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Penicillium expansum, *P. crustosum*, and *P. paneum* cause blue mold of sugar beet roots in Serbia

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SUMMARY

In this study, three *Penicillium* species, *P. expansum*, *P. crustosum*, and *P. paneum*, were identified in sugar beet roots with blue mold collected at harvest and from overwintering roots, marking a novel finding for Serbia. Notably, our study is the first to document *P. crustosum* as the causal agent of blue mold in sugar beet in the world. Pathogenicity tests on artificially inoculated sugar beet roots confirmed that all *Penicillium* isolates can induce rot, with *P. expansum* demonstrating the highest virulence, followed by *P. crustosum* and *P. paneum*. As *Penicillium* species are important postharvest pathogens linked to decay, economic losses and mycotoxin contamination, further research into their postharvest presence and impact is essential. This study presents the first analysis of *Penicillium* spp. on sugar beet in Serbia, aimed to characterize isolates both molecularly and morphologically, and to evaluate their pathogenic potential as postharvest pathogens. The results contribute to the current knowledge of *Penicillium* species capable of colonizing sugar beet roots, and expand our understanding of *Penicillium* spp. and their diversity and distribution in Serbia.

Keywords: sugar beet, blue mold, characterization, phylogeny, virulence

INTRODUCTION

The genus *Penicillium* Link comprises a diverse group of species that are widely distributed across a broad range of habitats worldwide. Many *Penicillium* species are commonly present in soil as saprobes, where their primary ecological role is decomposition of organic matter. Some species, however, are capable of infecting healthy plant tissue as wound pathogens, typically causing disease postharvest. These *Penicillium* spp. are characterized by the ability to produce pectolytic enzymes, which efficiently degrade plant cell walls and induce decay. They are also known for their abundant sporulation, ability to grow at low temperatures, and capacity to colonize various substrates, including plant products (Frisvad & Samson, 2004; Pitt & Hocking, 2009). The primary inoculum source of *Penicillium* spp. for cultivated plants is the soil, where these fungi can survive on organic debris. Increased plant susceptibility at harvest, inoculum present in soil adhering to plants, combined with wounds caused by harvest operations or other fungal infections, may provide entry points and

favor *Penicillium* infection leading to development of blue mold or Penicillium rot (Dugan & Strausbaugh, 2019; Fugate & Campbell, 2009).

In sugar beet (*Beta vulgaris* L.), which is an important industrial crop, *Penicillium* species have been frequently found to be associated with postharvest decay of roots. Liebe and Varrelmann (2016) found *Penicillium* only on stored sugar beet roots, while it was absent from freshly harvested roots. Kusstatscher et al. (2019) showed that species belonging to *Penicillium*, along with *Candida* and *Fusarium*, were the main disease indicators in the microbiome of decaying sugar beets. The reported *Penicillium* species on sugar beet include: *P. vulpinum* (Cooke & Massee) Seifert & Samson, *P. cellarum* C.A. Strausb. & Dugan, *P. cyclopium* Westling, *P. expansum* Link, *P. polonicum* K. Zaleski, *P. tulipae* Overy & Frisvad, and *P. paneum* Frisvad. Additionally, members of the closely related genus *Talaromyces* C.R. Benj. (formerly classified under *Penicillium*) reported on sugar beet include: *T. funiculosus* (Thom) Samson, Yilmaz, Frisvad & Seifert (syn. *P. funiculosum*), *T. variabilis* (Sopp) Samson, Yilmaz, Frisvad & Seifert (syn. *P. variabilis*), and *T. rugulosus* (Thom) Samson, N. Yilmaz, Frisvad & Seifert (Bugbee, 1975; Bugbee & Nielsen, 1978; Dugan & Strausbaugh, 2019; Fugate & Campbell, 2009; Liebe et al., 2016; Strausbaugh, 2018; Strausbaugh & Dugan, 2017). In addition, *P. roqueforti* Thom and *P. paneum* have been isolated from sugar beet fiber silage (Boysen et al., 2000). *P. vulpinum* (syn. *P. claviforme*) has been the most prevalent among *Penicillium* species in various regions of the USA (Bugbee, 1975; Fugate & Campbell, 2009). Recent studies have shown that *P. expansum* has emerged as the dominant species in Idaho and Japan (Strausbaugh, 2018; Uchino, 2001). The presence and prevalence of *Penicillium* species on stored sugar beet roots may be the result of environmental factors, such as temperature. For example, Liebe et al. (2016) described *P. paneum* as the dominant *Penicillium* species on roots stored at 20 °C, while Strausbaugh and Dugan (2017) observed the prevalence of *P. cellarum* on sugar beet roots stored at higher temperatures.

In Serbia, several *Penicillium* species that cause blue mold on pome fruits (apple, pear, quince and medlar), lemon, mandarin, nectarine and tomato, as well as on onion and garlic bulbs, have been described (Duduk et al., 2017, 2021; Stošić et al., 2021a, 2021b, 2025; Vico et al., 2014; Žebeljan et al., 2021a; Živković et al., 2021), yet there is no data available on their presence on sugar beet. The aim of this study was to identify and characterize *Penicillium* isolates obtained from sugar beet at harvest, as well as from overwintering roots, both molecularly and morphologically, according to Visagie et al. (2014). Additionally, in view of the fact that *Penicillium* species have been documented as postharvest pathogens, the pathogenic potential of the isolates was evaluated on sugar beet.

MATERIAL AND METHODS

Sample collection and isolation

Sugar beet roots with blue mold symptoms were collected in November 2020 and February 2021 from a field at Rimski Šančevi, Novi Sad, Serbia. Fungal isolation was performed by placing fragments of decayed sugar beet tissue on Potato Dextrose Agar (PDA) in Petri plates. Following incubation at room temperature for five days, the plates were examined and fragments of fungal colonies were transferred to sterile PDA to obtain pure cultures. Single spore cultures were obtained and preserved as conidial suspensions in 30% glycerol, 0.05% agar, and 0.05% Tween 20 (Sigma-Aldrich, USA) at -80 °C.

