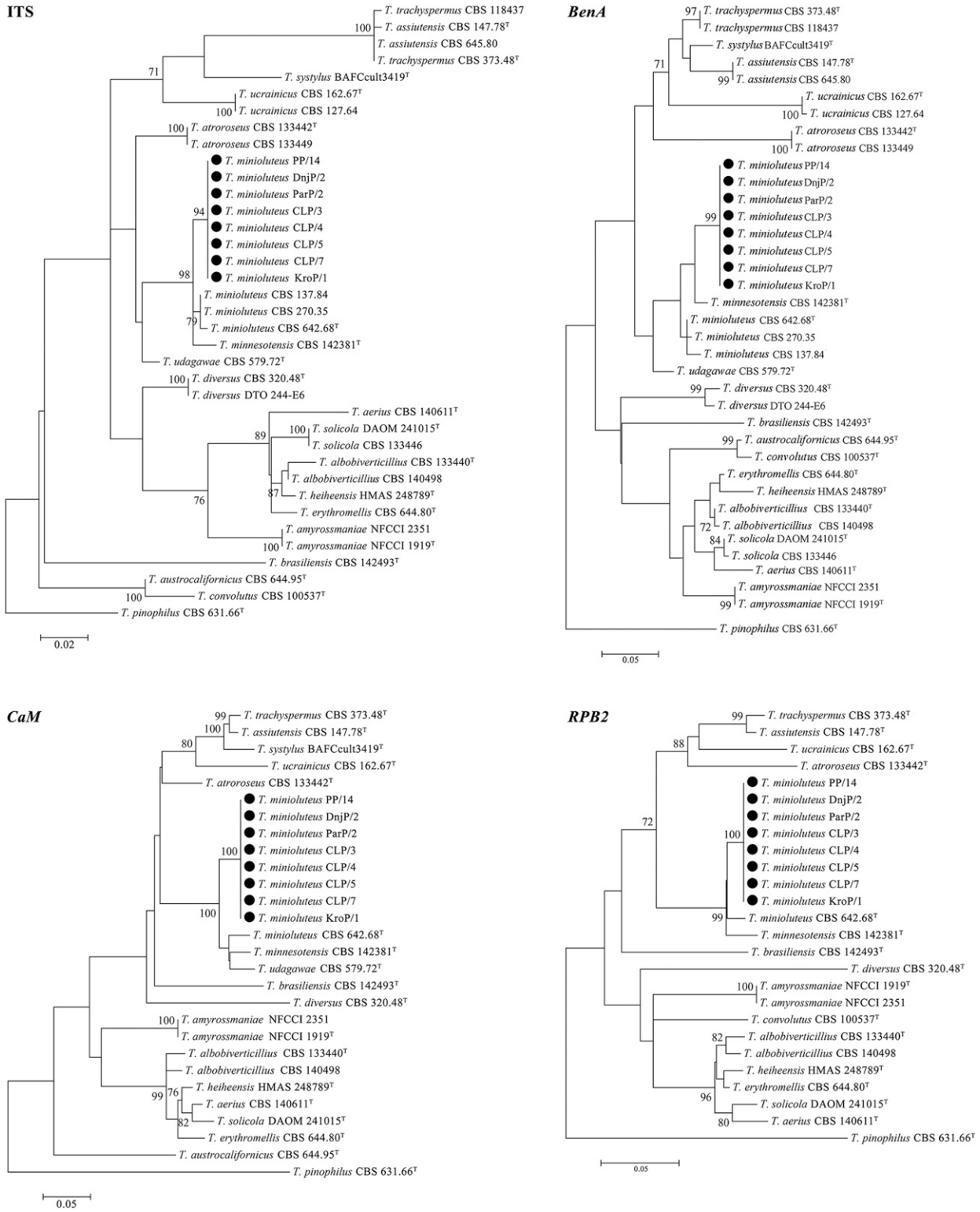

Fig. 3. Maximum likelihood phylogenetic trees of internal transcribed spacer (ITS) region, β-tubulin (*BenA*), calmodulin (*CaM*), and DNA-dependent RNA polymerase II second largest subunit (*RPB2*) genes of isolates from *Talaromyces* section *Trachyspermi*. Sequence of *Talaromyces pinophilus* (CBS 631.66^T) was used as an outgroup. Bootstrap analysis was performed with 1,000 replicates, and bootstrap values (>70%) are shown next to the relevant branches. *Talaromyces minioluteus* isolates from Serbia are highlighted with black circles.

inoculation, bright yellow mycelia were observed on quince, tomato, and onion. Ten days after inoculation, moderate sporulation was manifested on the surface of all fruits and bulbs, with dark green spores.

