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Biocontrol Potential and Catabolic Profile of Endophytic *Diaporthe eres* Strain 1420S from *Prunus domestica* L. in Poland—A Preliminary Study

Barbara Abramczyk [1,*], Anna Marzec-Grządziel [1], Jarosław Grządziel [1], Ewa Król [2], Anna Gałązka [1] and Wiesław Oleszek [3]

[1] Department of Agricultural Microbiology, Institute of Soil Science and Plant Cultivation—State Research Institute, Czartoryskich 8, 24-100 Puławy, Poland; agrzadziel@iung.pulawy.pl (A.M.-G.); jgrzadziel@iung.pulawy.pl (J.G.); agalazka@iung.pulawy.pl (A.G.)
[2] Department of Plant Protection, University of Life Sciences in Lublin, Leszczyńskiego 7, 20-069 Lublin, Poland; ewa.krol@up.lublin.pl
[3] Department of Biochemistry and Crop Quality, Institute of Soil Science and Plant Cultivation—State Research Institute, Czartoryskich 8, 24-100 Puławy, Poland; wieslaw.oleszek@iung.pulawy.pl
* Correspondence: babramczyk@iung.pulawy.pl; Tel.: +48-81-478-6960

Abstract: Recently, *Diaporthe* has been considered the most frequently isolated genera of endophytic fungi, having a broad spectrum of host plants and a worldwide distribution. The endophytic *Diaporthe* strain used in the present work came from the Fungal Collection of Phytopathology and Mycology Subdepartment, University of Life Sciences in Lublin (Poland), and was isolated from healthy *Prunus domestica* shoots during previous studies. Due to the possibility of using the *Diaporthe* endophytes as a promising option for plant disease management, the main goal of the research was to study the antagonistic effect of endophytic *Diaporthe* strain against six phytopathogens: *Verticillium dahliae*, *Botrytis cinerea*, *Fusarium avenaceum*, *F. sprotrichioides*, *Alternaria alternata*, and *Trichothecium roseum* based on the dual culture assay and to determine the catabolic profile of the endophyte by using Biolog FF Plates. The dual-culture test assay revealed the ability of the endophytic *Diaporthe* to limit the growth of all tested pathogens. The growth inhibition percentage ranged from 20% (*V. dahliae*) to 40% (*T. roseum*). A distinct zone of inhibition occurred between the endophytic *Diaporthe* and the pathogens *T. roseum*, *V. dahliae*, and *B. cinerea* in the co-growth combinations. As for the catabolic profile results, the most intensive utilization of carbon substrates was observed after 168 h of incubation. The growth of the analyzed strain was observed on 79 media containing carbohydrates, carboxylic acids, amino acids, amines and amides, polymers, and others. The most effective decomposition was observed in the polymers group, the least in amines and amides. Molecular identification indicated that this strain was closely related to the *Diaporthe eres* species complex.

Keywords: antagonistic activity; dual culture assay; Biolog FF Plates; carbon substrate utilization; *Phomopsis*-like morph; fruit trees

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1. Introduction

In recent decades, endophytic fungi have attracted the attention of scientists around the world due to their very positive effect on the host plants, stress resistance induction, growth promotion, plant protection, and metabolite production [1–6]. Some endophytic fungi colonize plant tissues without causing disease symptoms, and they occur in a wide range of plant species and habitats [7,8]. Recently, the use of endophytes as biocontrol agents has drawn special attention as an attractive option for management of some plant diseases, resulting in minimal impact on the environment [9]. Among endophytic fungi, *Diaporthe* is one of the most frequently isolated genera [10]. It has been widely investigated and has proved to produce a broad range of valuable compounds with different bioactivities, which was recently summarized in the extensive review by Xu et al. [11].These

authors showed that the genus *Diaporthe* and its anamorph *Phomopsis* are regarded as potential sources for producing diverse bioactive metabolites. In the past ten years, a total of 335 bioactive secondary metabolites have been isolated both from known species of *Diaporthe* and *Phomopsis* and from unidentified ones. Overall, there are 106 bioactive compounds derived from *Diaporthe* and 246 from *Phomopsis*, while 17 compounds are found in both of them. They are classified into polyketides, terpenoids, steroids, macrolides, ten-membered lactones, alkaloids, flavonoids, and fatty acids.

Diaporthe Nitschke, and its asexual *Phomopsis*-like morph have been often described as plant pathogens, saprobes, and endophytes in a wide range of plant hosts [10,12–17]. For many years, their identification has been based on morphological features and host-plant associations [12]. Recently, multi-locus DNA data analysis combined with morphological characterization and host associations has been employed for species delimitation in the genus [13,15,17–29]. Although several endophytic *Diaporthe* species have been described as endophytes on woody plants worldwide [3,20,30–35], there is no available information concerning endophytic *Diaporthe* on *Prunus domestica* in Poland or worldwide.

Since endophytic *Diaporthe* species are known as a valuable source of bioactive metabolites and are likely to be promising agents in the development of a biological plant protection products or bio fertilizers, the knowledge of its nutritional requirements and antagonistic activity against phytopathogens seems crucial. Therefore, the main goal of this research was (i) to test the antagonistic activity of endophytic *Diaporthe* strain against common phytopathogens based on the dual culture assay and (ii) to determine the catabolic profile of the strain, by using Biolog FF Plates.

2. Materials and Methods

2.1. Fungal Isolation

The endophytic *Diaporthe* strain was isolated from healthy *P. domestica* shoots during previous studies conducted on fungi from fruit plants in Poland [14]. The strain was isolated following the protocol described by Król [36]. Briefly, shoot fragments were cut into small pieces, then washed under running tap water, surface-disinfected for 30 s in a sodium hypochlorite solution (10%), and washed three times for 3 min in sterile distilled water, then plated on potato dextrose agar (PDA, Difco) and incubated at 25 °C in the dark for 5 days. When colonies formed, each mycelium was transferred to a new PDA Petri dish and a number was assigned. The selected isolate differed from the others by very poor sporulation after isolation, which is often associated with endophytic colonization of host plants. Single spore cultures were obtained as described previously [36,37].