Molecular identification and characterization

DNA extraction was performed from 7-day-old cultures of 15 obtained *Penicillium* spp. isolates grown on PDA using the CTAB protocol of Day and Shattock (1997). Using Bt2a/Bt2b primers, amplification of partial β tubulin region (*BenA*) was performed for all isolates (Glass & Donaldson, 1995). In nine selected isolates, nuclear ribosomal internal transcribed spacer region (ITS), partial gene for calmodulin (*CaM*), and RNA polymerase II second largest subunit (*RPB2*) were amplified using ITS1/ITS4, CMD5/CMD6, and 5F/7CR primers, respectively (Hong et al., 2006; Liu et al., 1999; Visagie et al., 2014; White et al., 1990). PCR reaction mix (25 µl) contained 1 µl of template DNA, 1xPCR Master Mix (Thermo Scientific, Vilnius, Lithuania) and 0.4 µM of each primer. Samples lacking DNA were used as negative controls. *BenA* and *CaM* amplifications were performed under the following conditions: initial denaturation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 45 s, 55 °C for 45 s and 72 °C for 60 s, and final elongation at 72 °C for 7 min. Conditions for amplification of the ITS region were as follows: initial denaturation at 95 °C for 2 min, followed by 35 cycles at 95 °C for 30 s, 55 °C for 30 s and 72 °C for 60 s, and final elongation at 72 °C for 10 min. Conditions for *RPB2* amplification were as follows:

initial denaturation at 94 °C for 5 min, followed by 5 cycles at 94 °C for 45 s, 50 °C for 45 s and 72 °C for 60 s, then 5 cycles at 94 °C for 45 s, 52 °C for 45 s and 72 °C for 60 s and 30 cycles at 94 °C for 45 s, 55 °C for 45 s and 72 °C for 60 s. The final elongation occurred for 7 min at 72 °C (Visagie et al. 2014). PCR products (5 µl) were analyzed on a 1.5% agarose gel, which was stained in ethidium bromide and visualized with a UV transilluminator. Amplified products were purified and sequenced in both directions with the primers applied in amplification. Sequences were assembled using Pregap4 from the Staden program package (Staden et al. 2000), manually inspected and compared to reference sequences available in the NCBI GenBank databases, then deposited in GenBank.

Multilocus sequence analysis and phylogeny

The obtained fungal DNA sequences were compared with those publicly available using BLAST (http://www.ncbi.nlm.nih.gov/). Related sequences and those of the closest species were retrieved from GenBank and aligned with sequences obtained in this study using ClustalX (Thompson et al., 1997), under MEGA version X (Kumar et al., 2018). Evolutionary history was inferred based on individual and combined analyses of four loci (ITS, *BenA*, *CaM*, and *RPB2*) of nine isolates from this study, reference isolates, and *P. expansum* CBS 325.48 or *P. paneum* CBS 101032 as outgroup for the two datasets, using the Maximum Likelihood (ML) method (MEGA X). The best nucleotide substitution model for ML was determined using the "find best model" option in MEGA X. Initial tree(s) for the heuristic search were obtained automatically by applying neighbor-joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach, and then selecting the topology with superior log likelihood value. To estimate the statistical significance of the inferred clades, 1,000 bootstraps were performed.

Morphological characterization

Colony morphology (appearance, presence of exudate, reverse color) and growth of eight selected *Penicillium* spp. isolates were analyzed on Malt Extract Agar (MEA), Czapek Yeast Extract Agar (CYA), and Yeast Extract Sucrose Agar (YES) according to Visagie et al. (2014), and additionally on PDA. Plates were inoculated with 1 µl of spore suspension (10^6 conidia/ml) of each isolate at three points, and three plates were used per medium. All inoculated plates were incubated in the dark at 24±2 °C. The morphology of conidiophores and conidia was evaluated from 7-10 day-old-cultures grown on MEA at 24±2 °C (Frisvad & Samson, 2004; Visagie et al., 2014), using the compound microscope Zeiss Axio Lab. Photographs of conidiophores and conidia were obtained using: Axiocam ERc 5s, Zeiss and conidia diameters were measured using ZEN 2 (blue edition) software.

Ehrlich test

The isolates were examined for the production of cyclopiazonic acid and other alkaloids according to Lund (1995). Filter paper (20 x 20 mm) was immersed in Ehrlich's reagent (2 g of 4-dimethylaminobenzaldehyde in 85 ml of 96% ethanol, with 15 ml of 10 N HCl) and placed on top of the mycelial side of agar plugs (three plugs per isolate, 8 mm in diameter) from 7-day-old cultures grown on CYA at 24±2 °C. After incubation for 2 to 10 minutes, color change was recorded. A positive reaction was indicated by the appearance of a violet ring, signifying the presence of cyclopiazonic acid or related alkaloids, while a pink, red or yellow ring indicated the production of other alkaloids (Frisvad & Samson, 2004).

Pathogenicity and virulence test

Fully developed healthy sugar beet roots were washed and surface-sanitized with 70% ethanol, then cut crosswise into 2 cm wide slices, sanitized with 70% ethanol and dried. Two holes per slice were made with the point of a finishing nail (10 mm deep and 3 mm in diameter), and inoculated by pipetting 40 µl of spore suspension into each hole. Seven-day-old cultures grown on PDA were used to prepare conidial suspensions (~10^6 spores/ml) in sterile distilled water with Tween 20 (0.05%). Four slices were used per isolate and control. Control slices were inoculated with 40 µl of Tween-treated sterile distilled water. Inoculated and control slices were placed in plastic containers and incubated under high humidity, at 24±2 °C in natural light/dark cycles. After 7 days of incubation, symptoms were recorded and lesion diameters measured. Fungal re-isolation was performed from lesions that developed on inoculated slices.

Statistical analysis

One-way ANOVA was used to determine whether there were significant differences in colony diameters among isolates of the same species, among different

species and/or among culture media, as well as to determine the difference in lesion size among isolates and species in the pathogenicity test. Post hoc Tukey's HSD test was used to evaluate differences that occurred in testing multiple groups. Statistical significance for all tests was defined at $p<0.05$. The statistical analyses were conducted with STATGRAPHICS (Centurion StatPoint 2005).

RESULTS

Penicillium identification and phylogeny

A total of 15 *Penicillium* isolates were obtained from sugar beet roots: five from samples collected at harvest in November 2020, and 10 from samples collected from overwintering roots in February 2021 (Table 1). *BenA* amplicons of the expected size (~500 bp) were obtained from all isolates, yielding 405-416 nt long sequences. Based on multiple sequence comparison and BLAST analyses of generated *BenA* gene sequences, three species were identified from sugar beet roots: *P. expansum* (seven isolates), *P. crustosum* Thom (three isolates), and *P. paneum* (five isolates). The *BenA* sequences of all *P. paneum* and *P. crustosum* isolates were identical to AY674387 of *P. paneum* ex-type CBS 101032 and AY674353 of *P. crustosum* ex-type CBS 115503, respectively, whereas *BenA* sequences of *P. expansum* isolates were 99-100% similar to AY674400 of *P. expansum* ex-type CBS 325.48 (four isolates were identical, and three isolates differed by 2 nt) (Visagie et al. 2014).

