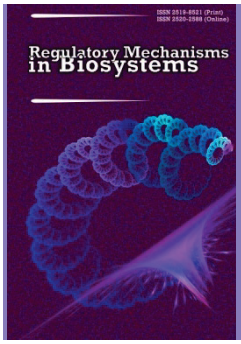

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.,
2022, 13(2), 130–136
doi: 10.15421/022218

Endophytic community of *Chaenomeles speciosa* fruits: Screening for biodiversity and antifungal activity

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Article info

Received 06.04.2022
Received in revised form 03.05.2022
Accepted 04.05.2022

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Lykholat, Y. V., Didur, O. O., Drehval, O. A., Khromykh, N. O., Sklyar, T. V., Lykholat, T. Y., Liashenko, O. V., & Kovalenko, I. M. (2022). Endophytic community of Chaenomeles speciosa fruits: Screening for biodiversity and antifungal activity. Regulatory Mechanisms in Biosystems, 13(2), 130–136. doi:10.15421/022218

Fruit crops of the genus *Chaenomeles* Lindl are considered today as a superfood due to accumulation of biologically active compounds with antioxidant ability and known health-promoting properties. Successful introduction of this non-traditional culture in the steppe zone of Ukraine characterised by an unfavourable climate suggests the functioning of effective protective mechanisms in plants, including those that can be provided by the influence of endophytic microorganisms. However, there is little information about the endophytic community of *Chaenomeles* plants. Herein, the current study was aimed to isolate the endophytic fungi from the *Ch. speciosa* fruits and evaluate their biological activities against the phytopathogens. The study was carried out based on the collection of the Botanical Garden of Oles Honchar Dnipro National University (Dnipro city, Ukraine). Three media, namely PDA, MPA, and Gause's medium were used for isolation of endophytic fungi. Colonies of isolates for identification were grown on PDA, Czapek's agar, and Czapek's yeast autolysate media. Six fungal endophytic isolates derived from both peel and pulp of *Ch. speciosa* fruits have been morphologically identified using macroscopic and microscopic techniques, and assigned to the genus *Penicillium* (sections *Chrysogena*, *Penicillium*, *Viridicata*), and genus *Talaromyces* (section *Talaromyces*). Species *P. expansum*, *P. viridicatum*, and *P. hirsutum* were identified among the peel isolates, while *P. chrysogenum*, *P. cyclopium*, and *P. purpurogenum* were among the pulp isolates. Antagonistic ability of the endophytic isolates against phytopathogenic fungi was evaluated using the dual culture method. The results showed moderate to high antifungal capacity of the endophytic isolates against the phytopathogenic strains of the *Fusarium* genus. The growth inhibition of *F. culmorum* mycelium due to the influence of endophytic isolates was 51.5–81.3%, and the inhibition of the growth of *F. oxysporum* colonies was in the range of 68.4–86.6% as compared with control. There were no significant differences in the antagonistic ability between endophytic isolates derived from the peel and pulp of the fruit. Taken together, our findings indicated the great potential of the endophytic fungi from *Ch. speciosa* fruits as a source for the development of biocontrol agents and discovery of new bioactive compounds.

Keywords: flowering quince fruits; endophytes; fungal isolates; antagonistic activity; penicilli; phytopathogens; growth inhibition.

Introduction

Endophytes are symbiotic microorganisms that are found in roots and stems (Lu et al., 2012), branches (Chen et al., 2019), leaves (Fan et al., 2020), fruits (Paul et al., 2012), flowers (Martins et al., 2021), and bark (Khan et al., 2016) of different plant species. Endophytes colonize inner plant tissues without causing symptoms of disease (Uzma et al., 2018; Baron & Rigobelo, 2021), while playing a positive role in plant growth promotion and enhanced resistance against stresses (Javed et al., 2019; Alam et al., 2021). The mechanisms of endophytic fungi influence can be varied, including enhancing nutrient solubilization in the plant rhizosphere (Mehta et al., 2019), promotion of plant growth (Poveda et al., 2021), acting as the biological control agents (Poveda & Baptista, 2021), or activating plant systemic resistances to biotic (Poveda et al., 2020) and abiotic (Cui et al., 2021) stresses. Endophytic fungi are not only novel sources of cytotoxic compounds, such as the anticarcinogenic molecules (Lykholat et al., 2016; Uzma et al., 2018) and antibacterial substances (Radic & Strukelj, 2012), but also the bio stimulants for essential oil biosynthesis (El Enshasy et al., 2019).