2.2. DNA Extraction, PCR Amplification, and Sequencing

DNA was extracted from the single spore mycelia growth on PDA at 25 °C for 7 days using the FastDNA®SPIN Kit and the FastPrep®Instrument (Qbiogene, Inc., Irvine, CA, USA), following the manufacturer's instructions. DNA amplification was performed in a 25 µL reaction volume containing 12.5 µL of DreamTaq™ Green PCR Master Mix (2X) (Thermo Scientific, Waltham, MA, USA), 1 µL of each primer (10 µM), 1 µL of genomic DNA (5 ng/µL), and 9.5 µL of purified water. For this research, 4 loci were amplified and sequenced: the internal transcribed spacer region (ITS) of the nuclear ribosomal RNA gene, part of the translation elongation factor 1-α gene (TEF1), part of the β-tubulin gene (TUB), and part of calmodulin gene (CAL). The ITS region was amplified using primers ITS1: TCCGTAGGTGAACCTGCGG, ITS4: TCCTCCGCTTATTGATATGC [38], Bt-2a: GGTAACCAAATCGGTGCTGCTTTC, and Bt-2b: ACCCTCAGTGTAGTGACCCTTGGC was used to amplify β-tubulin gene [39]. TEF1 was amplified with the primers EF1-728F: CATCGAGAAGTTCGAGAAGG, EF1-986R: TACTTGAAGGAACCCTTACC and calmodulin gene with CAL-228F: GAGTTCAAGGAGGCCTTCTCCC and CAL-737R: CATCTTCTGGCCATCATGG [40]. The PCR conditions were as follows: 95 °C for 3 min, followed by 39 cycles of 95 °C for 30 sec, 55 °C (ITS, CAL) or 58 °C (TEF1, TUB) for 50 sec, 72 °C for 1 min, and final extension at 72 °C for 10 min. The obtained PCR products were sequenced

at Genomed S.A. (Warsaw, Poland). The obtained DNA sequences of each region were deposited in the GenBank with the accession numbers MW664034 (ITS), OK506723 (TEF1), OK506724 (TUB), OK490500 (CAL). Bioinformatic analyses were made with the use of Bionumerics 7.6 (Applied Maths NV., Sint-Martens-Latem, Belgium) and SEED v.2.1.05 (Institute of Microbiology CAS, Prague, Czech Republic) software. The obtained sequences were compared to the NCBI GenBank.

2.3. Antagonistic Activity of Endophytic Diaporthe Strain Based on Dual Culture Assay

Antagonistic activity of endophytic *Diaporthe* strain was evaluated against common phytopathogens: *Verticillium dahliae* Kleb., *Botrytis cinerea* Pers., from the Fungal Collection of the Department of Agricultural Microbiology, Institute of Soil Science and Plant Cultivation, Pulawy, Poland, and *Fusarium avenaceum* (Fr.) Sacc., *F. sporotrichioides* Sherb., *Alternaria alternata* (Fr.) Keissl., *Trichothecium roseum* (Pers.) Link from the Fungal Collection of Phytopathology and Mycology Subdepartment, University of Life Sciences in Lublin, Poland. Endophyitic *Diaporthe* strain was tested using in vitro dual culture assays for its ability to inhibit the mycelial growth of common phytopathogens [41]. Five-milliliter plugs of endophyte and pathogen were co-cultured on PDA at the two opposite ends of the 90 mm plates, at a distance of 40 mm from each other. The plates were incubated at 25 °C for 16 days. The assay was performed in triplicate. The plates inoculated with pathogens alone served as controls. After 16 days of incubation, the radial growth of the pathogen in the presence of endophyte and the radial growth of the control colony (pathogen alone) were measured and the percentage inhibition (I%) of the average radial growth was calculated according to the following equation [41,42]:

$$I\% = [(RC - RT)/RC] \times 100 \quad (1)$$

I% = inhibition of radial mycelial growth;
RC = colony radial growth of the pathogen in control plate;
RT = colony radial growth of the pathogen in the presence of endophyte.

Data expressed as inhibition rate (%) of mycelial growth of all tested endophyte-pathogen combinations were analyzed using the GraphPad Prism 9.2.0.332 (GraphPad Holdings, San Diego, CA, USA) statistical package.

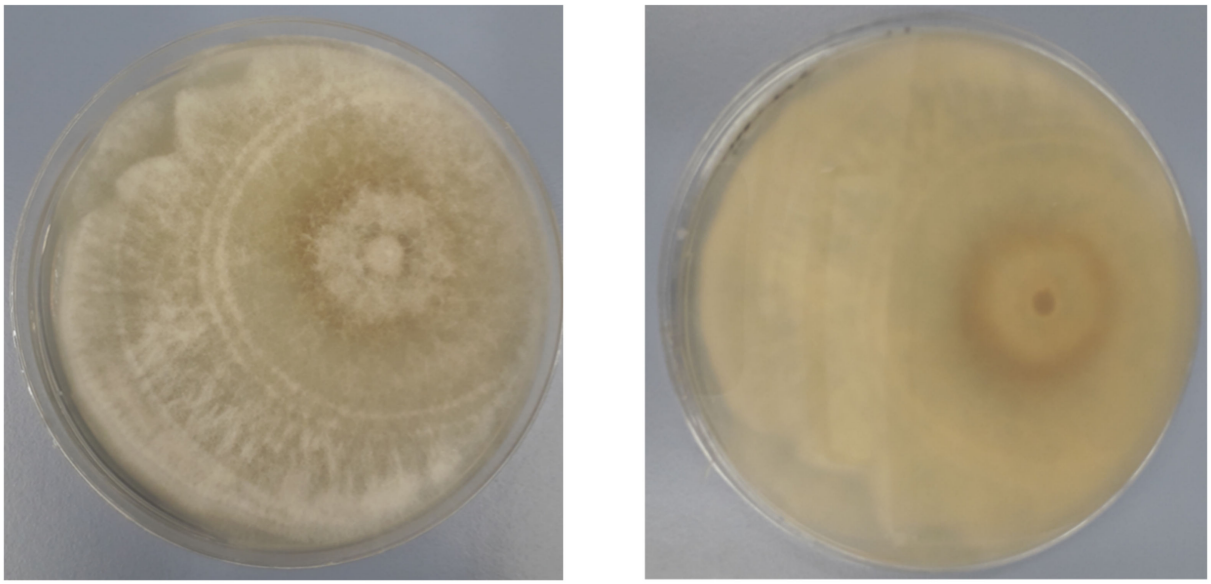
2.4. Application of Biolog FF Plates for Catabolic Profile of Diaporthe Isolate

The Biolog FF Plates (Biolog Inc., Hayward, CA, USA) containing 95 different carbon sources were used. The inoculation step was performed following the manufacturer's protocol. Mycelium from single spore cultures growth on PDA at 22 °C for 21 days was homogenized in inoculating fluid FF (IF-FF, Biolog Inc., Hayward, CA, USA), and the cell density was adjusted to 75%. Each well of the Biolog FF Plates was inoculated by 100 µL of the mycelium suspension, and the plates were incubated in an OmniLog incubator at 28 °C for 10 days. The experiment was done in three replicates. Each replicate was read every 15 min. The obtained results were analyzed using R package 4.0.3.

