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Growth and Antagonism of *Trichoderma* spp. and Conifer Pathogen *Heterobasidion annosum* s.l. *in Vitro* at Different Temperatures

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ABSTRACT

Variations in the radial growth rate of 24 isolates belonging to ten species of *Trichoderma*, three isolates of conifer pathogen *Heterobasidion annosum* s.s. and four isolates of *H. parviporum* were evaluated by incubation on a solid malt extract medium at a temperature of 4˚C, 15˚C and 21˚C. *Trichoderma* antagonism against *Heterobasidion* was investigated in dual culture *in vitro*. The slowest rate of growth was referable to all seven strains of *Heterobasidion* spp. All *Heterobasidion* spp. strains were overgrown by 63% of *Trichoderma* spp. strains after two weeks at 21˚C and by 33% of strains at 15˚C. 21% of *Trichoderma* strains did not grow and only four strains belonging to *T. koningii*, *T. viride* and *T. viridescens* demonstrated the ability to completely overgrow *Heterobasidion* spp. after two weeks incubation at 4˚C. According to the antagonistic efficiency, *Trichoderma* strains were divided into five groups with an Euclidean distance of 25. The groups contained isolates from different species. It was suggested that selected psychrotrophic fast growing *T. viride*, *T. koningii* and *T. viridescens* strains could be examined in different substrate conditions as suitable antagonist agents for the control of *H. annosum* and *H. parviporum*.

Keywords: *Trichoderma*; *Heterobasidion*; Antagonism; Growth Rate; Temperature

1. Introduction

Conifer root and butt rot disease caused by *Heterobasidion annosum* (Fr.) Bref. sensu lato is an economically important disease of coniferous trees in boreal and temperate forests [1]. The fungus spreads through aerial basidiospores to stump surfaces and wounds, and by mycelia via root contacts from tree to tree [2,3]. *Heterobasidion* spp. also has an anamorph stage preferably developing in laboratory conditions and forming conidia. Conidiospores also form on the stumps in moist weather and can survive in soil up to ten months [4]. *Heterobasidion* spp. grow at a wide range of temperature with a minimum of 2˚C [5].

A biocontrol method to reduce *H. annosum* s.l. infection is to apply the wood degrading fungus *Phlebiopsis gigantea* in a spore suspension directly on the freshly cut stumps immediately after cutting [6]. There are also a lot of other fungi examined for *Heterobasidion* biocontrol potential including *Trichoderma* spp. [7].

Trichoderma and its teleomorphic stage *Hypocrea* include cosmopolitan soil-borne species. At present, the International Subcommission on *Trichoderma* and *Hypocrea* Taxonomy lists 104 species (http://www.isth.info). *Trichoderma* are particularly prevalent in the humic layer of hardwood forests where they represent up to 3% of all fungal propagules [8]. Some strains are commonly used to control different plant diseases. Possible mechanisms involved in disease control by *Trichoderma* include mycoparasitism, antibiosis, competition, and induction of plant defence responses [9]. *Trichoderma* are also wood-colonising fungi which restrict the growth of *H. annosum in vitro* and under natural conditions [10,11]. There are a number of other investigations of antagonistic action of *Trichoderma* against *Heterobasidion* [12-16]. However, only one or a few strains per species of *Trichoderma* were involved [7].

In general, *Trichoderma* species are mesophylic. However, they possess different temperature minima, optima and maxima. Temperature also affects the metabolism features and antagonistic properties of *Trichoderma* spp. [17]. Goldfarb *et al*. [18] ascertained variation of growth rate substantially within and among species. The investigations supported the opinion that *Trichoderma* isolates

adapted to comparable or lower temperatures than that of plant pathogens provide superior disease control compared to *Trichoderma* isolates that are incapable of growth or activity at cold temperatures [19].

The aim of this work was to study the effect of temperature on antagonism of different *Trichoderma* isolates towards *H. annosum* s.l. with prospects of application in the biological control in boreal and temperate forests.

2. Materials and Methods

2.1. Fungal Strains

In total 24 *Trichoderma* spp. strains, as well as three *H. annosum* s.s. strains and four *H. parviporum* strains isolated primarily in Latvia were used (**Table 1**). For long-term storage, fungi were maintained frozen in liquid nitrogen in the Microbial Strain Collection of Latvia (MSCL) University of Latvia.