Recently, encouraging results have been obtained from the study of endophytic microorganisms in the context of sustainable agriculture and effective management of crops. We are talking about the olive tree (Nicoletti et al., 2020), citrus fruits (Tran et al., 2019), rice (Nayak et al., 2021), Cavendish bananas (Beltran-Garcia et al., 2021), and red pepper (Roy Choudhury et al., 2021). In this regard, one of the possible ways of environmentally safe control of phytopathogens is biological preparations, which are based on the endophytic fungi or bacteria, or their metabolites, as an alternative to chemical pesticides (Khan et al., 2018; Beltran-Garcia et al., 2021; Chen et al., 2022). Such drugs exhibit complex biological activity against pathogens (Zazharskyi et al., 2019, 2020), and are able to stay inside plants for a long time. These properties allow endophytic preparations to have a prolonged beneficial effect on plants and avoid competition from native microorganisms.

Little-studied potential sources of endophytic microbiota are fruit crops with high biological activity and antioxidant properties. Among such plants are the species of genus *Chaenomeles* Lindl. (subfamily Amygdaloideae, family Rosaceae), which were introduced into Europe in 1869 and domesticated in the northern countries as a fruit crop (Kikowska et al., 2019). The main active compounds of the *Chaenomeles* fruits are triterpenes and procyanidins, polyphenols, organic acids, alcohols, ketones, aldehydes, and vitamin C. In general, these plants are an important resource in traditional medicine due to their ability to improve human

health (Miao et al., 2018). The *Ch. speciosa* fruits are rich in diverse phytochemicals that bear strong anticancer, antioxidant, antiviral, antibacterial, anti-inflammation, antihyperlipidemic, anti-Parkinson effects (Huang et al., 2018), antihyperglycemic properties (Deng et al., 2020), and prevention of atherogenesis (Tang et al., 2010).

In the steppe zone of Ukraine, in a region with unfavourable climate and industrial pollution, several *Chaenomeles* species have been successfully introduced and bear fruit, accumulating a high level of the phenolic compounds and showing greater resistance to the common pathogens as compared to other fruit plants (Lykholat et al., 2019). Similarly, Ni et al. (2021) concluded low sensitivity of *Ch. sinensis* foliage to pathogens as only local cases of leaf spot disease caused by *Colletotrichum gloeosporioides* occurred. The relatively low level of pathogenic damage to *Chaenomeles* species may suggest that endophytic microorganisms have a protective function, though this requires confirmation. However, there is little information about the endophytic community of *Chaenomeles* plants, except for the great diversity of *Ch. speciosa* mycorrhizal fungi (Zhu et al., 2009). We have previously reported high antagonistic activity of the endophytic isolates from *Ch. speciosa* fruits against several common phytopathogens, as well as high antimicrobial ability of *Ch. cathayensis* and *Ch.* × *californica* fruit extracts against bacterial and fungal strains (Lykholat et al., 2021). The aim of this study was to discover and identify the diversity of endophytic fungi isolated from *Ch. speciosa* fruits, as well as to assess their antagonistic potential against common phytopathogens.

Material and methods

Fruits of the *Ch. speciosa* (Sweet) Nakai plants were taken in the first half of September 2021 from the Botanical Garden of Oles Honchar Dnipro National University (48°26′07" N, 35°02′34" E, Dnipro city, Ukraine). Here, plants of several *Chaenomeles* species have grown and borne fruit for about 25 years in a steppe climate with low precipitation (473 mm average, but 265 mm in dry years) and sharp temperature changes. Ripe *Ch. speciosa* fruits were collected, packed in plastic containers and taken to the laboratory immediately. The samples were washed with warm water, sterilized by sequentially immersion in 70% ethanol for 30 s and 4% sodium hypochlorite solution for 30 s, rinsed with sterile distilled water, and finally fired in a burner.

Fruit peel and pulp portions (about 2.0 g each) were taken with a sterile scalpel and ground in a sterile mortar by pestle with the addition of 10 mL of sodium chloride solution (0.5%). Aliquots (1 mL) of each suspension were inoculated on three different media, namely Potato Dextrose Agar (PDA), Meat Peptone Agar (MPA), and Gause's medium in sterilized Petri plates, following which the plates were kept at 28.0 ± 0.2 °C for 6 days. Then, the Petri plates were examined visually, and hyphae from distinct colonies were inoculated in the new Petri plates with PDA and incubated until colonies were formed. After incubation, pure cultures were transferred to PDA slants and stored at 4 ºC until they were used for further investigations. Colonies of endophytic isolates for subsequent identification were grown on PDA medium, Czapek's agar medium, and Czapek's yeast autolysate medium.

All fungal isolates were subjected to a preliminary morphological identification to the genus level following general taxonomic guides (Frisvad & Samson, 2004). Macroscopic and microscopic techniques were the base for the identification of endophytic microorganisms for the following characters: colony growth and texture, obverse and reverse colony colour, diffusible pigments and exudate production and microscopic characteristics (hyphae), with the help of taxonomic guides to the genus *Penicillium* (Frisvad & Samson, 2004) and genus *Talaromyces* (Yilmaz et al., 2014). Micrography of the slides prepared from a small portion of fungal mycelium and stained with methylene blue was carried out at 40x magnification using an Ulab XY-B2T LED microscope equipped with a trinocular attachment for connecting a digital camera-eyepiece.