ITS, *CaM*, and *RPB2* amplicons of expected sizes (~600, 580, 1000 bp, respectively) were obtained from nine selected *Penicillium* isolates, resulting in sequences 508-528 nt long for ITS, 435-480 nt long for *CaM*, and 918-1056 nt long for *RPB2*. The obtained sequences were deposited in NCBI GenBank under accession numbers provided in Table 1. The multilocus phylogeny confirmed the assignment of *Penicillium* isolates obtained in this study to *P. expansum*, *P. crustosum* and *P. paneum,* which formed well-supported clusters (Figure 1 a, b). Intraspecific variability within all three species was observed. Three well-supported subclades were formed within *P. expansum* and two within *P. paneum*, while separation within *P. crustosum* clade was supported with lower bootstrap values.

Table 1. *Penicillium* spp. from sugar beet roots.

Species	Isolate	Date of isolation	Accession numbers			
			BenA	ITS	*CaM*	*RPB2*
P. expansum	PSR464	February 2021	PQ614149	–	–	–
	PSR465/2*	February 2021	PQ614140	PQ606579	PQ614155	–
	PSR466/3P*	February 2021	PQ614141	PQ606580	PQ614156	PQ614164
	PSR467	February 2021	PQ614150	–	–	–
	PSR468/5*	February 2021	PQ614142	PQ606581	PQ614157	PQ614165
	PSR469	February 2021	PQ614151	–	–	–
	PSR471/8*	February 2021	PQ614143	PQ606582	PQ614158	PQ614166
P. crustosum	PSR1748*	November 2020	PQ614145	PQ606587	PQ614160	PQ614168
	PSR400/1z*	November 2020	PQ614144	PQ606586	PQ614159	PQ614167
	PSR470	February 2021	PQ614152	–	–	–
P. paneum	PSR401/1i*	November 2020	PQ614146	PQ606591	PQ614161	–
	PSR402	November 2020	PQ614147	PQ606592	PQ614162	–
	PSR403/3*	November 2020	PQ614148	PQ606593	PQ614163	PQ614169
	PSR463	February 2021	PQ614153	–	–	–
	PSR472	February 2021	PQ614154	–	–	–

Legend: *Isolates used for morphological characterization

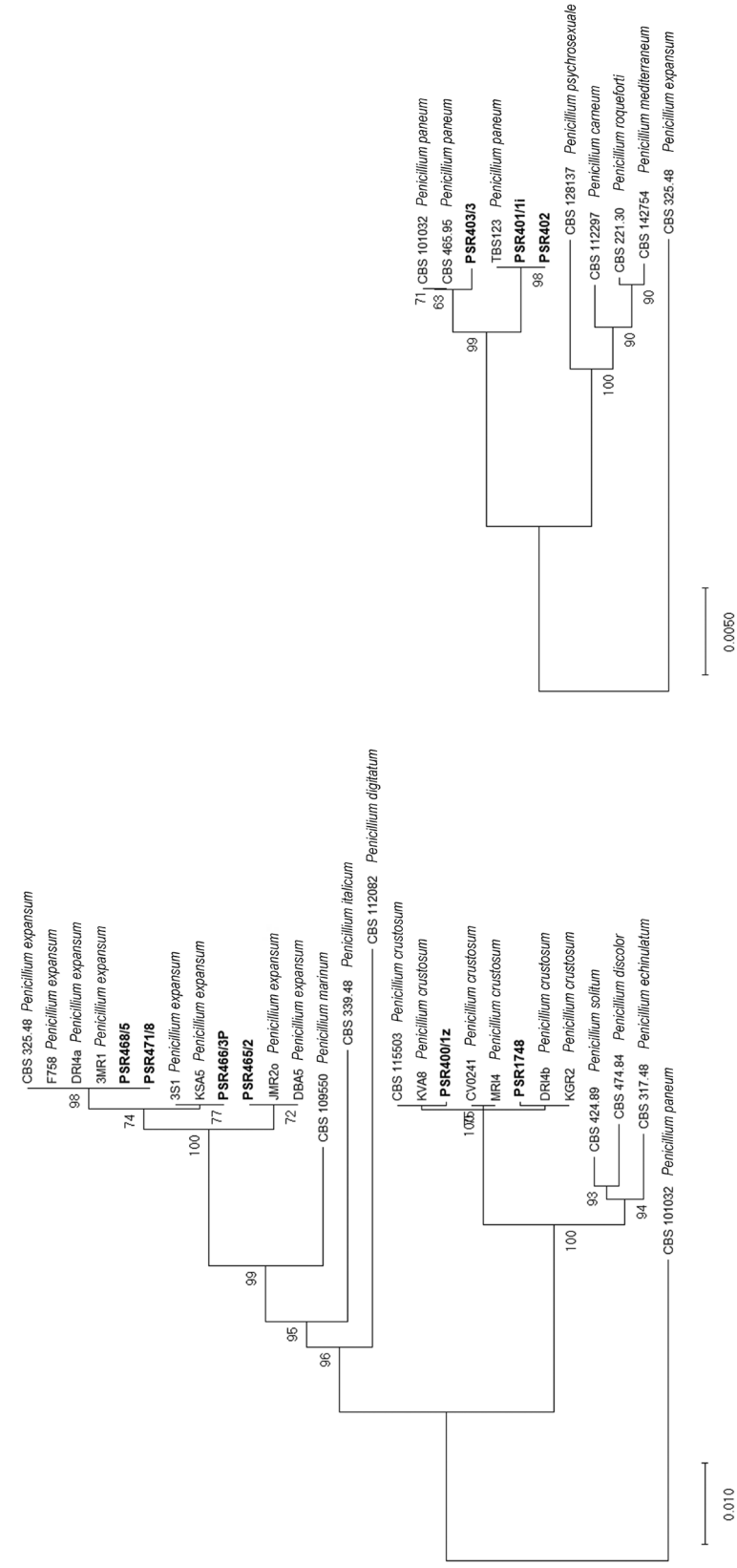

Figure 1. Phylogenetic relationships between *Penicillium expansum* and *P. crustosum* (a), and *P. paneum* (b), based on analysis of concatenated ITS, *BenA*, *CaM*, and *RPB2* sequences; inferred using the Maximum Likelihood method under the K2 + G model (a) and T92 model (b). The tree is rooted to *P. paneum* (a) and *P. expansum* (b). Numbers on the branches represent bootstrap values above 60% obtained from 1,000 replicates. Isolates obtained in this study are shown in bold. Scales show substitutions per site.