2.2. Molecular Identification of Species

A PCR was carried out with the DNA samples of 20 *Trichoderma* isolates. Genomic DNA of *Trichoderma* spp. isolates was extracted from mycelia using a PowerSoil™ DNA Isolation Kit (MO BIO Laboratories, USA). The rRNA gene region was amplified with primers

Table 1. *Trichoderma* and *Heterobasidion* spp. isolates used in this study and corresponding homologue sequences in the NCBI data base.

No. in MSCL	Species	Substrate of Isolation	Country of Origin	Length of Sequence, bp	Homologue Sequence, NCBI acc. no.	Query Coverage, %	Similarity, %
309	*T. asperellum*[a]	Soil	Latvia	268	GQ351595.1	100	95
335	*T. asperellum*[a]	Soil	Latvia	613	FJ004799.1	100	99
488	*T. asperellum*[a]	Biopreparation	Byelorussia	623	FJ004799.1	99	99
844	*T. asperellum*[a]	Soil	Latvia	573	FJ004799.1	99	99
966	*T. asperellum*[a]	Soil	Latvia	575	FJ605246.1	100	99
1011	*T. asperellum*[a]	Wastewater sludge	Latvia	614	EU280110.1	98	100
450	*T. citrinoviride*[a]	Soil	Latvia	594	HQ596981.1	100	98
867	*T. hamatum*[a]	Soil	Latvia	483	GQ220703.1	100	98
453	*T. harzianum*[a]	Soil	Latvia	584	HM176572.1	100	99
485	*T. koningii*[a]	Peat	Latvia	603	EU280128.1	100	99
1012	*T. koningii*[a]	Lake sapropel	Latvia	614	EU280128.1	100	99
1025	*T. koningii*	*Picea abies*, wood	Latvia	-	-	-	-
451	*T. longibrachiatum*[a]	Biopreparation	Estonia	644	EU280095.1	99	100
1024	*T. polysporum*	*P. abies*, wood	Sweden	-	-	-	-
883	*T. rossicum*[a]	Soil	Latvia	628	EU280089.1	100	99
585	*T. viride*[a]	Historical masonry wall	Latvia	615	DQ846665.1	98	99
845	*T. viride*[a]	Soil	Latvia	617	DQ846665.1	98	100
945	*T. viride*[a]	Soil	Latvia	573	FJ481123.1	100	99
946	*T. viride*[a]	Soil	Latvia	413	FJ872073.1	100	99
969	*T. viride*[a]	Soil	Latvia	573	FJ481123.1	100	99
1026	*T. viride*	*Alnus incana*, stem	Latvia	-	-	-	-
472	*T. viridescens*[a]	Rhododendron	Latvia	617	GU566274.1	100	100
538	*T. viridescens*[a]	Cranberry leaf	Latvia	265	GU934535.1	100	93
584	*Trichoderma* sp.	Historical masonry wall	Latvia	-	-	-	-
532	*H. annosum* s.s.	*Pinus sylvestris*, root	Latvia	-	-	-	-
1020	*H. annosum* s.s.	*P. sylvestris*	Latvia	-	-	-	-
1021	*H. annosum* s.s.	*P. sylvestris*	Latvia	-	-	-	-
980	*H. parviporum*	*P. sylvestris*, root	Latvia	-	-	-	-
981	*H. parviporum*	*P. sylvestris*	Latvia	-	-	-	-
1022	*H. parviporum*	*P. abies*, stem	Latvia	-	-	-	-
1023	*H. parviporum*	*P. abies*	Latvia	-	-	-	-

[a]: According to molecular identification presented in this paper.