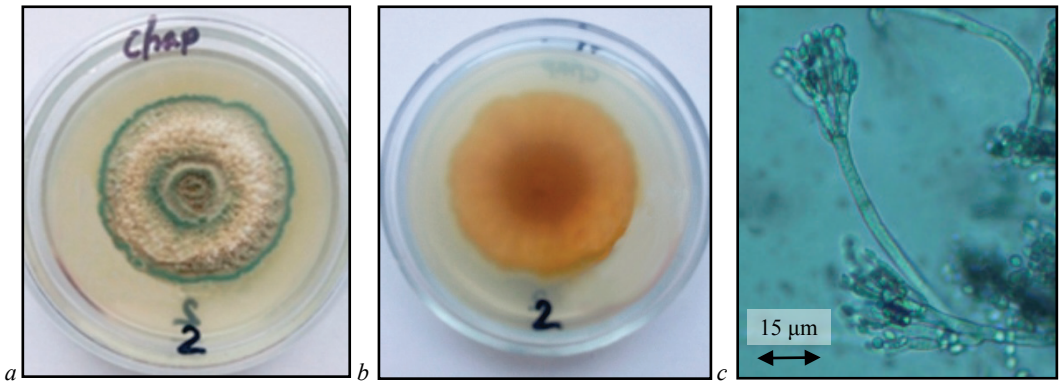
Antagonistic ability of the fungal isolates was evaluated by using the dual culture method (Mishra et al., 2017) against phytopathogenic fungi *Fusarium oxysporum* (strain IMB-F-54201) and *F. culmorum* (strain IMB-F-50716), both provided by the Microbiology and Virology Institute named after Zabolotny, Kyiv, Ukraine. Mycelium discs (7 mm in diameter) of actively growing 6-day-old cultures of pathogens and endophytic isolates were placed in Petri plates with PDA medium at an equal distance from the periphery; control plates were inoculated with pathogenic fungus only. Plates were incubated at 28.0 ± 0.2 °C for six days, and antagonistic ability of isolates was measured as the percent inhibition (PI %) based on the equation $PI\ \% = [(S_1 - S_2)/S_1] \times 100$, where, S_1 is the pathogen control colony area; S_2 is the pathogen colony area in presence of endophytic isolate.

All bioassays were carried out in quintuple replication. The data obtained were expressed as the mean ± standard deviation, and the differences between means were tested with Tukey's HSD. All differences were considered statistically significant at $P < 0.05$.

Results

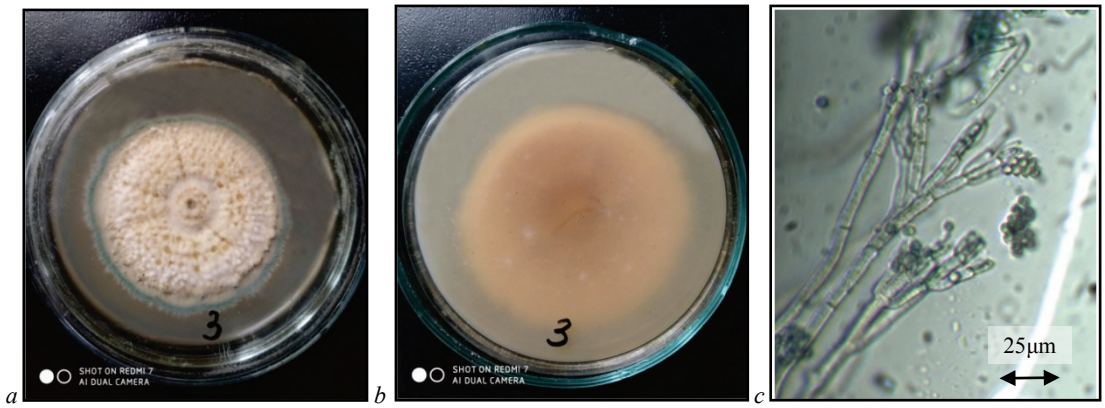
On the basis of tentative identification, most groups of endophytic fungi isolated from *Ch. speciosa* fruits were attributed to *Penicillium* spp. (6 isolates, both from peel and pulp). They were labeled isolate 2, isolate 3, isolate 4, isolate 13, isolate 14, and isolate 17, followed by identification using macroscopic and microscopic techniques.