Cross sections (stalk-calyx axis) of the inoculated hosts revealed that in onion bulb fungi were sporulating inside the wound and slowly progressing toward the midpoint of the bulb (Fig. 5H); in orange fruit fungi reached the center of the fruit (Fig. 5D); in tomato fruit, the pericarp wall was weakened, deformed, and perforated, and fungi reached the locular cavity (Fig. 5F); and quince fruit was the most severely affected with substantial decay of the pulp into the core (Fig. 5B). Koch's postulates were fulfilled by reisolation (100%) from the inoculated quince, tomato, and orange fruits and onion bulbs. Reisolates were not obtained from inoculated potato tubers.

Discussion

In this study we presented the first results of the 3-year investigation on the presence of *T. minioluteus* in Serbia on quince, tomato, and orange fruits, onion bulbs, and potato tubers. Based on the literature review, these are the first records of *T. minioluteus* on the listed hosts. Also, this is the first report of this species as a postharvest plant pathogen on quince, tomato, and orange fruits and onion bulbs.

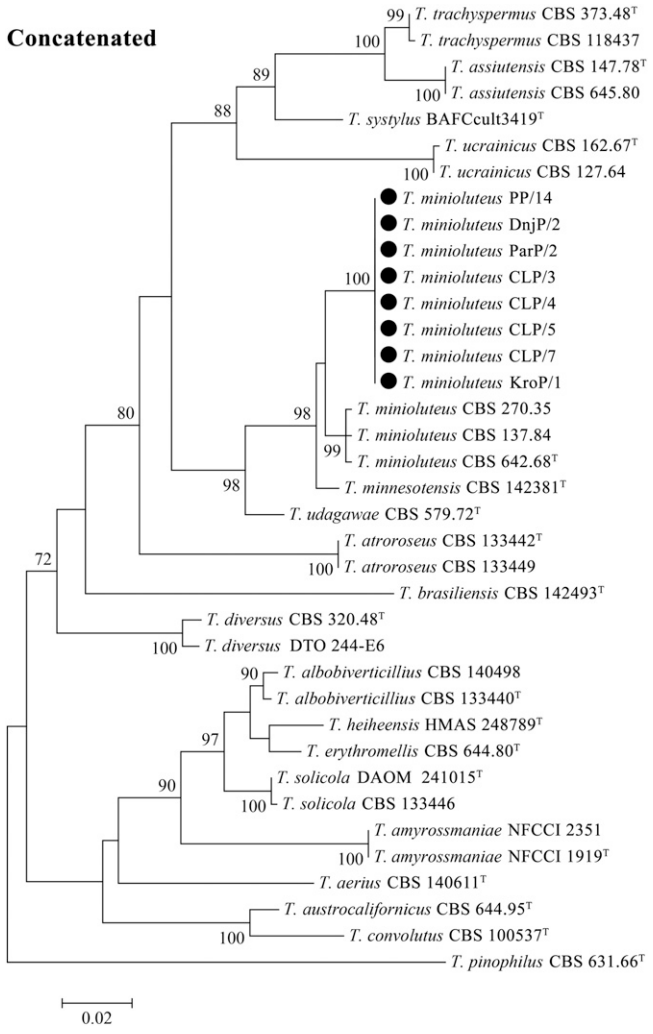

Fig. 4. Maximum likelihood combined phylogenetic tree using internal transcribed spacer region, β-tubulin, calmodulin, and DNA-dependent RNA polymerase II second largest subunit genes of isolates from *Talaromyces* section *Trachyspermi*. Sequence of *Talaromyces pinophilus* (CBS 631.66^T) was used as an outgroup. Bootstrap analysis was performed with 1,000 replicates, and bootstrap values (>70%) are shown next to the relevant branches. The Serbian *Talaromyces minioluteus* isolates are highlighted with black circles.