3. Results

3.1. Identification of Endophytic Diaporthe Strain

The endophyte grown on PDA medium at 25 °C formed a colony typical of the genus *Diaporthe* with creamy white compact mycelium forming characteristic rings with darker beige pigmentation at the center (Figure 1a,b) but sporulation of this strain was not observed.



(**a**) (**b**)

Figure 1. Fourteen-day-old culture of endophytic *Diaporthe* on PDA (**a**) avers; (**b**) reverse (photo by B. Abramczyk).

The endophyte was further identified based on ITS, TEF, TUB, and CAL genes. The obtained sequences were blasted against the nucleotide database of NCBI, and the closest related species were obtained. The endophytic *Diaporthe* strain was assigned to *D. eres* with 100% of similarity for all analyzed loci. The results of the BLASTn analysis are shown in Table 1.

Table 1. Species most closely related to endophytic *Diaporthe* based on ITS, TEF, TUB, and CAL sequences using BLASTn analysis.

LOCUS	GenBank Accession No.	Closest Related Species	Similarity [%]	Coverage [%]
ITS	MW664034	*D. eres* EU571099	100	99.8
TEF	OK506723	*D. eres* JF461475	100	100
TUB	OK506724	*D. eres* MG281207	100	100
CAL	OK490500	*D. eres* MH051294	100	100

3.2. Antagonistic Activity

The radial growth inhibition percentage (I%) was calculated for each endophyte-pathogen combination and summarized in Table 2.

Table 2. Mycelial growth inhibition percentage (I%) after 16 days of incubation.

Pathogen	Inhibition Rate (I%)
A. alternata	32.04
B. cinerea	29.20
F. avenaceum	33.40
F. sporotrichioides	27.84
T. roseum	40.68
V. dahliae	20.68

Dual culture assay revealed the inhibition percentage of endophytic *D. eres* strain against all tested plant pathogens but there was no complete inhibition of growth observed in any dual culture combination. The I% ranged between 20.68 % and 40.68%. The highest

I% was observed in combination with *T. roseum* and the lowest I% with *V. dahliae* as compared to the control (Table 2, Figure 2). Moreover, a distinct zone of inhibition occurred between the *Diaporthe* endophyte and the pathogens *T. roseum*, *V. dahliae*, and *B. cinerea* in the co-growth combinations (Figure 3a,b,d). However, in the remaining co-growth combinations, the contact between the hyphae of the pathogen and the endophyte was observed (Figure 3c,e,f).

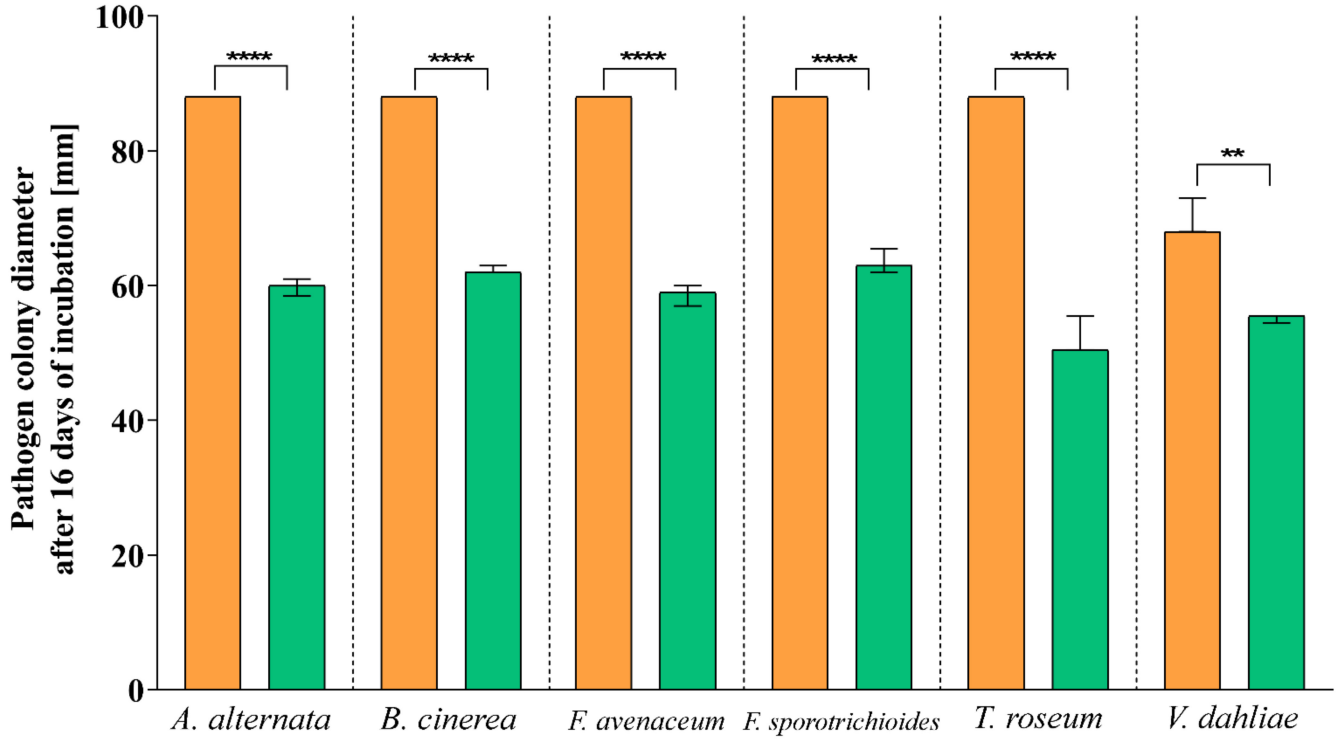
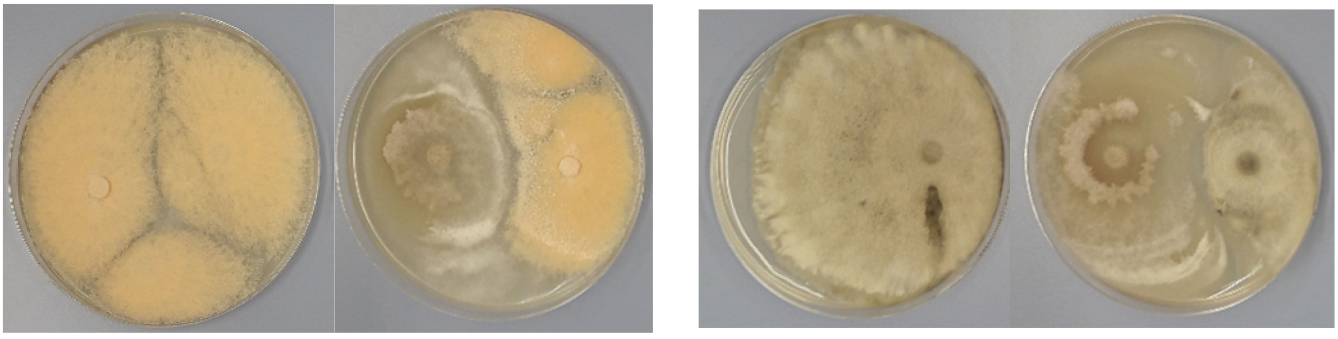