ITS1F [20] and ITS4 [21]. The reactions in Eppendorf Mastercycler Personal were carried out in 25 µl volume. The mixture contained 0.2 µl Hot Start *Taq* DNA Polymerase, 2.5 µl 10X Hot Start PCR Buffer, 2.5 µl dNTP Mix, 2 mM each, 2 µl 25 mM $MgCl_2$ (all reagents from Fermentas, Lithuania), 0.5 µl of each 25 µM primer (OPERON Biotechnologies), 15.43 µl sterile distilled water and 1 µl of DNA template. The PCR conditions were as follows: the initial denaturation step of 4 min at 95˚C, 40 s of denaturation at 95˚C, 40 s of annealing at 52˚C, 1 min of primer extension at 72˚C (30 cycles) and final extension 10 min at 72˚C. PCR products (20 - 25 µl) were purified using 0.5 µl Exonuclease I (20 $u \cdot \mu l^{-1}$) and 2.0 µL Shrimp Alkaline Phosphatase (1 $u \cdot \mu l^{-1}$) (both from Fermentas, Lithuania). Samples were further processed in a PCR machine 30 min at 37˚C, 15 min at 85˚C. Both strands of the amplification products were sequenced by using an ABI Prism Big Dye terminator cycle sequencing ready reaction kit (version 3.1, Applied Biosystems, Foster City, USA) and primers ITS1F and ITS4. Sequencing of strains 309 - 845 and 1011 - 1012 was done in CBS, Utrecht, the Netherlands. Sequencing of strains 867 - 969 was done in the Biomedical Study and Research Center at the University of Latvia. Obtained sequences were analyzed using Staden Package 1.6.0. release. The resulting consensus sequences were used in the BLASTN homology search against The National Center for Biotechnology Information (NCBI) nucleotide database (http://www.ncbi.nlm.nih.gov).

2.3. Growth Kinetics and Antagonism Assay

Paired cultures of *Trichoderma* and *Heterobasidion* spp. were grown from inocula placed 5 cm apart, on malt extract agar (MEA; Becton Dickinson, USA) at 4˚C, 15˚C and 21˚C. In controls, single cultures were grown in the centre of Petri dishes. Radii of fungal colonies have been recorded daily after 3rd - 28th days. The rate of colony growth was calculated from measurements of radii after three days long incubation of fungi at 21˚C and 15˚C or after 10 days of incubation at 4˚C when the growth corresponded to the exponential growth phase. The results were expressed in $mm \cdot h^{-1}$. The experiment was performed twice. The efficiency of *Trichoderma* in suppressing radial growth was calculated as follows: $(C-T)/C \times 100$, where C is radial growth measurement of the pathogen in the control and T is radial growth of the pathogen in the presence of *Trichoderma* [22].

2.4. Conidiospore Germination

Conidia from *H. annosum* 1020 were obtained from fully sporulated agar cultures. Droplets (100 µl) of spore suspension (about 10^7 conidia$\cdot ml^{-1}$) were transferred to sterile cellophane strips and placed onto a surface of *Trichoderma* spp. growing in the MEA medium for three days. Germinated spores were counted after one, two and three days of incubation at 21˚C using a microscope. Germination of 100 randomly selected conidia in each droplet was evaluated with a microscope at ×600 magnification. Enumeration of germinated conidia was performed in five replicates (on five cellophane strips). A conidium was considered germinated, if the length of the germ tube was not shorter than the diameter of the conidium. The experiment was repeated three times. An average of measurements from 15 replicates was calculated.

2.5. Statistical Analysis

The significance of the difference in values was determined through ANOVA analysis at a significance level of 0.05. The efficiency of different *Trichoderma* strains in suppressing of *Heterobasidion* spp. was analysed using hierarchial cluster analysis with complete linkage method and Euclidean distance matrix.

3. Results

3.1. Identity of *Trichoderma* Species

According to molecular identification, six *Trichoderma* strains proved to be *T. asperellum* and five belonged to *T. viride*, two strains belonged to *T. koningii* and two belonged to *T. viridescens* but five further species (*T. citrinoviride*, *T. hamatum*, *T. harzianum*, *T. longibrachiatum* and *T. rossicum*) were represented by single isolates (**Table 1**).

3.2. Growth and Antagonism between Trichoderma and *Heterobasidion* spp., *in Vitro*

17% of the investigated *Trichoderma* spp. strains grew at all the temperatures tested (4˚C, 15˚C, and 21˚C), but the growth was comparatively slow at 4˚C. The observed mean radial growth rate was from less than 0.010 (five *Trichoderma* spp. strains) to 0.139 (*T. viride* 969) $mm \cdot h^{-1}$ for *Trichoderma* spp. incubated at 4˚C, from 0.094 (*Trichoderma* sp. 584) to 0.260 (*T. viride* 1026) $mm \cdot h^{-1}$ at 15˚C and from 0.187 (*T. rossicum* 883) to 0.451 (*T. koningii* 1025) $mm \cdot h^{-1}$ at 21˚C. The growth rate of *Heterobasidion* strains was slower and ranged only from less than 0.010 (*H. annosum* 1020 and 1021) to 0.021 (*H. parviporum* 1023), from 0.017 (*H. annosum* 1020) to 0.045 (*H. parviporum* 1022) and from 0.061 (*H. annosum* 1020) to 0.133 (*H. parviporum* 981) correspondingly at 4˚C, 15˚C and 21˚C.