Colony characteristics and micromorphology

Penicillium expansum isolates formed blue-green to green colonies with white margins on all media. On PDA, colonies were mostly fasciculate, with or without concentric zones, and radially sulcate. Exudate was clear (PSR468/5 and PSR471/8) or yellow (PSR465/2 and PSR466/3P). Colony reverse was cream to pale yellow (PSR468/5 and PSR471/8) or intense yellow with a brown ring around the centre (PSR465/2 and PSR466/3P). On MEA, *P. expansum* formed fasciculate colonies, some with concentric zones and radially sulcate. Abundant clear exudate droplets were present in all colonies. Colony reverse was cream (PSR468/5 and PSR471/8) or yellow with a light brown ring (PSR465/2 and PSR466/3P). On CYA, colonies were concentrically fasciculate and weakly radially sulcate, rarely velutinous, and exudate was absent. Colony reverse was salmon pink (PSR468/5 and PSR471/8) or salmon cream (PSR465/2 and PSR466/3P). On YES, colonies were mostly fasciculate, radially sulcate, and exudate was clear to yellow. Colony reverse was pale yellow to intense yellow. Isolates formed terverticillata condiophores with smooth walled stipes. Conidia were blue-green in color, globose (mean diam 3.23±0.37 µm), with smooth walls, and formed in chains on ampulliform phialides (Figure 2; Table 2).

Penicillium crustosum isolates formed dull green colonies with white margins on all media. Colonies on PDA and MEA were velutinous to slightly fasciculate and radially sulcate, and became crustose after 10 days. Clear exudate was present. Colony reverse was yellow. On CYA, *P. crustosum* formed velutinous colonies with clear exudate. Colony reverse was

Table 2. Conidial dimensions and colony diameters of *Penicillium expansum*, *P. crustosum*, and *P. paneum* isolates on different media, 7 dpi, in the dark at 24±2 °C

Isolate	Conidia (µm ± SD, n=50)	Colony diameter on different culture media (mm ± SD)			
		PDA	MEA	CYA	YES
Penicillium expansum					
PSR465/2	3.18±0.34	18.83±0.49a*	20.76±0.54b	45±0.82a	35.94±1.25a
PSR466/3P	3.45±0.28	21.36±0.49b	19.83±0.51a	46.11±0.78ab	44.31±3.31c
PSR468/5	3.11±0.40	27.18±0.40c	29.10±0.57c	46.73±0.90b	42.37±4.19bc
PSR471/8	3.18±0.33	27.27±0.47c	29.22±0.44c	47.11±0.78b	40.44±3.78b
Mean	**3.23±0.37**	**22.42±3.55a****	**23.22±4.19a**	**46.45±1.02a**	**40.69±4.48a**
Penicillium crustosum					
PSR1748	3.08±0.23	34.23±0.97b	28.53±0.82b	44.00±1.48a	41.89±1.47a
PSR400/1z	3.06±0.31	30.27±1.05a	26.03±0.89a	44.33±0.52a	40.54±3.14a
Mean	**3.07±0.27**	**32.25±2,22b**	**27.28±1.51b**	**44.11±1.20a**	**41.30±2.4a**
Penicillium paneum					
PSR401/1i	3.92±0.18	45.33±2.72a	45.37±1.99a	41.54±0.88a	59.40±2.16b
PSR403/3	3.82±0.18	53.8±1.81b	52.93±1.68b	61.82±1.08b	47.14±8.11a
Mean	**3.88±0.19**	**49.57±4.80c**	**49.15±4.16c**	**50.83±10.15b**	**53.48±8.39b**

* Numbers labelled with different letters represent means of colony diameters that are significantly different among isolates of the same species according to Tukey's HSD test ($p<0.05$).

** Bolded numbers labelled with different letters represent means of colony diameters that are significantly different among species according to Tukey's HSD test ($p<0.05$).

cream yellow. On YES, colonies were fasciculate, radially sulcate and dense. Exudate was absent. Colony reverse was yellow to intense yellow. Isolates formed terverticillata conidiophores with rough walled stipes. Conidia were dull green to blue-green, globose (mean diam 3.07±0.27 µm), with smooth walls, and formed in chains on ampulliform phialides (Figure 3, Table 2).