Figure 2. Diameter of the colonies of the tested pathogens after 16 days of co-growth with the endophyte. The graph presents pathogen colony diameter after 16 days of incubation in positive control (orange) and in antagonistic test plate (green). The statistical analysis was prepared with T-Student Test, $\alpha = 0.05$. Statistically significant differences are marked as: **** $p < 0.0001$, ** $p < 0.01$.



(**a**) (**b**)

Figure 3. *Cont.*

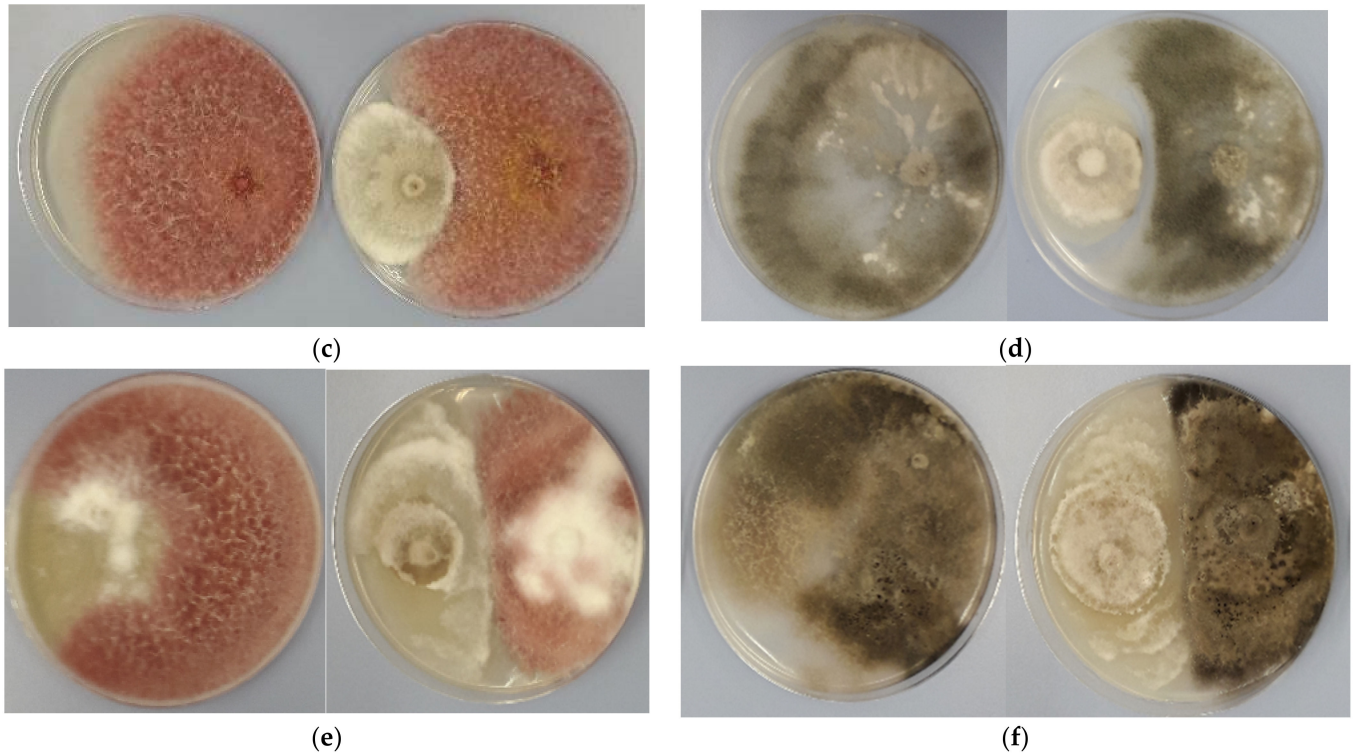


Figure 3. Dual culture assay between endophytic *Diaporthe* against 6 plant pathogens (**a**–**f**): (**a**) on the left—*T. roseum* as a control, on the right—*D. eres* (endophyte) with *T. roseum*; (**b**) on the left—*V. dahliae* as a control, on the right—*D. eres* (endophyte) with *V. dahliae*; (**c**) on the left—*F. sporotrichioides* as a control, on the right—*D. eres* (endophyte) with *F. sporotrichioides*; (**d**) on the left—*B. cinerea* as a control, on the right—*D. eres* (endophyte) with *B. cinerea*; (**e**) on the left—*F. avenaceum* as a control, on the right—*D. eres* (endophyte) with *F. avenaceum*; (**f**) on the left—*A. alternata* as a control, on the right—*D. eres* (endophyte) with *A. alternata*; (photo by B. Abramczyk).