Examining the differences among the species (**Figure 1**, **Table 2**), we can see that the slowest rate of growth

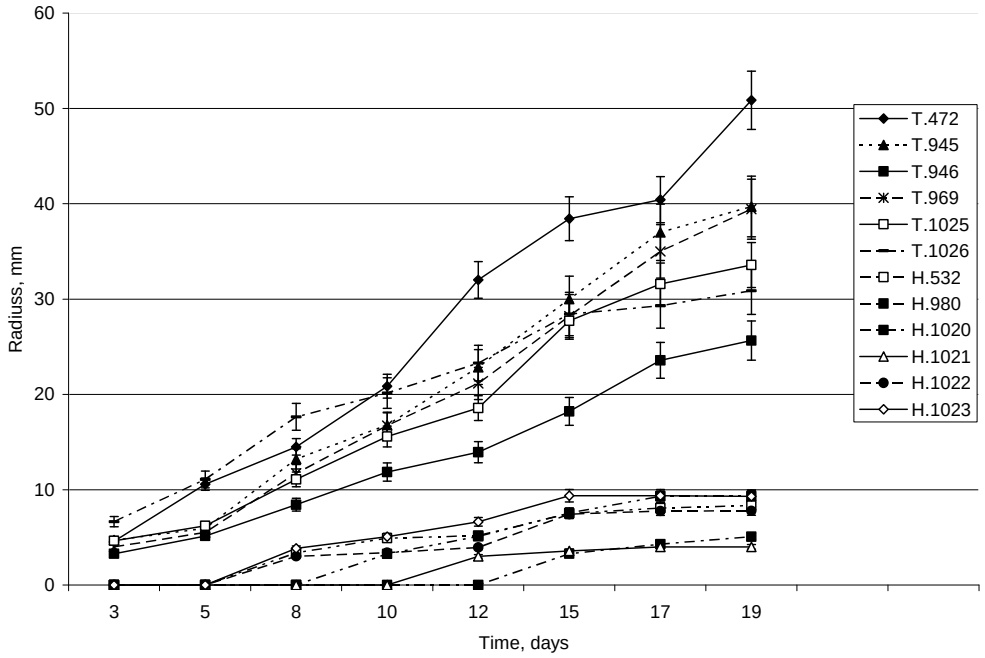

Figure 1. Mycelial radii of *Trichoderma* (T.) and *Heterobasidion* (H.) colonies during incubation on MEA at 4˚C. Isolate numbers are shown in Table 1. Data are presented as means for each variant. Error bars indicate SD.

Table 2. Radial growth rate of colonies of *Trichoderma* and *Heterobasidion* species on MEA at different temperatures.

Species	Number of Strains	Rate of Growth, mm·h^{-1} ±SD		
		21˚C	15˚C	4˚C
T. asperellum	6	0.269 ± 0.015^{c}	0.158 ± 0.017^{d,e}	0.016 ± 0.026^{a,b}
T. citrinoviride	1	0.326 ± 0.008^{d}	0.155 ± 0.008^{d}	0.055 ± 0.006^{d}
T. hamatum	1	0.293 ± 0.009^{c}	0.181 ± 0.010^{e}	0.039 ± 0.005^{c}
T. harzianum	2	0.274 ± 0.011^{c}	0.152 ± 0.027^{c,d,e}	0.014 ± 0.014^{a,b}
T. koningii	3	0.344 ± 0.077^{c,d}	0.194 ± 0.040^{d,e}	0.080 ± 0.015^{e}
T. longibrachiatum	1	0.284 ± 0.005^{c}	0.185 ± 0.009^{e}	<0.010^{a}
T. polysporum	1	0.194 ± 0.005^{b}	0.117 ± 0.006^{b}	0.055 ± 0.008^{d}
T. rossicum	1	0.187 ± 0.007^{b}	0.138 ± 0.005^{c}	0.064 ± 0.006^{d}
T. viride	6	0.338 ± 0.039^{d}	0.219 ± 0.031^{e}	0.080 ± 0.009^{e}
T. viridescens	2	0.284 ± 0.014^{c}	0.184 ± 0.009^{e}	0.073 ± 0.032^{c,d,e}
H. annosum s.s.	3	0.085 ± 0.019^{a}	0.033 ± 0.012^{a}	0.009 ± 0.008^{a}
H. parviporum	4	0.110 ± 0.014^{a}	0.041 ± 0.003^{a}	0.018 ± 0.005^{b}

