INFLUENCE OF TEMPERATURE ON THE ANTAGONISM OF *TRICHODERMA* SPP. AGAINST *HETEROBASIDION ANNOSUM* S.L. *IN VITRO*

Laura Alksne[1], Zaiga Petriņa[1], Daina Eze[1], Vizma Nikolajeva[1], Tālis Gaitnieks[2]

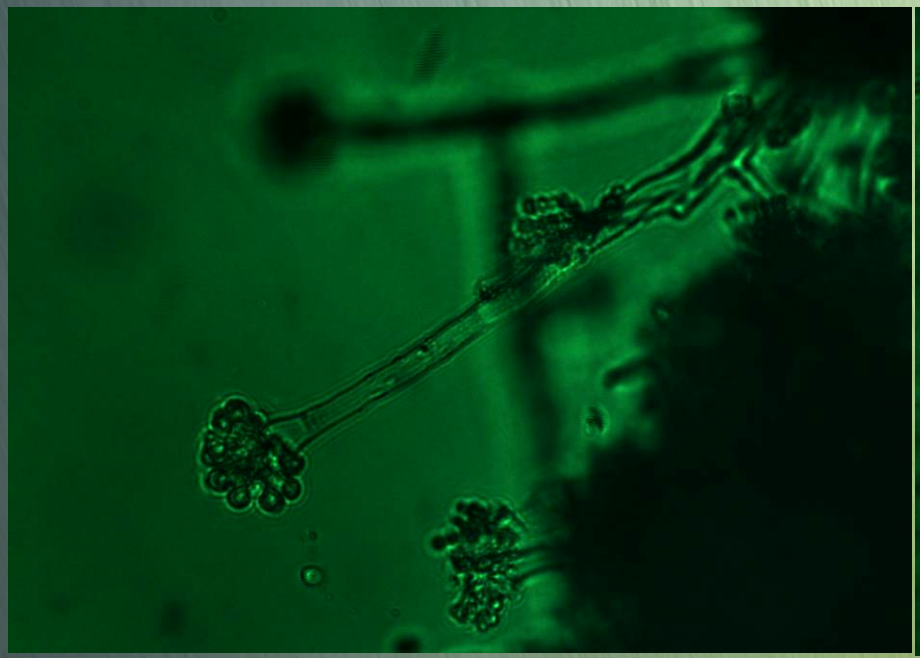
1 Faculty of Biology, University of Latvia