3.3. BIOLOG Analysis

The most intensive mean utilization of carbon substrates was observed after 168 h of incubation; therefore, this hour was chosen to analyze the results. The growth of the analyzed strain was observed on 79 media containing 11 amino acids (L-Alanine, Ala-Gly, L-Asparagine, L-Aspartic Acid, L-Glutamic Acid, Gly-Glu, L-Ornithine, L-Phenylalanine, L-Proline, L-Serine, L-Threonine), 39 carbohydrates (N-Acetyl-D-Galactosamine, N-Acetyl-D-Glucosamine, N-Acetyl-b-D-Mannosamine, Adonitol, D-Arabinose, L-Arabinose, D-Arabitol, Arbutin, D-Cellobiose, D-Fructose, L-Fucose, b-Gentiobiose, D-Glucose, myo-Inositol, a-D-Lactose, Lactulose, Maltitol, D-Maltose, Maltotriose, D-Mannitol, D-Melezitose, a-Methyl-D-Galactoside, b-Methyl-D-Galactoside, a-Methyl-D-Glucoside, b-Methyl-D-Glucoside, Palatinose, D-Psicose, D-Raffinose, L-Rhamnose, D-Ribose, Sedoheptulosan, D-Sorbitol, Stachyose, Sucrose, D-Tagatose, D-Trehalose, Turanose, Xylitol, D-Xylose), 14 carboxylic acids (D-Galacturonic Acid, D-Gluconic Acid, D-Glucuronic Acid, 2-Keto-D-Gluconic Acid, b-Hydroxy-Butyric Acid, a-Keto-Glutaric Acid, L-Lactic Acid, D-Malic Acid, L-Malic Acid, Quinic Acid, D-Saccharic Acid, Sebacic Acid, Succinic Acid, N-Acetyl-L-Glutamic Acid), 8 miscellaneous (a-D-Glucose-1-Phosphate, Glycerol, D-Salicin, Bromo-Succinic Acid, D-Lactic Acid Methyl Ester, Adenosine, Uridine, Adenosine-5′-Monophosphate), 3 amines and amides (L-Alaninamide, Ethanolamine, Putrescine), and 4 polymers (Tween 80, a-Cyclodextrin, b-Cyclodextrin, Glycogen) (Figure 4).

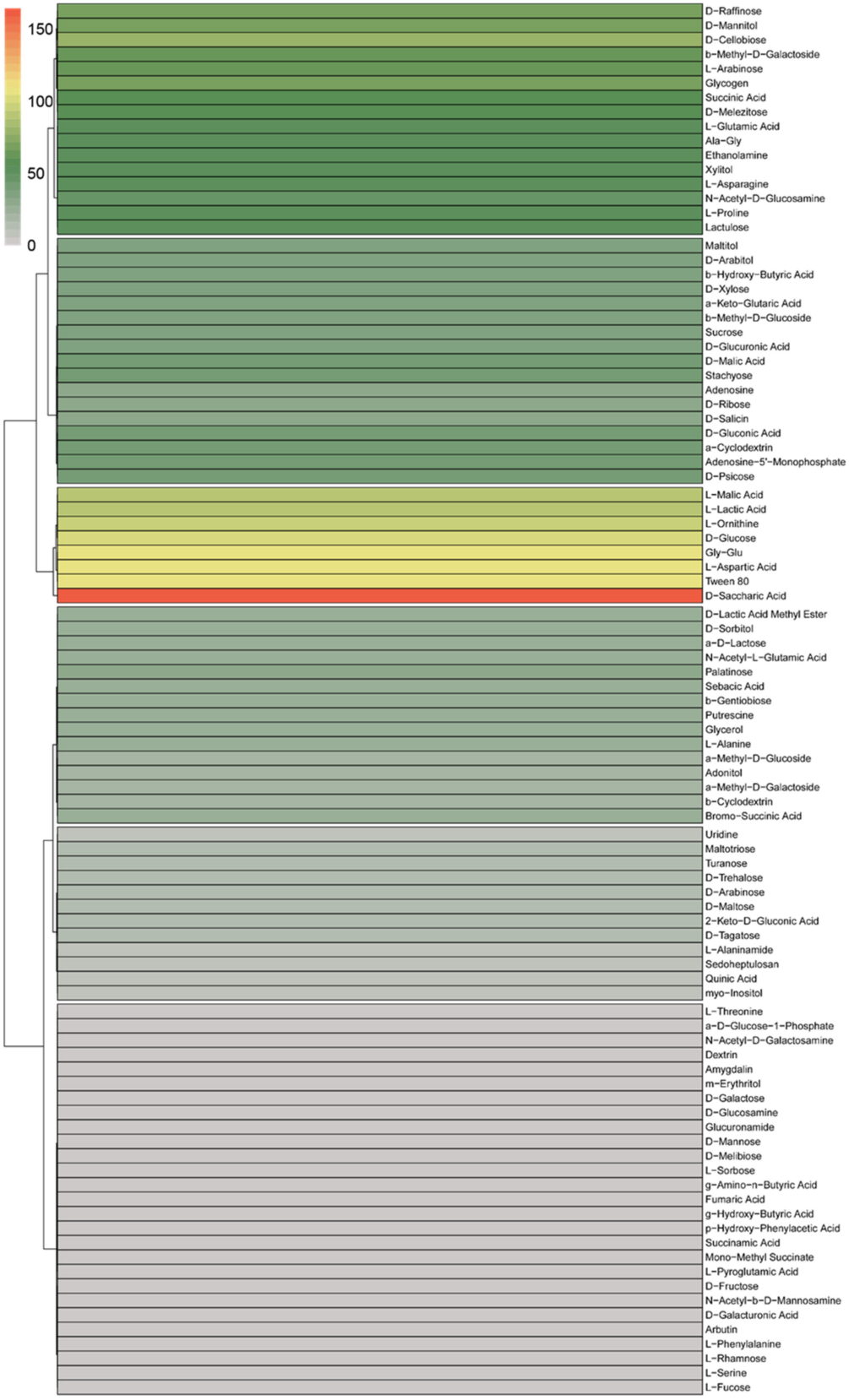


Figure 4. The heatmap presenting utilization of all substrates by analyzed endophytic strain (168 h of incubation).