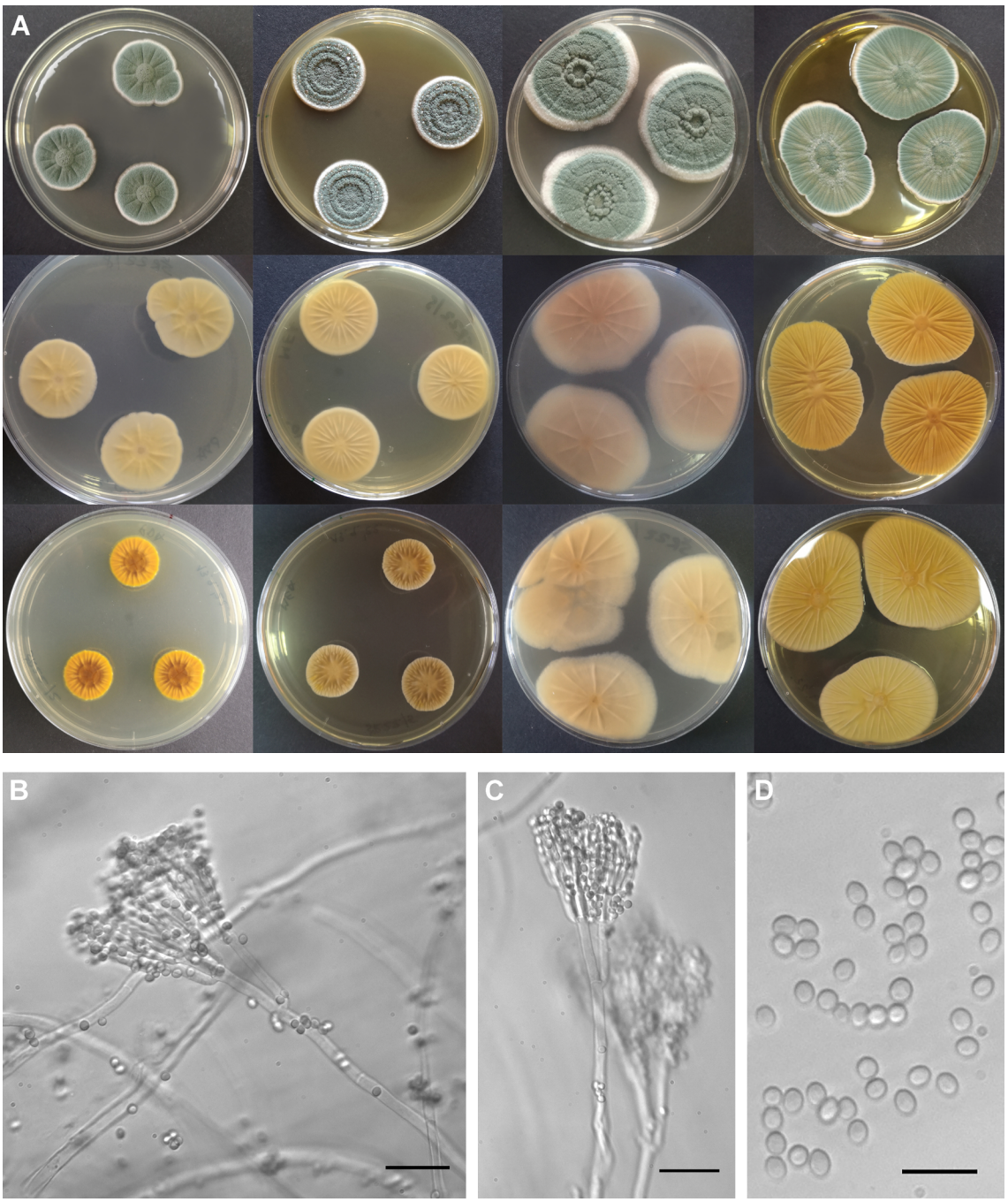

Figure 2. *Penicillium expansum*. A. Colonies on PDA, MEA, CYA and YES (left to right): obverse (top row) and reverse (middle row) of isolate PSR471/8; reverse of isolate PSR466/3P (bottom row). B, C. Conidiophores (Scale bars = 20 µm). D. Conidia (Scale bar = 10 µm).

Penicillium paneum formed blue-green to green colonies with olive brown centre on CYA and YES. On PDA and MEA, colonies were velutinous, slightly radially sulcate with white margins, with clear exudate droplets present. Colony reverse was cream. On CYA, colonies were velutinous with white margins. Yellow to brown exudate was present, and reverse was cream. On YES, colonies were radially sulcate and dense, and exudate was absent. Colony reverse was cream orange. The isolates formed terverticillata and, occasionally, biverticillata conidiophores with very rough walled stipes. Conidia were blue-green to green, globose (mean diam 3.88±0.19 µm), with smooth walls, and formed in chains on ampulliform phialides (Figure 4, Table 2).

Colony growth and Ehrlich test

Colony growth of *P. expansum*, *P. crustosum,* and *P. paneum* on four media is presented in Table 2. *P. paneum* had the fastest colony growth on all media ($p<0.05$). YES was the most favourable growth media for