Isolate 2 (Derived from the fruit peel). Colonies on PDA medium were white at the beginning of growth, later became green with a white marginal zone up to 2 mm wide (Fig. 1). On Czapek's agar medium, fast growing colonies (4.5–5.0 cm) were grey-green with a yellow tinge and with a grey-green marginal zone; reverse was light orange-brown. On Czapek's yeast autolysate medium, colonies 3.0–3.5 cm in diameter were radially striated, mealy texture, grey-green with a white marginal zone; reverse was yellow-brown. The conidiophores were terverticillate, 125 × 4.0 µm, rough when the conidia matured; rami 45 × 3.5 µm; metulae 12 × 3.5 µm. The phialides were bottle-shaped, 8.5 × 2.5 µm, tapering towards the apex into short distinct collulum; conidia were elliptical, 3.7–4.0 µm, or almost spherical, 2.5–3.0 µm in diameter, with a smooth shell. The above set of features made it possible to attribute the endophytic isolate 2 to *Penicillium expansum* Link (subgenus *Penicillium* section *Penicillium* series *Expansa*).



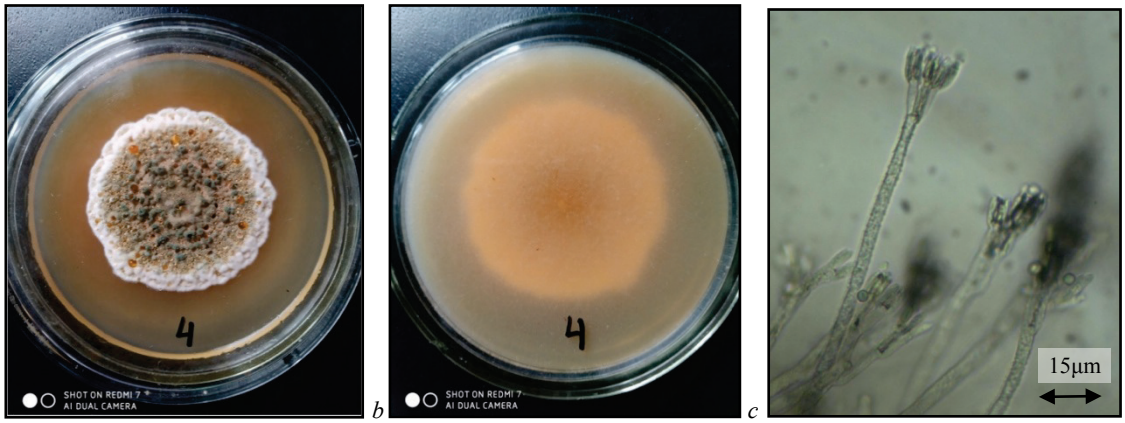
a *b* *c*

Fig. 1. Morphological identification of endophytic fungal strains of *Penicillium expansum* Link from *Ch. speciosa* fruits peel: *a* – surface of culture on CZA medium, *b* – reverse colour of culture, *c* – conidiophore and conidia under the light microscope



a b c

Fig. 2. Morphological identification of endophytic fungal strains of *Penicillium viridicatum* Westling from *Ch. speciosa* fruits peel: *a* – surface of culture on CZA medium, *b* – reverse colour of culture, *c* – conidiophore and conidia under the light microscope



a b c

Fig. 3. Morphological identification of endophytic fungal strains of *Penicillium hirsutum* Dierckx from *Ch. speciosa* fruits peel: *a* – surface of culture on CZA medium, *b* – reverse colour of culture, *c* – conidiophore and conidia under the light microscope

Isolate 3 (Derived from the fruit peel). On Czapek's medium, fast-growing colonies (4.5–5.0 cm) were yellowish-green with a bluish-green marginal zone, had abundant exudate with numerous light yellow drops (Fig. 2). The reverse was light brown, with aging the colonies darkened, the medium around the colonies turned brown. On Czapek's yeast autolysate medium, the colonies were radially folded, grey-green with a brown tinge, 2.0–3.5 cm in diameter; reverse was yellow or orange-brown. Conidiophores were asymmetric biverticillate, 161.4 × 4.78 µm, rough, with one or more rami extending from the central axis, metulae 10.77 × 4.16 µm. The phialides were ampullo-shaped, 11.8 × 3.0 µm; conidia elliptical, 3.73–2.83 µm or spherical, 2.91–3.58 µm in diameter, with a smooth shell. The above set of features made it possible to attribute the fungal isolate 3 to *Penicillium viridicatum* Westling (subgenus *Penicillium* section *Viridicata*).