Biolog FF Plate analysis using the OmniLog platform and Retrospect software allows observation of the changes that occur over time and the kinetics of the decomposition of

individual substrates. The results of the decomposition of the substrates were analyzed at 24 h intervals (24 h to 168 h). After 24 h, decomposition was observed in 19 substrates (20%), after 48 h, 22 substrates (23.16%); after 72 h, 29 substrates (30.53%); after 96 h, 46 substrates (48.42%); after 120 h, 59 substrates (62.10%); after 144 h, 74 substrates (77.90%); after 168 h, 79 substrates (83.16%). The wells in which the fastest growth (before 12 h of incubation) was observed were: Tween 80, D-Arabinose, L-Arabinose, D-Arabitol, D-Cellobiose, a-Cyclodextrin, b-Cyclodextrin, D-Fructose, D-Galacturonic Acid, D-Glucose, Maltotriose, D-Ribose, D-Xylose, L-Malic Acid, Quinic Acid, Sebacic Acid, L-Glutamic Acid, Uridine, and Adenosine-5′-Monophosphate. The decomposition of N-Acetyl-D-Galactosamine, Dextrin, m-Erythritol, D-Galactose, D-Glucosamine, Glucuronamide, D-Mannose, L-Sorbose, g-Amino-n-Butyric Acid, Fumaric Acid, g-Hydroxy-Butyric Acid, p-Hydroxy-Phenylacetic Acid, Succinamic Acid, Mono-Methyl Succinate, L-Phenylalanine, L-Pyroglutamic Acid, L-Threonine characterized the smallest efficiency (mean efficiency < 1). The highest decomposition was observed in the well with D-Saccharic Acid (mean efficiency > 50) (Figure 4).

The most effective decomposition of analyzed substrates after 240 h of incubation was observed in polymers (mean efficiency = 61.40) and amino acids groups (mean efficiency = 63.54), and the least, amines and amides (mean efficiency = 33.78) (Figure 5).

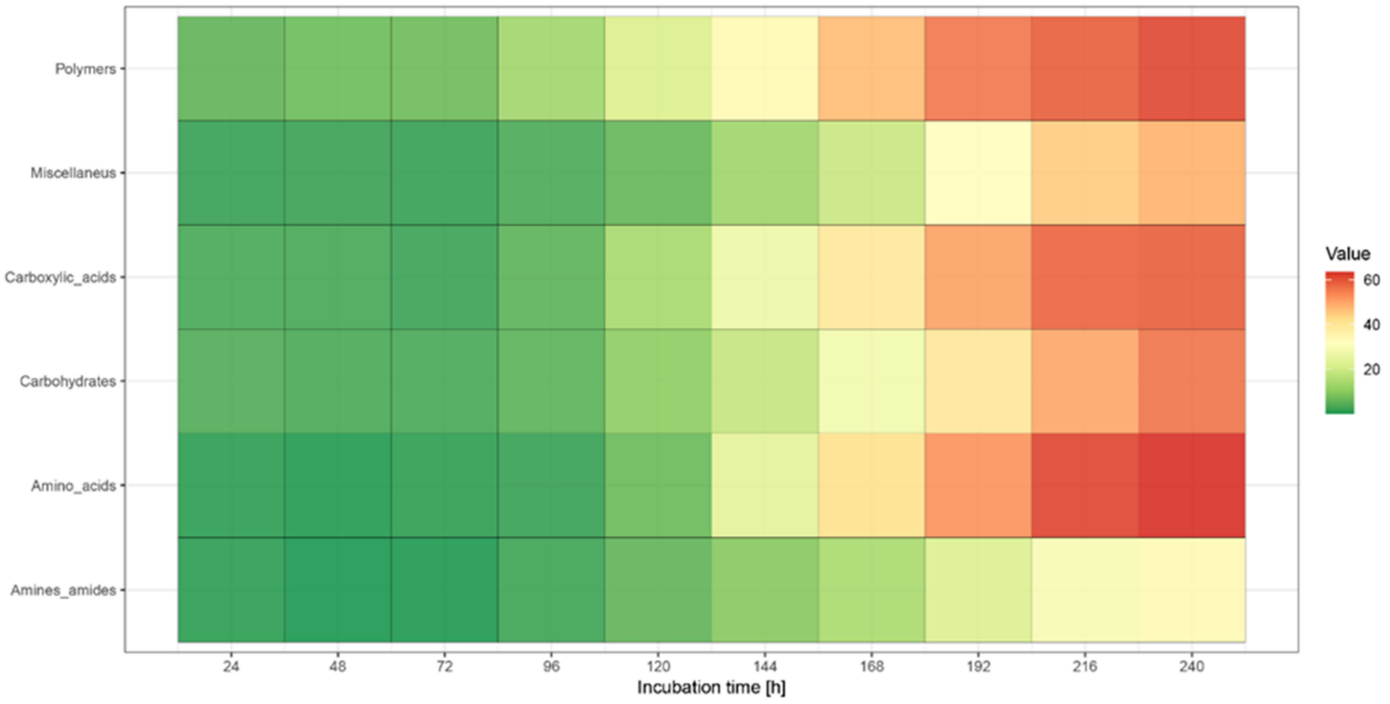


Figure 5. Heatmap presenting utilization of different groups of substrates by analyzed endophytic strain.

4. Discussion

Excessive use of agrochemicals has contributed to the environmental pollution and development of pathogen resistance. Therefore, alternative methods to combat pathogens and sustain agriculture are important and have received more attention in the past decade [9]. Endophytes that reside asymptomatically within a plant can make the chemical intensive crop production system more sustainable as they have the ability to enhance plant growth and increase plant fitness by providing biotic and abiotic stress tolerance and have the potential to provide a source of candidate strains for biocontrol applications [6,9,43].