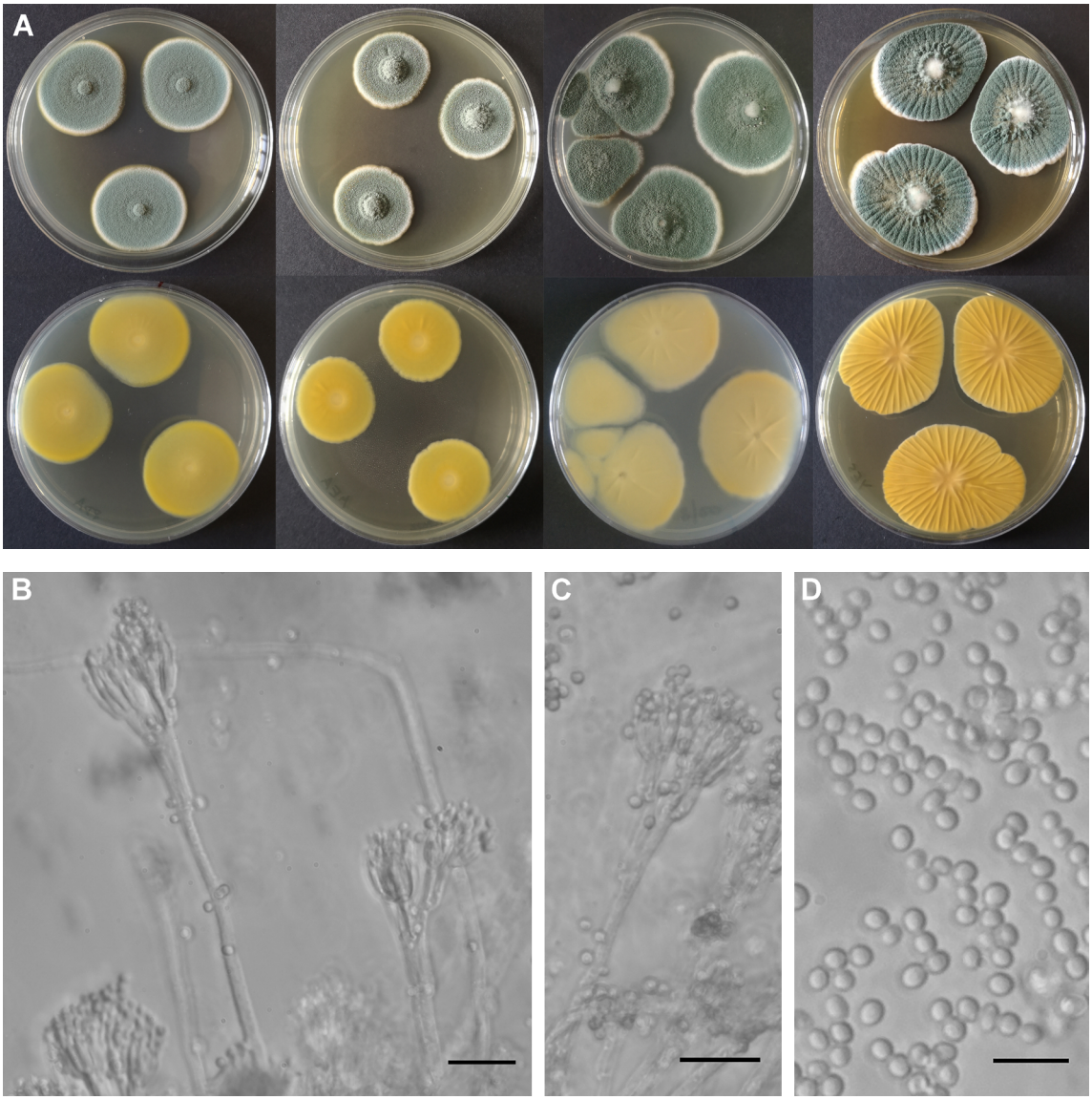

Figure 3. *Penicillium crustosum*. A. Colonies on PDA, MEA, CYA and YES (left to right): obverse (top row) and reverse (bottom row) of isolate PSR1748. B, C. Conidiophores (Scale bars = 20 µm). D. Conidia (Scale bar = 10 µm).

P. paneum, and CYA for *P. expansum* and *P. crustosum*. On PDA and MEA, *P. crustosum* growth was faster than that of *P. expansum* ($p<0.05$), while the growth of these two species on CYA and YES was comparable. Variability in colony growth was observed among isolates of *P. expansum* and *P. paneum* on all media ($p<0.05$), but it was found only on PDA and MEA among *P. crustosum* isolates ($p<0.05$). *P. expansum* isolates, which differed in reverse color on PDA, MEA, and CYA, also differed in colony growth on these media (PSR468/5 and PSR471/8 had faster growth than PSR465/2 and PSR466/3P).

In the Ehrlich test, *P. crustosum* and *P. expansum* isolates formed a yellow ring, while no color change was

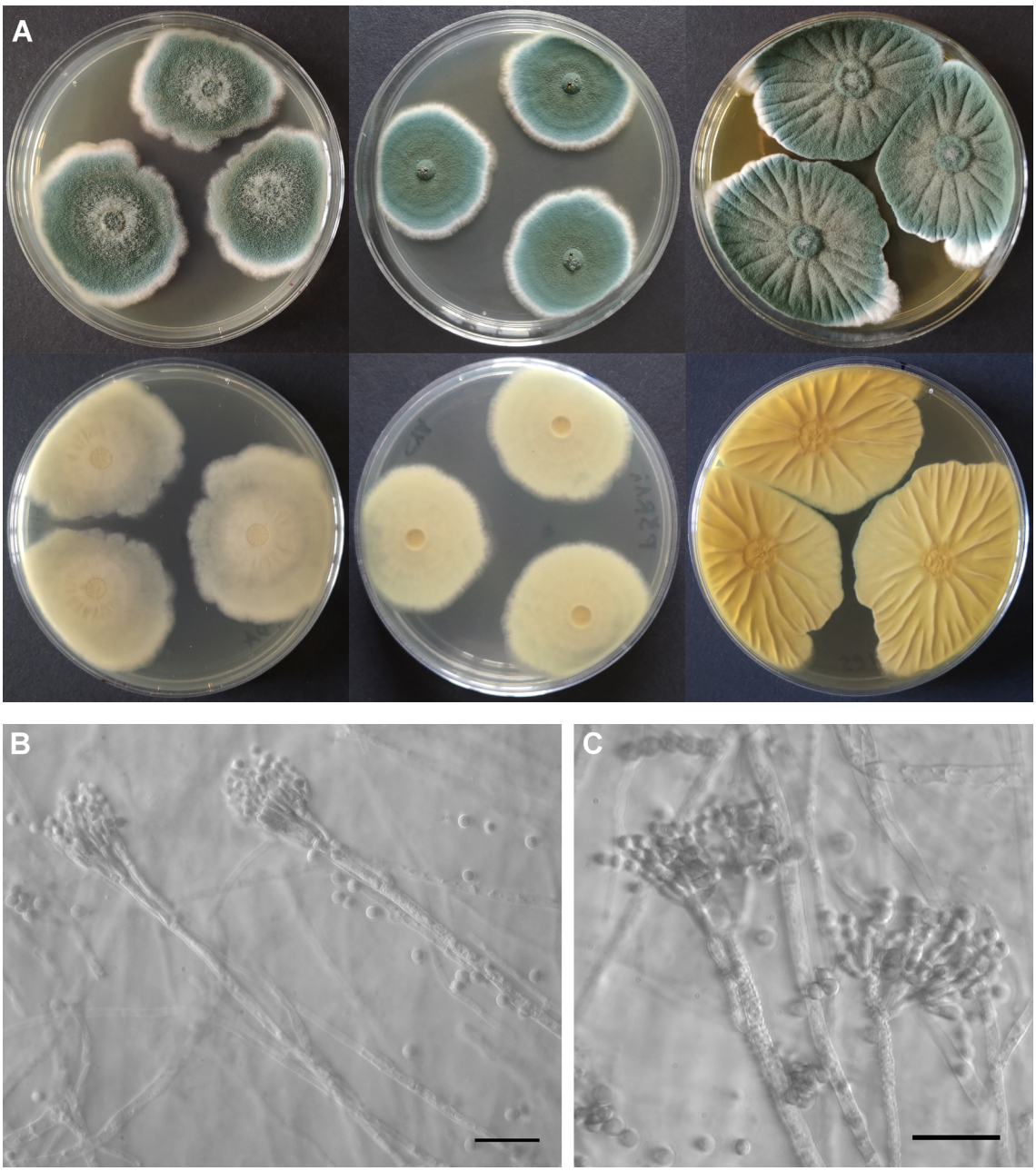

Figure 4. *Penicillium paneum*. A. Colonies on MEA, CYA and YES (left to right): obverse (top row) and reverse (bottom row) of isolate PSR403/3. B, C. Conidiophores and conidia (Scale bar = 20 μm).

observed in *P. paneum* isolates. The observed reactions indicated that *P. crustosum* and *P. expansum* isolates did not produce cyclopiazonic acid, but produced other alkaloids.