Isolate 4 (Derived from the fruit peel). On Czapek's medium, colonies are fast growing (4.5–5.0 cm), velutinous to fasciculate, beige with a white marginal zone, later green; exudate is abundant, in numerous drops from orange to dark brown, after evaporation of which crater-like traces remain; reverse yellow-brown with a pink tint (Fig. 3). On Czapek's yeast autolysate medium, colonies (2.5–4.0 cm) were velutinous, radiate-folded, and grey-green with a white marginal zone; exudate was abundant, light yellow, reverse yellow-brown. Conidiophores were biverticillate, with one ramus extending from the central axis, 162.62 × 3.9 µm, rough; conidia and phialides were smooth. Tassels were asymmetrical; ramus cylindrical, 16–27 × 3.2–4.0 µm; metulae slightly widened towards apex 10.92 × 3.02 µm, in whorls of 4–6 pieces; sterigmata 9.99 × 2.28 µm, numerous, nearly parallel, in whorls of 6–10; conidia were spherical, 3.83 µm in diameter. The above set of features made it possible to attribute the fungal isolate 4 to *Penicillium hirsutum* Dierckx (subgenus *Penicillium* section *Viridicata* series *Corymbifera*).

Isolate 13 (Derived from the fruit pulp). On Czapek's medium, colonies were 4.0–5.0 cm in diameter, velutinous, slightly downy, and radially striated (Fig. 4). The conidial zone was bluish-green with a sterile area of vegetative yellowish mycelium in the center. Exudate was abundant, in numerous drops of light yellow colour; reverse orange tint. On Czapek's yeast autolysate medium, colonies were 3.0–3.5 cm in diameter, fast growing, velutinous, slightly downy, and radially striated; conidial zone of bluish-grey-green hues, with a growing white edge. Exudate was abundant, light lemon in colour; reverse was light yellow, the medium around the colony is coloured light yellow. Conidiophores 160–210 × 3.0–3.6 µm, with a smooth shell, asymmetric biverticillate, with one or more rami extending from the main axis, 15–25 × 3.0–3.5 µm. Metulae were 12–16 × 2.5–3.5 µm, 2–5 per whorl; phialides 8.0–10.0 × 2.0–2.5 µm, narrowed towards the apex, 4–6 in compact whorls; conidia almost spherical, 3.0–3.6 × 2.8–3.5 µm, smooth. The above set of features made it possible to attribute the fungal isolate 13 to *Penicillium chrysogenum* Thom (subgenus *Penicillium* section *Chrysogena* series *Chrysogena*).

Isolate 14 (Derived from the fruit pulp). Colonies on Czapek's medium were velutinous, grey-blue with a white marginal zone, fast growing, up to 3.5 cm in diameter; reverse was yellow (Fig. 5). On Czapek yeast autolysate, colonies (3.0–3.5 cm) were velutinous, grey-bluish green with a white marginal zone; reverse light yellow; exudate was in small drops of light yellow colour. Conidiophores 165.98 × 4.78 µm were smooth, biverticillate, with one or more rami, and terverticillate. Metulae were 13.46 × 3.84 µm; sterigmata lanceolate, 6.9–7.8 × 2.53 µm; conidia were spherical, 2.46–3.53 µm in diameter, with a smooth shell. The above set of features made it possible to attribute the fungal isolate 14 to *Penicillium cyclopium* Westling (subgenus *Penicillium* section *Viridicata* series *Viridicata*).

Isolate 17 (Derived from the fruit pulp). On Czapek's medium, colonies (1.5–3.0 cm in diameter) were fast-growing, at first light yellow, with the formation of conidia, yellowish-grey-green with a white marginal zone, velutinous, abundantly spore-bearing; reverse was red with a cinnabar-red border, the medium around the colonies was red (Fig. 6). On Czapek's yeast autolysate medium, colonies were up to 3.0 cm in diameter, uniform, velutinous, dirty green in the center, near the edge of a greenish-blue colour with a yellowish edge; reverse dark red; exudate in

small few drops. Conidiophores 146.2 × 3.71 µm were smooth, biverticillate, symmetrical, and compact. Metulae were 10.90 × 2.66 µm, 3–4 per whorl; phialides 13.35 × 2.20 µm; conidia elliptical, 2.89 × 2.16 µm, or spherical, 2.12 µm in diameter, with a smooth shell. The above set of features made it possible to attribute the fungal isolate 17 to *Penicillium purpurogenum* Stoll. However, in accordance with modern taxonomy, this fungal species is classified as *Talaromyces purpurogenus* (Stoll) (genus *Talaromyces* section *Talaromyces*).