Our research describes for the first time the endophytic *D. eres*, whose occurrence on *P. domestica* in Poland has been previously reported [14] but its characterization has not been performed before. The identification of the strain was confirmed based on morphological features and sequence data analysis of ITS, TEF, TUB, and CAL genes in accordance with other scientists' research where the delimitation of species within the genus *Diaporthe* only

proved satisfactory once morphology combined with multi-gene DNA sequence data were analyzed [13,15,17–29].

The antagonistic activity test revealed the ability of *D. eres* to reduce the growth of all tested phytopathogens with the mycelial growth inhibition percent (I%) ranging from 20.68% to 40.68%. The highest I% was observed in combination with *T. roseum* (40.68%), followed by *F. avenaceum (33.40%)* and *A. alternata* (32.04%), a slightly lower I% with *B. cinerea* (29.20%) and *F. sporotrichioides* (27.84%), whereas the lowest I% was recorded for *V. dahliae* (20.68%). The literature contains information about the occurrence of endophytic *D. eres* on other species of woody plants, including the branches of *Fagus sylvatica* in Poland, Germany [44], and Switzerland [45,46]; leaves of *Acer macrophyllum* in British Columbia [46,47]; barks of *Taxus chinensis* in China [48]; leaves and branches of *Quercus cerris* in Italy [49]; and leaves of *Larix koempferi* and *Pinus densiflora* in Korea [50]. However, the authors cited did not investigate the antagonistic activity of these strains.

In our study, the type of interaction between the endophytic *Diaporthe* strain and pathogens was the limitation of mycelium growth by contact, which suggests that competition for space was the main mechanism of action [51]. However, endophytic fungus also formed the inhibition zones with some pathogens in dual cultures. This fact indicates that the endophyte might reduce the pathogen growth by producing certain metabolites, rather than by competition or parasitism. In fact, the antagonistic properties of microorganisms that act as biocontrol agents are based on the activation of multiple mechanisms. They can control pathogens by competing for nutrients and space, by mycoparasitism, antibiosis, or metabolite production, by affecting the pathogen, or by promoting plant growth and defense mechanisms [52–54].

Several studies have investigated the in vitro activity of other *Diaporthe* endophytes against various plant pathogens. Santos et al. [6] demonstrated the ability of *Diaporthe* endophytes isolated from leaves of *Sapindus saponaria* to reduce the growth of phytopathogens *Fusarium solani*, *Moniliophthora perniciosa*, and *Glomeralla* sp. The endophytic strain with the best result was *Diaporthe citri* with a 67.50 % inhibition rate against *F. solani* followed by *Diaporthe phaseolorum* with 64.80 % against *M. perniciosa*. In another study, Santos et al. [55] proved an inhibitory effect of new endophytic *Diaporthe* species (*D. terebinthifolii* and *D. endophytica*) from medicinal plants *Schinus terebinthifolius* and *Maytenus ilicifolia*, respectively, described earlier by Gomes et al. [13] against citrus pathogen *Phyllosticta citricarpa* in vitro and in detached fruits. Moreover, the endophytic *Diaporthe* strains from *Pachystachys lutea* were effective against *F. oxysporum* and *Colletotrichum* sp. [51] and the antifungal activity of *D. citri*, isolated from *Mikania glomerata*, was verified by in vitro tests against *Fusarium solani* and *Didymella bryoniae* [56]. Similarly *Diaporthe phaseolorum* isolated from *Espeletia* sp. demonstrated significant activity against the plant pathogen *Phytophthora infestans* [57]. Furthermore, the antifungal effects of *D. phaseolorum* strains on *Ganoderma boninense* were established using the dual culture test [58]. Some compounds obtained from *Diaporthe* have also demonstrated potent antifungal and antibacterial activity against Gram-positive and Gram-negative bacteria [51,56,59,60]. On the other hand, Tanney et al. [60] showed antifungal activity of *Diaporthe maritima* from *Picea* spp., and their crude extracts on the growth of the biotrophic pathogen *Microbotryum violaceum* and the yeast *Saccharommyces cerevisiae*. Among endophytic fungi, *Diaporthe longicolla* exhibited moderate activity with growth inhibition ranging between 11.20% and 61.95% against the tested phytopathogens [61].

The possibility of pathogen control by the *Phomopsis*-like morph has also been found. Antifungal metabolites of *Phomopsis* sp. from *Gossypium hirsutum* limited the growth of pathogenic fungi: *Sclerotinia sclerotiorum*, *Bipolaris maydis*, *Fusarium oxysporum*, *Botrytis cinerea*, *Bipolaris sorokiniana*, *Gaeumannomyces graminis* var. *tritici*, and *Rhizoctonia cerealis* [62]. On the other hand, *Phomopsis oblonga* (anamorph of *Diaporthe eres*) colonizing *Ulmus* tree overgrows the mycelium of pathogens *Ophiostoma ulmi* and *Ophiostoma novo-ulmi.* It is possible that through the colonization of *Phomopsis oblonga* bark and phloem of elms is un-attractive for the invasion and reproduction of Dutch Elm Disease vectors, namely *Scoltus* spp. Thus, the beetles do not spread conidia of *Ophiostoma* spp. to other

living trees [30,63]. The defense of insects most likely relies on the action of fungal toxins. It can be assumed that *D. eres* also produces such toxins in beech leaves [45].

The use of antagonistic endophytes as biocontrol agents is an attractive option for the management of some plant diseases, resulting in a minimal impact on the environment. When testing endophytic fungi for antagonistic potential, it is important to determine the nutritional preference of the fungus with a view to discovering a promising candidate for the future commercial production of new endophyte-based biopreparations. In our preliminary studies, the Biolog FF Plates method was used to determine the ability of endophytic *D. eres* strain to use different carbon sources. This system is a rapid method for the analysis of the catabolic potential of a fungal community or fungal strain pure culture based on their abilities to utilize 95 carbon substrates [64]. The BIOLOG method has been applied previously to assess functional diversity and nutritional profiles of individual fungal species [65,66], to test the nutritional competition between fungal pathogens and beneficial strains [67], and to distinguish closely related cultures [64,68–70].

Although the BIOLOG system has been used in many studies on the physiological characteristics of different fungal species, as far as we know, no data are available on the use of such method to study the biology of *Diaporthe* species.