Data are presented as means for all strains of each species at each temperature. Significant difference ($P < 0.05$) is established in all of the rows. The means in the same column with different superscripts are significantly ($P < 0.05$) different.

was referable to all seven strains of two *Heterobasidion* species. Only some strains formed a sterile zone between antagonistic colonies. For example, it was in the case of *T. asperellum* 1011 and *H. parviporum* 981 (**Figure 2**). We also observed changes in the *Trichoderma* pigmentation from green to yellow near of the pathogen while the pathogen colony restricted their growth. No inhibition zone formed, for example, between colonies of *T. rossicum* 883 and *H. parviporum* 981 (**Figures 3(a)** and **(b)**) at any incubation temperature.

Heterobasidion spp. strains were overgrown (100% efficiency of *Trichoderma)* by 63% of *Trichoderma* spp.

(a) (b) (b)

Figure 2. Dual growth of *T. asperellum* 1011 (on the left) and *H. parviporum* 981 (on the right) after one week (a), two weeks (b) and four weeks (c) incubation at 15˚C.

(a) (b) (b)

Figure 3. Dual growth of *T. rossicum* 883 (on the left) and *H. parviporum* 981 (on the right) after one week (a) and four weeks (b) incubation at 21˚C, and (c) *T. asperellum* 309 (on the left) and *H. parviporum* 981 (on the right) after four weeks incubation at 4˚C.

strains after two weeks at 21˚C and by 33% of strains at 15˚C (**Table 3**). The restriction of *Heterobasidion* was especially difficult and slow at 4˚C. 21% of *Trichoderma* strains did not grow and only four strains (*T. koningii* 585, *T. viride* 595 and 969, and *T. viridescens* 472) clustered in the group (**Figure 4**) what demonstrated the ability to completely overgrow *Heterobasidion* spp. also after two weeks incubation at 4˚C. *T. asperellum* 309 was not able to grow at 4 ˚C and allowed to extend *H. parviporum* 981 (**Figure 3(c)**) with minimal restriction.

3.3. *Trichoderma* Impact on the Germination of *Heterobasidion* Species

All eight of the randomly chosen *Trichoderma* spp. strains (*T. koningii* 1025, *T. polysporum* 1024, *T. viride* 585, 945, 946, 969 and 1026, and *T. viridescens* 472) caused inhibition of *H. annosum* 1020 conidia germination after one day of incubation (data not shown). After two days, the inhibition effect was retained by only four *T. viride* strains and *T. polysporum* 1024. Both *T. viride* 585 and *T. viride* 969 demonstrated the ability to significantly ($P < 0.05$) slowing down the conidiospore germination of pathogen (**Table 4**), especially on the first day.

Table 3. *In vitro* efficiency of *Trichoderma* isolates in suppressing linear growth of *Heterobasidion* spp. in paired cultures measured after two weeks of growth at different temperatures.