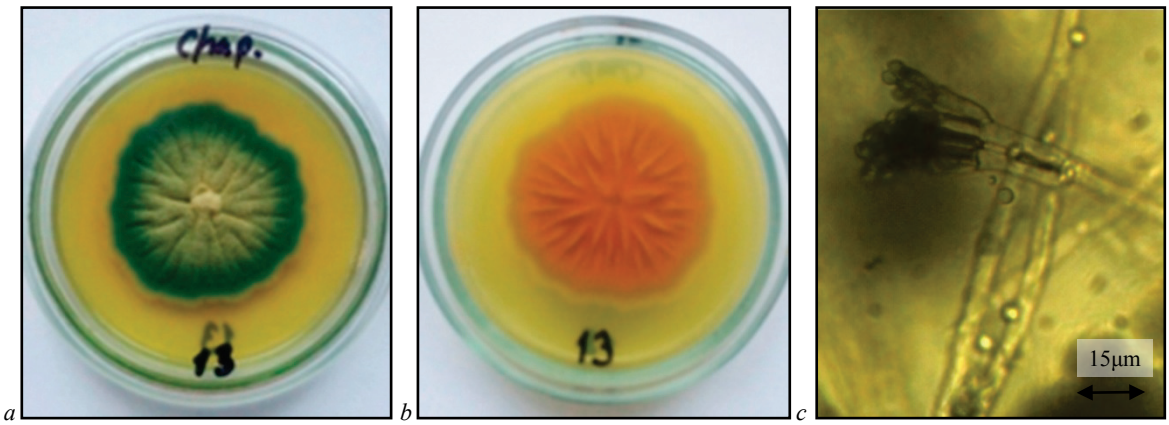


Fig. 4. Morphological identification of endophytic fungal strains of *Penicillium chrysogenum* Thom from *Ch. speciosa* fruits pulp: *a* – surface of culture on CZA medium, *b* – reverse colour of culture, *c* – conidiophore and conidia under the light microscope

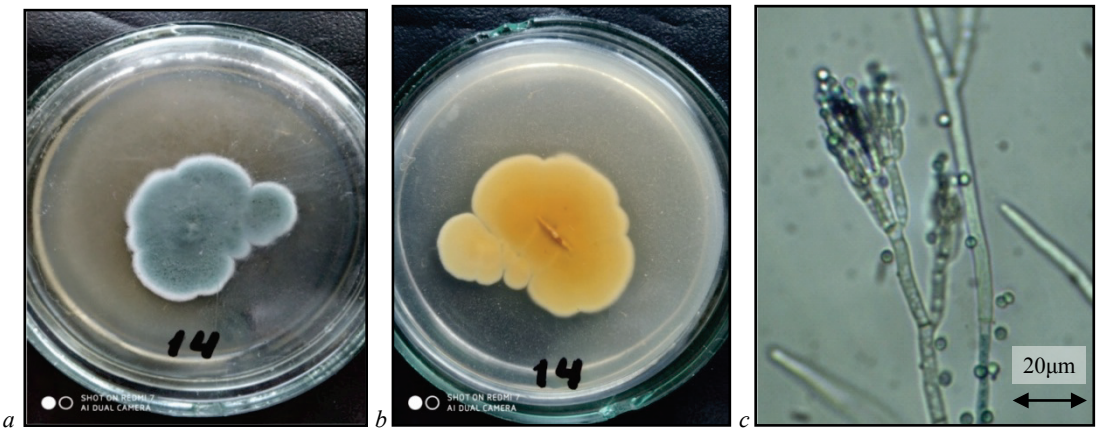


Fig. 5. Morphological identification of endophytic fungal strains of *Penicillium cyclopium* Westling from *Ch. speciosa* fruits pulp: *a* – surface of culture on CZA medium, *b* – reverse colour of culture, *c* – conidiophore and conidia under the light microscope

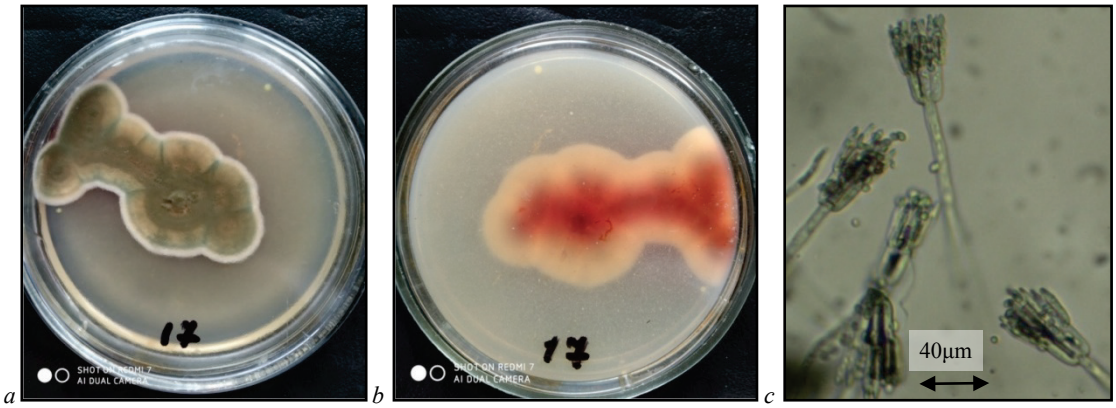


Fig. 6. Morphological identification of endophytic fungal strains of *Penicillium purpurogenum* Stoll from *Ch. speciosa* fruits pulp: *a* – surface of culture on CZA medium, *b* – reverse colour of culture, *c* – conidiophore and conidia under the light microscope

According to the results of dual culture assay, endophytic isolates from *Ch. speciosa* peel and pulp exhibited moderate to high antagonistic activity against two different phytopathogenic strains (Table 1).