Our preliminary study revealed that the endophytic *D. eres* strain has the ability to utilize a broad range of carbon sources. This may contribute to the polymorphic nature of the fungus, being one of the reasons why *Diaporthe* spp. Colonize a wide range of host plants and can be found in various climate zones around the world [10,12]. De la Cruz et al. [68] observed the same trend for the marine fungus *Dendryphiella* during the investigation of carbon source utilization by two marine species of this fungus. In our study, the endophytic strain mainly degraded carbohydrates and carboxylic acids, which are the main sources of metabolic changes in cells [71], but other carbon sources such as amino acids and polymers have also been consumed. Similarly, in the study by Barrera et al. [66] on the carbon assimilation by 10 strains from the genus *Cladorrhinum*, carbohydrates were mainly utilized by most strains. Only three strains, *C. foecundissimum* CBS 180.66, *C. samala* INTA-AR 1, and *C. bulbillosum* INTA-AR 54, used mainly esters or polymers.

Species of *Diaporthe* are a major group of endophytes in stems and leaves of angiosperms in both tropical and temperate ecosystems [12,31,72,73]. It has been shown that some of them have the ability to switch between life-styles. The same species can be found on the same or other hosts in different life modes [12]. Examples of phytopathogens reported as endophytes comprise some *Diaporthe* species, frequently present in asymptomatic tissues of a wide variety of plant species [74]. For instance, *D. eres* occurs as pathogen on a wide range of plant hosts throughout the world, including economically important fruit trees and ornamentals [13,23,75] and as endophyte on *P. domestica* in a previous [14] and the present study. Furthermore, *D. foeniculina*, generally accepted as an opportunistic pathogen on various herbaceous weeds, ornamentals, and fruit trees [22], has also been found as an endophyte occurring on a wide range of tropical trees [22]. Huang et al. [20] observed that some *Diaporthe* species associated with citrus in China acted as opportunistic plant pathogens as well. Moreover, the study by Dong et al. [76] revealed the existence of several previously known pathogenic *Diaporthe* species as endophytes on citrus. For instance, *D. limonicola* isolated form C. grandis "Tomentosa" leaves as endophyte was first reported as a dieback pathogen of lemon trees in Europe [77]. Two other species, *D. masirevicii* and *D. perseae*, have been reported as pathogens on several other hosts [78–81]. Results obtained by Sessa et al. [74] have demonstrated that healthy apple, pear, blueberry, and peach shoots can host many known endophytic fungi, including *Diaporthe*, along with potential wood disease-causing fungi that should be regarded as latent pathogens [74]. *D. amygdali*, the well-known and highly frequent pathogen associated with constriction canker in peach shoots, and *D. eres*, associated with cankers in young apple trees, were not recovered as latent pathogens from any of the hosts. Possible explanations for these notorious absences could be that these pathogenic species have a very short latent stage and therefore would be less likely to be present in the sampled asymptomatic twigs, or that

they are actually outcompeted by true endophytes in young intact branches [74]. Although *Diaporthe* was also a highly frequent genus, represented by 10 species, two of the most well-known pathogenic species *D. amygdali* and *D. eres* were not detected in asymptomatic twigs, and only three species (*D. infecunda*, *D. serafiniae* and *D. oxe*) could be considered as latent pathogens [74]. Identification of previously known pathogenic species as endophytes might reveal the opportunistic pathogenic nature of *Diaporthe* species [82].

Although the species of *Diaporthe* can be found occupying different life modes in nature, so far, the factor that drives them into pathogenicity from endophytes has not been confirmed [20]. Mostly, the outcome of interactions relies on the environmental factors and the genotype of both the host and the interacting microorganism [83]. Furthermore, the successful colonization by endophytes is affected by the plant tissue type, the microbial taxon and strain type, and other biotic and abiotic factors [84]. As a result of adaptation to these different environmental conditions, different fungi forming distinctive endophytic communities are specific to each environmental condition and tissue type. Besides geographic areas and plant tissues exert influences on endophytic fungal communities, isolation strategies (e.g., media nutrient composition) often gives preference to certain fungal groups [85]. Host preference is an important parameter for both parasitic and symbiotic plant–fungal interactions [86]. Some species of *Diaporthe* may be either pathogenic or harmless endophytes, depending on the type of host and the health of the host [13]. In general, changing of lifestyle from endophytic to pathogenic or vice versa when colonizing its host might be due to the disruption of a balanced communication with its host factor [87]. Therefore, different environmental pressures can select endophytes with different physiological abilities, such as enzyme production and antagonistic activity against phytopathogens of economically important plant cultivars [51]. The ability to enter and thrive in host tissues makes endophytes unique, showing multidimensional interactions within the host plant [88].

The number of reports of endophytic *Diaporthe* species is high, and works have focused on their potential as producers of novel enzymes and secondary metabolites with antibiotic, fungicidal, or anticarcinogenic activity [74]. Our current knowledge concerning ecology and biology of endophytic *Diaporthe* species is just the "tip of the iceberg" and much similar work should be focused on collecting more endophytic *Diaporthe* from wide geographic regions. Since concept of species in the genus *Diaporthe* has now been more clearly defined based on multi-locus phylogeny, new species recognition and species redefinition are the main areas for future taxonomic work on *Diaporthe* [89].

5. Conclusions

Sequence analyzes confirmed that the endophytic strain inhabiting *P. domestica* shoots belongs to the *D. eres* species complex. The research using the BIOLOG system focused mainly on comparing several strains within the same or different species. Therefore, our preliminary research using only one *Diaporthe* strain should be treated as an introduction to further analyses of a larger group of this species. The species where only one isolate was tested should be studied in more detail. Further studies are necessary to understand factors that determine the pathogenicity of endophytic *Diaporthe* strains towards fruit trees. Therefore, better knowledge of endophytic *Diaporthe* communities that thrive in phyllosphere of orchard plants is needed. This would be useful for achieving a better understanding of factors that influence *Diaporthe* endophytes composition, the structure and the ability to switch life modes in order to predict their response to climate change. By identifying factors that play a dominant role in the structure of *Diaporthe* endophytes communities in orchard plants, novel strategies could be developed for mitigating the impacts of climate change and for improving the fruit farming.

Since the endophytic *D. eres* strain could be considered a promising option for plant disease management in the future, further research is required to investigate more isolates of this species for their metabolite production and biocontrol potential.

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