Mold symptoms on the various fruits and vegetables used in this study, very similar to those produced by penicillia, were observed during our survey, and their causal agent was identified as *T. minioluteus*. It can be explained by the fact that many species currently placed in the genus *Talaromyces* were previously known as *Penicillium* species. These symptoms included tissue discoloration, brown, usually circular spots, and soft and watery decayed host surface. Later, white and yellow mycelia were observed on diseased tissue, followed by dark green sporulation.

There are several reasons why *T. minioluteus* has been detected on stored crops at low incidence. *T. minioluteus* is a species with cosmopolitan distribution but in many studies was isolated at low frequencies compared with other fungi (Hujslová et al. 2010; Magnoli et al. 1998; Nesci et al. 2006; Sage et al. 2004). Low frequencies can be a complicating factor in isolation of this species from stored fruit and vegetables. We can assume that *T. minioluteus* is a weak pathogen, compared with some plant pathogenic penicillia (e.g., *P. expansum*, *P. digitatum*, and *P. italicum*). Weaker growth of this fungus on nutrient-rich artificial media such as CYA, MEA, and YES has also been reported (Yilmaz et al. 2014). As a consequence of these

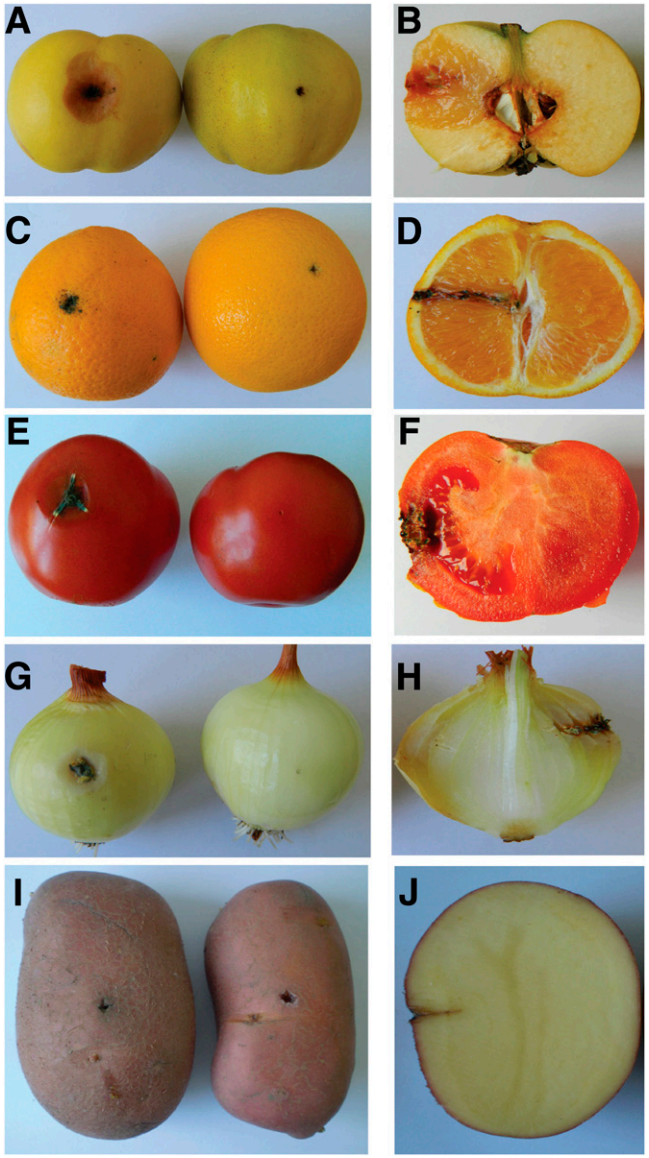

Fig. 5. Pathogenicity on hosts inoculated with corresponding *Talaromyces minioluteus* isolates after 7 days of incubation: **A,** quince fruit; **C,** orange fruit; **E,** tomato fruit; **G,** onion bulb; and **I,** potato tuber. Hosts inoculated with pathogen are to the left and controls to the right. Cross-sections of the inoculated hosts: **B,** quince fruit; **D,** orange fruit; **F,** tomato fruit; **H,** onion bulb; and **J,** potato tuber.