Strain of Trichoderma	21˚C	15˚C	4˚C
T. asperellum 309	72.2 ± 9.0	66.4 ± 12.2	5.0 ± 5.0
T. asperellum 335	77.5 ± 3.8	62.7 ± 11.4	0.0 ± 0.0
T. asperellum 488	74.6 ± 10.2	66.9 ± 10.2	0.0 ± 0.0
T. asperellum 844	88.2 ± 10.2	69.9 ± 8.3	0.0 ± 0.0
T. asperellum 966	100.0 ± 0.0	90.3 ± 5.3	47.7 ± 5.1
T. asperellum 1011	76.8 ± 11.2	60.8 ± 11.5	0.0 ± 0.0
T. citrinoviride 450	100.0 ± 0.0	81.9 ± 5.5	29.3 ± 11.8
T. hamatum 867	90.5 ± 3.8	80.4 ± 10.2	21.7 ± 4.7
T. harzianum 453	100.0 ± 0.0	75.4 ± 7.9	12.8 ± 10.8
T. koningii 485	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
T. koningii 1012	100.0 ± 0.0	100.0 ± 0.0	47.3 ± 9.4
T. koningii 1025	100.0 ± 0.0	100.0 ± 0.0	55.2 ± 9.3
T. longibrachiatum 451	78.1 ± 6.4	68.8 ± 7.2	0.0 ± 0.0
T. polysporum 1024	66.7 ± 11.5	55.6 ± 5.9	34.9 ± 3.7
T. rossicum 883	55.9 ± 12.4	43.7 ± 6.8	32.1 ± 8.2
T. viride 585	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
T. viride 845	100.0 ± 0.0	85.9 ± 8.1	47.2 ± 1.5
T. viride 945	100.0 ± 0.0	100.0 ± 0.0	45.6 ± 14.3
T. viride 946	100.0 ± 0.0	94.7 ± 4.1	51.7 ± 2.4
T. viride 969	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
T. viride 1026	100.0 ± 0.0	100.0 ± 0.0	36.7 ± 11.6
T. viridescens 472	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
T. viridescens 538	100.0 ± 0.0	95.3 ± 4.1	9.6 ± 4.0
Trichoderma sp. 584	100.0 ± 0.0	81.9 ± 8.5	7.0 ± 6.3

Data are presented as means of efficiency according to three *Heterobasidion* strains (H. 521, H. 980 and H. 981) ± SD. The efficiency of *Trichoderma* was calculated as follows: $(C-T)/C\times 100$, where C is radial growth measurement of the pathogen in the control and T is radial growth of the pathogen in the presence of *Trichoderma*.

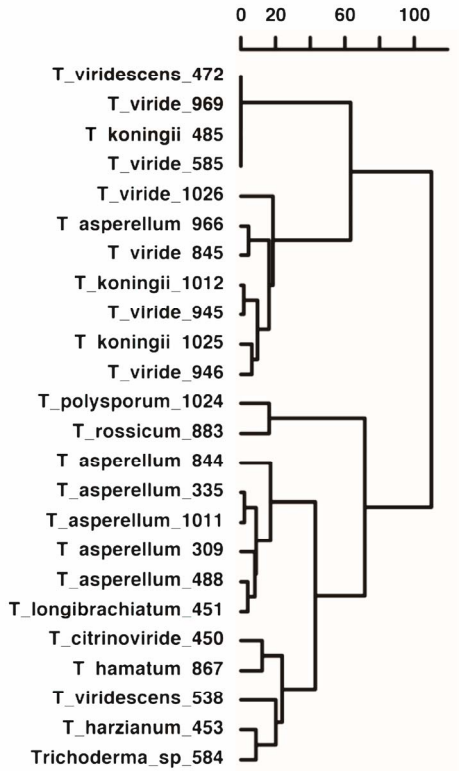

Figure 4. Dendrogram, based on the Euclidean distance, of the *Trichoderma* strains according to efficiency in suppressing linear growth of *Heterobasidion* spp. in paired cultures at a temperature of 21˚C, 15˚C and 4˚C (Table 3).

Table 4. Percentage of germinated *H. annosum* 1020 conidia ± SD affected by *Trichoderma* colonies.

Variant	Time, Days		
	1	2	3
Control	31.1 ± 1.1	ND	ND
T. viride 585	1.1 ± 0.8[a]	10.0 ± 3.8[a]	ND
T. viride 969	1.7 ± 2.5[a]	10.9 ± 1.0[a]	22.8 ± 3.6[a]

[a]Significant difference in comparison with control ($P < 0.05$). ND—the mycelium was formed and therefore the enumeration of germinated conidia was not possible.

4. Discussion

4.1. Growth of Fungi at Different Temperatures

Our studies were carried out to determine the biocontrol potential of different *Trichoderma* strains against *H. annosum* and *H. parviporum in vitro* at different temperatures with emphasis on psychrotrophic growth at low temperature. It is known that the intensive infection of conifers with root rot occurs when the mean day temperature exceeds 5˚C because then conditions are suitable for germination of *Heterobasidion* basidiospores [2]. An optimum for *H. annosum* is located at 17˚C and 22˚C and for *H. parviporum* at 27˚C. The slowest growth takes place at 2˚C for both species (0.65 mm·d^{-1} for *H. parviporum* and 0.60 mm·d^{-1} for *H. annosum*). Our absolute values always were about 30% - 40% lower (**Figure 1**) and the obtained means differed significantly ($P < 0.05$) between species only at 4˚C (**Table 2**).