Pathogenicity and virulence of *Penicillium expansum*, *P. crustosum* and *P. paneum* on sugar beet

All tested isolates induced light brown rot on inoculated sugar beet slices, with sporulation mainly present at the inoculation site, confirming their pathogenicity. Control slices remained symptomless. Fungi reisolated from inoculated sugar beet slices exhibited identical morphological characteristics as the original isolates. The most virulent *Penicillium* species was *P. expansum* (average lesion size 20.8 mm), followed by moderately virulent *P. crustosum* (14.06 mm), and less virulent *P. paneum* (10 mm) ($p<0.05$). Considering isolates of the same species, differences in virulence were observed within *P. crustosum* (isolate PSR1748 was more virulent than PSR400/1z and PSR470), while no difference in virulence was noted within *P. paneum* and *P. expansum* (with the exception of PSR469) (Figure 5).

DISCUSSION

In this study, *P. expansum*, *P. crustosum*, and *P. paneum* were identified on sugar beet roots grown in Serbia. Two species, *P. crustosum* and *P. paneum*, were isolated at harvest, while *P. expansum* was dominant on overwintering sugar beet roots, followed by *P. crustosum* and *P. paneum*. The presence of these three species on sugar beet is a novel finding for Serbia, while *P. crustosum* had not been previously reported in scientific literature on sugar beet.

Penicillium expansum (section *Penicillium*) is the most prevalent and economically significant causal agent of blue mold in stored apples and pears globally (Jurick & Cox, 2017: Rosenberger, 2014). This species has also been isolated from a wide variety of other fruits, as well as vegetables, meat, cheese, grains, apple juice, dry fish, etc (Pitt & Hocking, 2009). *Penicillium expansum* has also been reported as a postharvest pathogen of sugar beet (Strausbaugh, 2018; Uchino, 2001). In this study, *P. expansum*, a well-documented pome fruit pathogen in Serbia (Žebeljan et al., 2021a), was isolated from overwintering sugar beet roots, revealing sugar beet as a novel host of this species in Serbia.

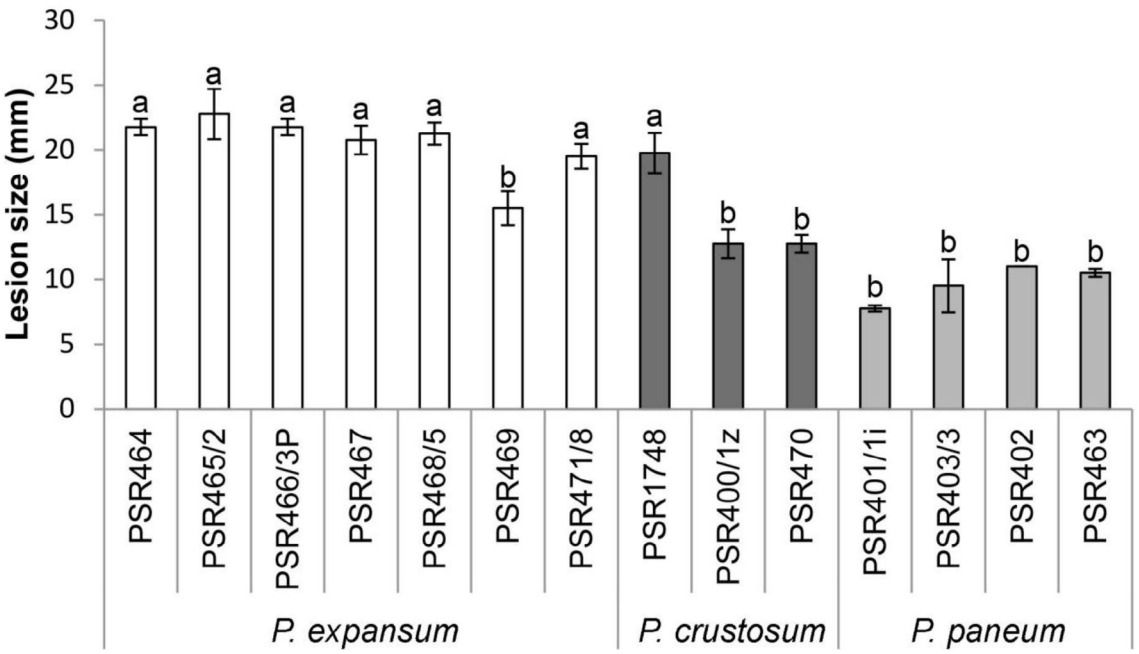

Figure 5. Virulence of *Penicillium expansum*, *P. crustosum*, and *P. paneum* on sugar beet slices. Numbers labelled with different letters represent means of lesion size that are significantly different among isolates according to Tukey's HSD test ($p<0.05$). Vertical bars represent standard errors of the mean.

The second identified species, *P. crustosum* (section *Fasciculata*), is a ubiquitous spoilage fungus, which has been isolated from cereal and animal feed samples such as maize, processed meats, cheese, biscuits, cakes and fruit juices (Pitt & Hocking, 2009). *Penicillium crustosum* has also been frequently isolated worldwide from pome fruits with blue mold (Andersen & Thrane, 2006; Louw & Korsten, 2014; Sholberg & Haag, 1996; Žebeljan et al., 2021a). In this study, *P. crustosum* was isolated from sugar beet roots collected at harvest, as well as from overwintering sugar beet roots, which represents the first report of this *Penicillium* species on sugar beet worldwide.

The morphological characteristics and growth rates of *P. expansum* and *P. crustosum* isolates from sugar beet were consistent with previously published descriptions for each species (Frisvad & Samson, 2004; Pitt & Hocking, 2009). Interestingly, when compared to previously characterized *P. expansum* and *P. crustosum* isolates from pome fruits from Serbia (Žebeljan et al., 2021a), similarities were observed in the presence of subgroups within *P. expansum* isolates from sugar beet based on their morphology (colony reverse on PDA, MEA and CYA), growth and phylogeny, as well as within *P. crustosum* based on *RPB2* locus and virulence. These results confirmed the morpho-genetic diversity within *P. expansum* and *P. crustosum*, opening opportunities for further studies of their populations across various hosts in Serbia.