characteristics, other related plant pathogenic species, such as some penicillia, can colonize different plant hosts more rapidly than *T. minioluteus* and can completely cover it. Quintanilla (1985) noticed hyphae of *P. samsonii* (= *T. minioluteus*) on heavily contaminated apple fruit with *P. expansum* after prolonged incubation. Also, infections of the stored crops are often mixed, in which several different species were reported on one host (Borecka 1977; Cole and Wood 1970; Cunningham and Taverner 2007), making the identification of the causal agents based solely on the produced symptoms challenging, if not impossible. It is also difficult to distinguish the symptoms caused by *Penicillium* from the symptoms induced by *Talaromyces* species on stored fruits and vegetables. Mukhtar et al. (2019) determined *T. funiculosus* as causal agent of peach fruit core rot but emphasized that its infection "can be confused with *Penicillium* postharvest decay." All of the above-specified arguments are the reasons why we obtained and operated with a limited number of *T. minioluteus* isolates in this study.

Morphological characteristics and radial growth measurements on different media of *T. minioluteus* isolates from this study varied to a small degree in comparison with previous descriptions (Frisvad et al. 1990; Van Reenen-Hoekstra et al. 1990; Visagie 2012; Yilmaz et al. 2014). Out of all tested media, significant difference ($P \leq 0.05$) among isolates has not been determined only on MEA and CREA. There were no significant differences in culture appearance on the same type of medium between isolates originating from different hosts. Also, there were no significant differences in growth and colony morphology between nonpathogenic isolate KroP/1 and other tested pathogenic isolates. *T. minioluteus* belongs to the section *Trachyspermi*, whose members have restricted growth on CYA, YES, and DG18 and slightly faster growth on MEA (Yilmaz et al. 2014). Growth of our isolates was slightly faster on YES than on MEA. This is different than in Yilmaz et al. (2014) but is in agreement with the findings of Van Reenen-Hoekstra et al. (1990) and Visagie (2012). CREA growth results were consistent with Visagie (2012) and higher than in Yilmaz et al. (2014). The radial growth measurements on DG18 and OA were similar to those reported in the literature (Yilmaz et al. 2014). All of our isolates had more abundant sporulation on all media and slightly faster growth on MEA and YES than isolates from the listed literature sources (Frisvad et al. 1990; Van Reenen-Hoekstra et al. 1990; Visagie 2012; Yilmaz et al. 2014). Culture appearances of isolates from this work do not differ between each other, but there are some distinctions when compared with the previous descriptions of this species, mostly in colony textures. On CYA, our isolates were velutinous, which is in agreement with descriptions of Visagie (2012). In Yilmaz et al. (2014), *T. minioluteus* on CYA is floccose and concentrically sulcate. Funiculose texture of tested isolates on MEA is similar to Yilmaz et al. (2014) and different from velutinous cultures of Van Reenen-Hoekstra et al. (1990) and Visagie (2012). On DG18, our cultures were velutinous, which was different than concentrically sulcate colonies in Yilmaz et al. (2014). Our isolates had loosely funiculose texture on OA, whereas in Yilmaz et al. (2014) *T. minioluteus* was slimy in appearance.

T. minioluteus isolates from this study were tested for growth on CYA at five different temperatures for determination of minimal and maximal temperature growth limits. There was no growth at 5 and 37°C, which is in agreement with Visagie (2012) and Yilmaz et al. (2014). The strongest growth was observed at the temperature of 30°C, which is congruent with the results of Visagie (2012). *Talaromyces* is a mesophilic genus, generally (Houbraken et al. 2014), and our assay confirmed that *T. minioluteus* is a mesophilic species. The growth of our isolates was favored by higher tested temperatures, at 25 and 30°C. Different temperatures also had an impact on the culture appearance and sporulation of all isolates. Unfortunately, due to a lack of data from other authors about the growth and appearance of the colonies at 15°C, we are unable to make comparison about this observation. We can only state that the appearance of Serbian *T. minioluteus* colonies at 30°C differed from the ones in Visagie (2012). Our cultures were plane and velutinous at 30°C, whereas the colonies in Visagie (2012) were slightly raised at the center and floccose. These results can help in anticipation of the possible natural or man-made habitats where *T. minioluteus* can be detected. It is possible to find it in the markets, where the average temperature is near to the optimal growth temperature. Contrary, it is less likely to encounter *T. minioluteus* in cold storage rooms, where the temperatures are usually 0 to 5°C, and this species cannot grow at 5°C in vitro, which was confirmed in this and other studies (Visagie 2012; Yilmaz et al. 2014).