Generally, the tested strain of *F. culmorum* was more resistant to the antagonistic action of most endophytic isolates as compared to *F. oxysporum* strain. Fungal endophytic isolate *P. cyclopium* caused the greatest inhibition of *F. culmorum* colony growth, exceeding the effect of other isolates by 1.2–1.6 times (Fig. 7). As for the *F. oxysporum* strain, the highest inhibition of colony growth was found due to the action of *P. purpurogenum* and *P. hirsutum*, while *P. cyclopium* antagonistic activity was the lowest. In total, endophytic isolate *P. chrysogenum* showed the least antagonistic activity against the tested phytopathogenic strains.

Discussion

Endophytic fungi belonging to the very diverse and cosmopolitan genus *Penicillium* have a wide range of existence within plants, including roots, stems and leaves (Perrone & Susca, 2017; Baron & Rigobelo,

2021). In this regard, our findings of the fungal endophytic isolates in the peel and pulp of *Ch. speciosa* fruits correspond to the known data. Endophytic isolates of *Ch. speciosa* fruits were derived both from peel and pulp; however, they were not the same penicilli. Species *P. expansum*, *P. viridicatum*, and *P. hirsutum* were identified among the peel isolates, while *P. chrysogenum*, *P. cyclopium*, and *P. purpurogenum* were among the pulp isolates. A similar confinement of endophytic fungi of the genus *Penicillium* to living in roots, stems and leaves was shown for the shrub *Securinega suffruticosa* (Pall.) Rehd (Du et al., 2020).

Antagonistic ability of the endophytic isolates against fungal strains of the *Fusarium* genus, tested by the dual culture method, varied relative to both isolates and pathogenic species. In general, all penicilli achieved greater inhibition of *F. oxysporum* colony growth, with the exception of *P. cyclopium*, which was more effective at inhibiting *F. culmorum* colony growth. Differences in antagonistic ability between endophytic isolates from the peel and pulp of *Ch. speciosa* fruits were not identified. In general, the antagonistic capacity of all studied species of the genus *Penicillium* against phytopathogens exceeded 50% of the growth of control colonies. Of these, *P. cyclopium*, derived from fruit pulp, achieved the greatest inhibition of *F. culmorum* mycelial growth and significantly exceeded the efficacy of other endophytic isolates. At the same time, the growth of *F. oxysporum* colonies was greatly inhibited due to the influence of two other endophytic species, namely *P. hirsutum* and *P. purpurogenum*. The level of antagonistic activity of endophytic isolates from *Ch. speciosa* fruits is comparable to or exceeds the known data on the effectiveness of endophytes. Du et al. (2020) reported colony growth inhibition of *Colletotrichum siamense* only by 3.6–26.3% under the influence of endophytic fungi from *Securinega suffruticosa* roots, stems and leaves. The endophytic fungi isolated from the tissues of *Chloranthus japonicus* exhibited good antagonistic capacities (the percent inhibition of mycelial growth ranged 47.72–88.18%) against different pathogens (An et al., 2020). Thus, antagonistic ability of the endophytic isolates derived from the peel and pulp of *Ch. speciosa* fruits and assigned to the genus *Pelicillium* can be generally assessed as high.

Table 1

Antagonistic activity of the fungal endophytic isolates of genus *Penicillium* from *Ch. speciosa* fruits (% of inhibition by dual culture assay, x ± SD, n = 5)

Identified fungal endophytic isolates	Tested fungal pathogens	
	Fusarium culmorum (IMB-F-50716)	*Fusarium oxysporum* (IMB-F-54201)
P. expansum (isolate 2)	67.30 ± 2.22^{a}	74.57 ± 0.67^{a}
P. viridicatum (isolate 3)	61.01 ± 0.61^{b}	73.82 ± 0.89^{a}
P. hirsutum (isolate 4)	51.53 ± 1.65^{c}	80.16 ± 0.67^{b}
P. chrysogenum (isolate 13)	NA	73.76 ± 0.16^{a}
P. cyclopium (isolate 14)	81.29 ± 0.19^{d}	68.39 ± 0.42^{c}
P. purpurogenum (isolate 17)	66.08 ± 0.79^{a}	86.63 ± 0.33^{d}

Note: values with different superscripts in each column are significantly different according to Tukey's test ($P < 0.05$); NA – no activity.