The significant influence of the temperature ($P < 0.05$) was estimated on the growth rate of different *Trichoderma* strains and species allowing their division into three, four and five groups respectively at a temperature of 21˚C, 15˚C and 4˚C (**Table 2**). Already Danielson and Davey [23] recognized that some *Trichoderma* species are typical for cool geographic regions (*T. viride*, *T. polysporum*) and others are typical for warm climatic regions (*T. koningii*, *T. hamatum*, *T. harzianum*, *T. pseudokoningii*, *T. saturnisporum*). Today, *T. polysporum* is identified as one of the psychrotrophic *Trichoderma* species present in temperate [18] and arctic environments [24]. Our fungal strains were isolated from the temperate region mainly from Latvia (**Table 1**). In addition, our single *T. polysporum* strain (origin—Sweden) showed a high rate of growth (0.055 mm·h^{-1}) at 4˚C. However, three other species, *T. viride*, *T. koningii* and *T. viridescens*, demonstrated a growth rate of 0.073 - 0.080 mm·h^{-1} and overcame *T. polysporum* (**Table 2**).

Jaklitsch *et al*. [25] estimated that *T. viride* and *T. viridescens* is characterised by north- and south-temperate distribution, relatively slow growth and temperature optimum of about 25˚C. They showed very little variation in growth rate among their many isolates that corresponds to the results of our study. Lieckfeldt *et al*. [26] found that *T. viride* has an optimum temperature of 22.5˚C but *T. asperellum* has an optimum of 30˚C. We did not attempt to estimate optimum temperature, however, all six of our investigated *T. viride* strains showed significantly faster growth ($P < 0.05$) than all six of *T. asperellum* strains at all of the used temperature regimes.

4.2. Fungal Antagonism at Different Temperatures

The interaction between *Trichoderma* and *Heterobasidion* spp. was highly dependent on temperature. It is recognized that different *Trichoderma* spp. strains have different thermal requirements for biocontrol [27,28]. Köhl and Schlösser [29] established the potential of some *Trichoderma* isolates to decay sclerotia of *Botrytis cinerea* at low temperatures, even at 5˚C. In the present investigation we found out that all 24 of the investigated *Trichoderma* strains showed antagonistic activity against *Heterobasidion*. The number of *Trichoderma* strains overgrowing pathogens decreased with a decrease in temperature from 21˚C to 15˚C and especially to 4˚C (**Table 3**). According to the antagonistic efficiency, *Tricho-*

derma strains were divided into five groups with an Euclidean distance of 25 (**Figure 4**). The groups contained isolates from different species.

We did not find any *Trichoderma* strain that demonstrated a significant difference in antagonism against several strains of *Heterobasidion*. This is in contrary to the investigation of Bell *et al*. [30], which showed that a single isolate of the antagonist can be highly effective against one isolate (in particular *Rhizoctonia solani*) but may have only minimal effects on other isolates of the same species. Napierala-Filipiak and Werner [31], who studied interactions between 42 mycorrhizal fungi and six strains of *Heterobasidion* spp., ascertained that although antagonism is dependent on growth rates of fungi, the fungi do not display antagonistic properties to all strains of the pathogen.

Obtained results show that *Trichoderma* strains inhibited germination of *Heterobasidion* especially in the beginning (**Table 4**). A direct influence of hyphae and macromolecular compounds on the conidia was excluded by plating cellophane strips onto the surface of the colonies. Therefore, we can assume that soluble low molecular and/or volatile substances produced this impact.

Possible mechanisms of antagonism among our isolates of *Trichoderma* and *Heterobasidion* such as mycoparasitism and antibiosis will be studied in our further experiments. The experiments will also include investigation of the ability of *Trichoderma* to replace *Heterobasidion* in different substrate conditions including wood media.

It was suggested that selected psychrotrophic fast growing *T. viride*, *T. koningii* and *T. viridescens* strains could be examined in different substrate conditions as suitable antagonist agents for the control of *H. annosum* and *H. parviporum*.

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