Micromorphological features and conidial dimensions of our *T. minioluteus* isolates were in agreement with previous results (Frisvad et al. 1990; Van Reenen-Hoekstra et al. 1990; Visagie 2012; Yilmaz et al. 2014). Our isolates had conidiophores with biverticillate branching type, smooth stipes, acerose phialides, and smooth-walled, ellipsoidal to subglobose conidia. The sexual stage was not observed among Serbian isolates of *T. minioluteus*, which is consistent with the result of Yilmaz et al. (2014).

Results of the enzyme production tests from this research indicate that our isolates of *T. minioluteus* originating from different hosts showed similar reactions and growth intensities. Lipase and esterase

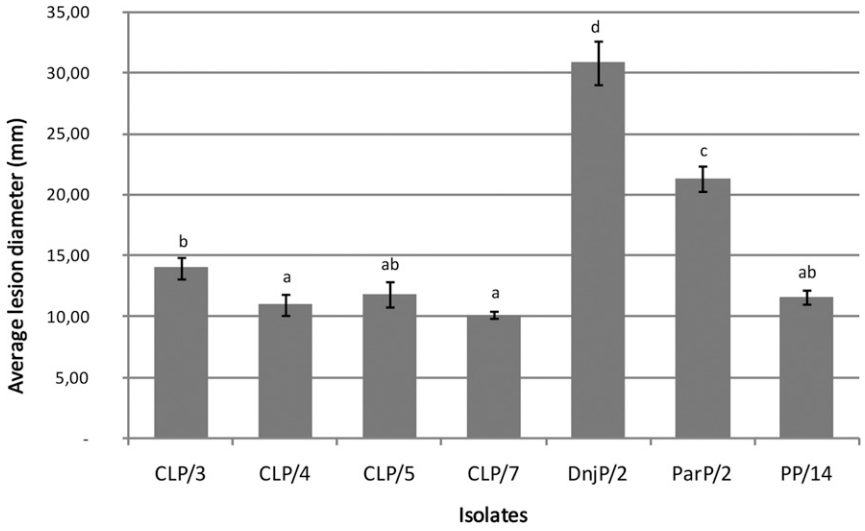

Fig. 6. Pathogenicity of *Talaromyces minioluteus* isolates on onion bulbs, potato tubers, and quince, tomato, and orange fruits. Numbers with the same letters represent values that are not significantly different according to Tukey's HSD test ($P \leq 0.05$). Vertical error bars indicate standard deviation of the mean.

production tests were negative, and the amylase production test was positive. Comparison with previous data are not possible because lipase, esterase, and amylase production have not been reported for *T. minioluteus*. Fungi produce many enzymes (e.g., lipases, proteases, carbohydrases, and esterases) that have different functions in growth and development, host infection, deterioration of food, and degradation of organic matter (Filtenborg et al. 1996; Hankin and Anagnostakis 1975). More detailed and comprehensive analyses using modern techniques should be performed to examine the enzyme production of *T. minioluteus*. Previous reports on lipase, esterase, and amylase production by other *Talaromyces* spp. indicate that these enzymes have a considerable biotechnological potential: from starch bioconversion (Bunni et al. 1989; Xian et al. 2015), use in the food and paper industry (Tuohy et al. 1994), use in the agricultural and food sectors (Crepin et al. 2003; Garcia-Conesa et al. 2004), and utilization in laundry detergents (Romdhane et al. 2010).

Minor differences in various morphological and physiological features of *T. minioluteus* are reported in this study, compared with literature data (Frisvad et al. 1990; Van Reenen-Hoekstra et al. 1990; Visagie 2012; Yilmaz et al. 2014). Like penicillia (Guerche et al. 2004; Visagie et al. 2014b), *T. minioluteus* manifests phenotypic plasticity, so variation in micro- and macromorphological characters is expected due to the differences in the environmental factors or loss of the typical features over the years through extensive subculturing. The interpretation of morphological characters often depends on the experience of the researchers, because taxonomy and identification of species from *Penicillium* and *Talaromyces* are quite difficult (Dupont et al. 2006; Pitt and Hocking 2009; Pitt and Samson 1990).