Penicillium paneum (section *Roquefortorum*), known to occur on baled grass silage, has been reported as a significant contaminant of cereal grains, and has also been described as a pathogen of apple fruit (Boysen et al., 2000; Frisvad & Samson, 2004; O'Brien et al. 2008; Yin et al. 2017). Previously reported in Serbia on grape marc (Jovicic-Petrovic et al. 2016), *P. paneum* has now been identified as the causal agent of blue mold of sugar beet. In this study, we observed that *P. paneum* is clearly distinguishable from the other two *Penicillium* species by its larger conidia and more rapid growth, all consistent with the species description (An et al. 2009; Frisvad & Samson, 2004; O'Brien et al., 2008). Described as a postharvest pathogen of sugar beet, *P. paneum* dominates under storage conditions at 20°C (Liebe et al. 2016).

Penicillium is the most diverse genus of soil fungi (Visagie et al., 2014). Some species are opportunistic plant pathogens that colonize plants at the end of the growing season, affecting senescent plants or their parts, and are therefore often encountered postharvest. To assess the pathogenic potential of *Penicillium* isolates obtained at harvest or from overwintering sugar beet roots, we conducted artificial inoculation tests on sugar beet under conditions that maximally favor pathogenicity. Our results have shown that *P. expansum* isolates were the most virulent, which is consistent with previous findings (Strausbaugh, 2018). In comparison, *P. crustosum* displayed moderate virulence, while *P. paneum* showed lower virulence. Overall, the higher virulence of *P. expansum* compared to other *Penicillium* species, such as *P. crustosum,* was also observed on other plant species, such as apples (Louw & Korsten, 2014; Sanderson & Spotts, 1995; Žebeljan et al., 2021a). Research on the *Penicillium*-apple pathosystem has shown that *P. expansum* induces more intense and dynamic metabolic changes than *P. crustosum*, aiding the pathogen to overcome host defenses. This results in significantly reduced levels of phenolics and glutathione during *P. expansum*-mediated decay, compared to *P. crustosum*-mediated decay (Žebeljan et al. 2019, 2021b), leading to higher virulence of *P. expansum* and thus faster decay of the infected apple fruit.

Sugar beet roots stored in piles are vulnerable to *Penicillium* infection. Prolonged storage periods under higher temperatures can increase the likelihood of infection events. *Penicillium* species are characterized by abundant sporulation, which enhances their dispersal and survival, serving as an evolutionary strategy that allows their persistence in agricultural soils and on crop residues. In this study, the most virulent species, *P. expansum*, as well as *P. crustosum* and *P. paneum*, were isolated from overwintering sugar beet, indicating that inoculum persists in the roots throughout the winter. Although *Penicillium* species are generally less virulent than other postharvest pathogens of sugar beet, such as *Phoma betae* or *Botrytis cinerea*, their abundant sporulation can produce substantial airborne inoculum, potentially leading to destructive blue mold under prolonged or improper storage conditions for sugar beet (Fugate & Campbell, 2009). While *P. crustosum* is less virulent than *P. expansum*, it demonstrates a significant capacity to produce large quantities of conidia (Pitt & Hocking, 2009). The occurrence and persistence of less virulent *Penicillium* species, such as *P. crustosum*, may therefore be supported by the ability to sporulate abundantly (Žebeljan et al., 2021a).

Colonization by *Penicillium* species can negatively impact sugar beet by causing root decay, significantly reducing sucrose content, and introducing the risk of mycotoxin contamination (Fugate & Campbell, 2009). Though this study has identified three *Penicillium* species colonizing sugar beet, further detailed research is needed to determine their prevalence and impact

both as postharvest pathogens of sugar beet roots or as contaminants of sugar beet pulp silage in Serbia. However, based on their presence and pathogenic potential, these species are the likely contributors to postharvest losses in sugar beet. Additionally, the three species identified on sugar beet, *P. expansum*, *P. crustosum*, and *P. paneum*, are toxigenic. *P. expansum* is the primary producer of patulin, and also produces citrinin, roquefortine C and other mycotoxins; *P. crustosum* produces penitrem A, roquefortine C and other mycotoxins, while *P. paneum* produces roquefortine C and patulin (Perrone & Susca, 2017). The production of cyclopiazonic acid, a mycotoxin, is used for *Penicillium* spp. characterization (Frisvad & Samson, 2004; Visagie et al., 2014). Although the production of this extrolite is typically associated with *P. expansum* and *P. crustosum*, the Ehrlich test performed in this study showed that *P. expansum* and *P. crustosum* isolates from sugar beet did not produce cyclopiazonic acid, suggesting a variability in production across different substrates. However, other alkaloids were produced. Nevertheless, colonization by toxigenic species is particularly concerning if the infected roots are used as livestock feed. Although *P. paneum* has been detected in hard-pressed beet fibers, the impact on contamination remains unconfirmed (Boysen et al., 2000). Boudra et al. (2015) found low mycotoxin levels in sugar beet pulp, with roquefortine C detected in only a few samples and no detectable patulin. These findings suggest that further studies under diverse conditions and involving a broader range of mycotoxins are needed to verify the low-risk mycotoxin status of sugar beet pulp silage.

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Penicillium expansum, *P. crustosum* i *P. paneum* prouzrokovači plave truleži šećerne repe u Srbiji

REZIME

U ovom istraživanju kao prouzrokovači plave truleži korena šećerne repe identifikovane su tri vrste iz roda *Penicillium: P. expansum*, *P. crustosum* i *P. paneum*, što predstavlja novi nalaz za Srbiju. Osim toga, naročito je značajno da je *P. crustosum* prvi put opisan kao prouzrokovač plave truleži korena šećerne repe u svetu. Izolati *Penicillium* spp. iz korenova šećerne repe prikupljenih u toku vađenja i iz prezimelih korenova molekularno i morfološki su okarakterisani i procenjen je njihov potencijal kao postžetvenih patogena. Test patogenosti na veštački inokulisanim korenovima potvrdili su da sve izolovane *Penicillium* spp. mogu izazvati trulež korena šećerne repe, pri čemu je najvirulentnija vrsta bila *P. expansum*, a zatim *P. crustosum* i *P. paneum*. Pošto su vrste roda *Penicillium* značajni postžetveni patogeni koji mogu dovesti do ekonomskih gubitaka i kontaminacije mikotoksinima, dalja istraživanja njihovog prisustva i uticaja nakon vađenja repe, u toku čuvanja, su od suštinskog značaja. Dobijeni rezultati doprinose znanju o vrstama roda *Penicillium* koje mogu kolonizovati koren šećerne repe i proširuju naše razumevanje raznovrsnosti vrsta ovog roda u Srbiji.

Ključne reči: šećerna repa, plava trulež, karakterizacija, filogenija, virulentnost