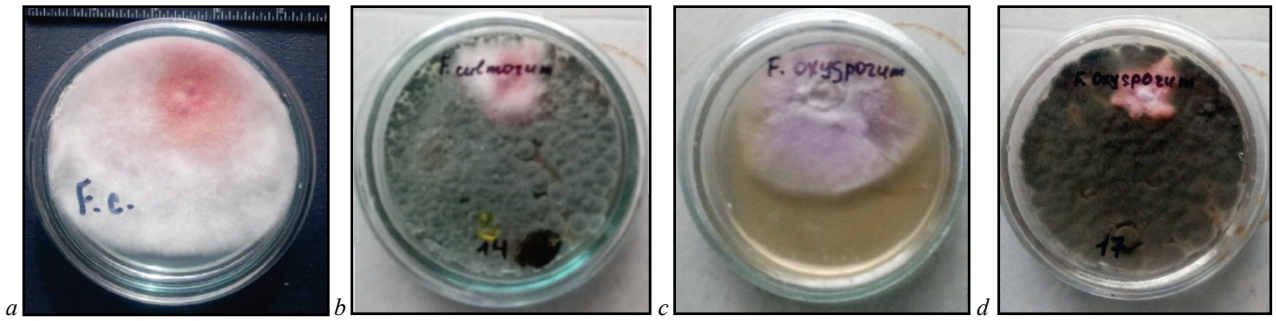


Fig. 7. Antagonistic activity of *Ch. speciosa* isolates as observed in dual culture assay on PDA medium: *a* – control (*F. culmorum*), *b* – *Penicillium cyclopium* (isolate 14) against *F. culmorum*, *c* – control (*F. oxysporum*), *d* – *Penicillium purpurogenum* (isolate 17) against *F. oxysporum*

Species of the genus *Fusarium* used as the test cultures in our study are known to be phytopathogenic organisms causing significant crop damage. Seepe et al. (2021) reported that various *Fusarium* species cause disease in many economically important crops, including cereals, potatoes, tomatoes, bananas, mangoes and others. Phytopathogenic influence of *Fusarium* species has also been noted for ornamental plants, namely orchids (Srivastava et al., 2018). Therefore, the endophytic isolates from *Ch. speciosa* fruits belonging to the genus *Penicillium* and showing significant antagonism against *Fusarium* fungi can have a potential application in the biological control of phytopathogens and in the creation of antifungal drugs.

Various aspects of the enormous biotechnological potential of genus *Penicillium* species, including those isolated by us from the peel and pulp of *Ch. speciosa* fruits, have been reported. For example, *P. chrysogenum* (isolate 13 in our work) has shown the ability to synthesize penicillin and xanthocillin X, treatment of pulp mill waste, production of polyamine oxidase, phospho-gluconate dehydrogenase, and glucose oxidase (Frisvad & Samson, 2004), and biogenesis of silver nano-nanoparticles (Abd El Aty et al., 2020). The species *P. purpurogenum* (isolate 17 in our work) has been shown as a potentially effective tool in the food industry (Zavaleta & Eyzaguirre, 2016) and production of the natural pigments (Tsiailanis et al., 2018; Pandit et al., 2020; Parul et al., 2020). We reported earlier (Lykholat et al., 2021) on the production of several compounds with known antimicrobial activity and their detection in the culture medium and mycelium of endophytic fungi from *Ch. speciosa* fruits. Of these, *P. expansum* produced (4-hexyl-2,5-dioxo-2,5-dihydro-3-furanyl)acetic acid, diisooctyl phthalate, 11-hexadecyn-1-ol, and 2,2-dihydroxymalonic acid. *Penicillium chrysogenum* produced various phthalates, eicosyl and isopropyl ethers of hexadecanoic acid, and 4-butoxy-2-butanone. The diverse endophytic penicilli with high antifungal activity identified in this study may provide a rich resource of secondary metabolites and should be investigated further.

Conclusion

In this study, the diversity and antagonistic activity of the endophytic fungi from the peel and pulp of *Ch. speciosa* fruits were investigated for the first time. Our results illustrated that the fruits of these plants harboured an abundant fungal endophytic community representing a taxonomic diversity within the genus *Penicillium*, including three sections, namely *Chrysogena*, *Penicillium*, and *Viridicata*. In addition, *P. purpurogenum*, which is currently assigned to a separate genus *Talaromyces* and called *T. purpurogenus* Stoll (section *Talaromyces*), was found in the pulp of *Ch. speciosa* fruits. Strong inhibitory activities of the fungal endophytic isolates from the peel and pulp of *Ch. speciosa* fruits against pathogenic microorganisms *Fusarium culmorum* (51.5–81.3% colony growth inhibition) and *F. oxysporum* (68.4–86.6% colony growth inhibition) were established. The study results indicate the expediency of further investigation of the antimicrobial potential of fruit-associated endophytes. Overall, our findings also indicate that the endophytic fungi from *Ch. speciosa* fruits have promising research value and may be of potential interest in screening bio-control agents and discovery of new bioactive compounds.

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