Molecular identification and phylogeny based on the sequences of four genetic markers (ITS, *BenA*, *CaM*, and *RPB2*) supported the morphological identification of Serbian *T. minioluteus* isolates. Species-level identification of our isolates as *T. minioluteus* was easily achieved using each of the four genetic loci. We had very rare ambiguities in BLAST searches of the obtained four genetic marker sequences. Although ITS is the universal genetic barcode for fungi (Schoch et al. 2012), *BenA* has better resolution in species identification in *Penicillium* and *Talaromyces*, and it is proposed as a secondary molecular marker for these taxa (Visagie et al. 2014b; Yilmaz et al. 2014). Yilmaz et al. (2014) stated in their paper that amplification of *RPB2* can be very challenging, which we can confirm. We followed their protocol but could not manage to amplify *RPB2* using touch-up PCR. Modification of the protocol with a standard PCR cycle and annealing temperature of 60°C for primer pair RPB2-5F and RPB2-7CR in our case resulted in clear and satisfying sequences of *RPB2*. There were no problems in this study with amplification of the calmodulin with CMD5/CMD6 primers, contrary to the experience of Yilmaz et al. (2014). Isolates from this work were grouped together as a single distant branch in each of the four single-gene phylogenetic trees, within the same clade as other reference *T. minioluteus* isolates with high bootstrap support. Phylogenetic tree topologies obtained for all amplified loci were similar to the ones previously published for the section *Trachyspermi* (Rajeshkumar et al. 2019).

Multilocus phylogenetic analyses were performed with all four sequenced loci, and they confirmed the results of single-gene phylogenetic analyses. In the combined data set, Serbian isolates of *T. minioluteus* were placed jointly in a phylogenetic tree as a single distant branch with high bootstrap support. Reference isolates of *T. minioluteus* and the isolate of *T. minnesotensis* CBS 142381T were placed in the same clade with our isolates. Topology of the multilocus phylogenetic tree was congruent with the topology of the multigene phylogenetic tree of the section *Trachyspermi* in the work of Rajeshkumar et al. (2019). Nevertheless, more characterized isolates are necessary for thorough insight in phylogenetic relationships of this species.

Results of our research confirmed that Serbian isolates of *T. minioluteus* are pathogenic on quince, tomato, and orange fruits and onion bulbs. Pathogenicity of the isolate from potato tuber was not confirmed. Although saprobic, this isolate did not have different phenotypic and molecular characteristics in comparison with the pathogenic isolates from other hosts. A statistically significant difference ($P \leq 0.05$) has been determined in the degree of necroses on different hosts. The largest lesions were manifested on quince fruits, whereas the smallest lesions were recorded on onion bulbs. Symptoms on artificially inoculated hosts were identical to those from natural infected fruits and vegetables, except on orange fruit, for which visible surface necroses were not observed.

Pathogenicity of *T. minioluteus* (= *P. minioluteum*) on stored fruits and vegetables was only confirmed by Viñas et al. (1993) on apple. In fact, their isolates obtained from apple fruits were among the most resistant to imazalil (Viñas et al. 1993). Palou et al. (2010, 2013) isolated *T. minioluteus* (referred there as *P. minioluteum*) from pomegranate, among other fungal species. They tested the pathogenicity of *T. minioluteus* on pomegranate, but the outcome was negative. Other *Talaromyces* spp. as proven postharvest plant pathogens include recent discoveries of *T. rugulosus* on grapes (Li et al. 2019) and *T. funiculosus* on peach (Mukhtar et al. 2019). The latter two *Talaromyces* species were also reported in Serbia, on a fresh-sliced mix of ready-to-use salads (Kocić-Tanackov et al. 2010), but their pathogenicity on the hosts was not examined.

The results of our research provide valuable information and indicate the significance of *T. minioluteus*. To the best of our knowledge, this is the first report of this species in Serbia. More importantly, these are the first records of *T. minioluteus* as a postharvest plant pathogen on quince, tomato, and orange fruits and onion bulbs. Our future research should be directed at investigating control measures of this pathogen using biological and other alternative control methods.

Acknowledgments

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