2 Latvian State Forest Research Institute “Silava”

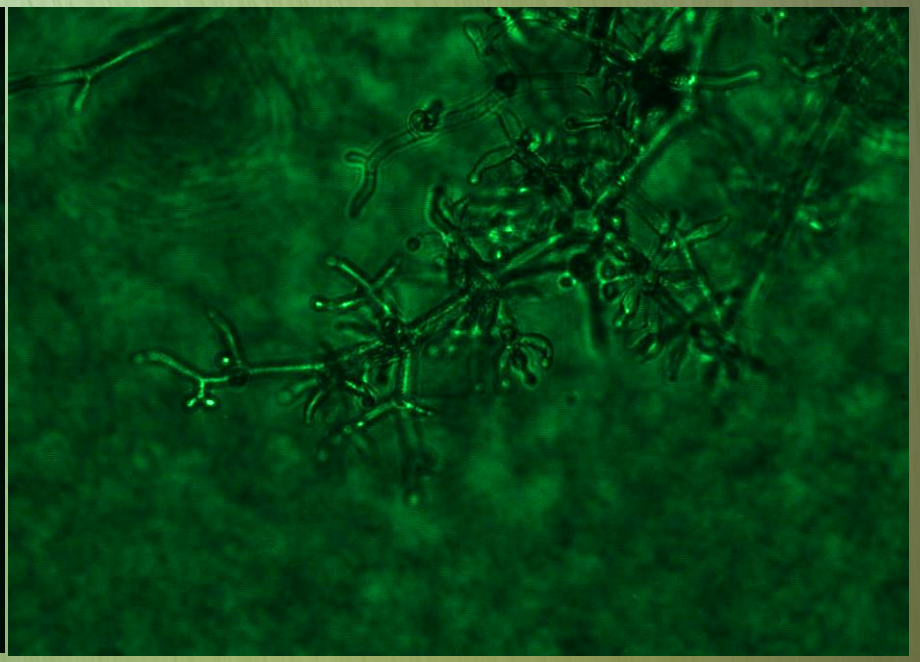
The main goal

Find *Trichoderma* spp. :

- **with antagonistic influence against *Heterobasidion annosum* s.l.;**
- **efficiency in low temperatures**



Heterobasidion annosum conidiophore with conidia in 400x increase



Trichoderma sp. conidiophore with conidia in 400x increase

Trichoderma spp.

- Found in soil, on dead trees, needles, wet or decomposing wood
- Concentrated near the plant root system
- Survives in soil for decades
- Usually the fastest growing at 25-30 °C
- Grows relatively fast and emits specific type of antibiotic substances
- Strong antagonists against soil pathogenic fungi
- Usually gets the most antagonistic properties at 15-20 ° C
- Saprophytic fungi, which are used as biological agents for protection against plant diseases

Heterobasidion annosum s.l. (*H. annosum* s.s., *H. parviporum*)

- Causes the conifer root rot pathogen infection
- Widespread in the northern hemisphere
- Fruiting bodies are on the lower surface of infected roots
- The primary source of infection in healthy forest plantations - spores
- Mycelium growth begins at 0-20 ℃
- Preserves viability in degrading wood

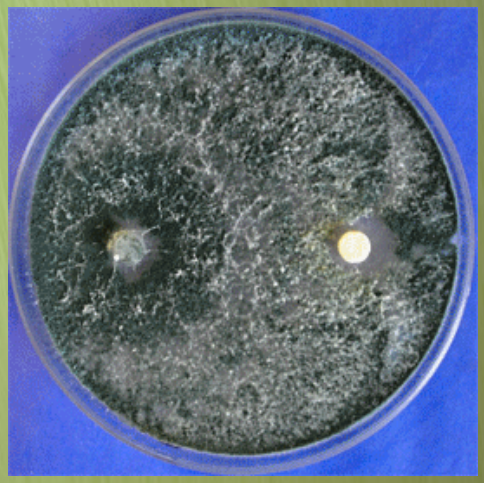
Materials and methods

- For the characterization of antagonistic properties against *Heterobasidion annosum* (3 strains) and *H. parviporum* (4 strains) twenty four *Trichoderma* spp. strains, isolated mainly in Latvia, were estimated (Table 1).

Table 1. *Trichoderma* spp. and *Heterobasidion annosum* s.l. isolates used in this study

Number in MSCL	Species	Substrate of isolation	Country of origin
309	*T. asperellum*	Soil	Latvia
335	*T. asperellum*	Soil	Latvia
450	*T. citrinoviride*	Soil	Latvia
451	*T. longibrachiatum*	Biopreparation	Estonia
453	*T. harzianum*	Soil	Latvia
472	*T. viridescens*	Rhododendron	Latvia
485	*T. koningii*	Peat	Latvia
488	*T. asperellum*	Biopreparation	Byelorussian
538	*T. viridescens*	Cranberry leaf	Latvia
584	*Trichoderma* sp.	Historical masonry wall	Latvia
585	*T. viride*	Historical masonry wall	Latvia
844	*T. asperellum*	Soil	Latvia
845	*T. viride*	Soil	Latvia
867	*T. hamatum*	Soil	Latvia
883	*T. rossicum*	Soil	Latvia
945	*T. viride*	Soil	Latvia
946	*T. viride*	Soil	Latvia
966	*T. asperellum*	Soil	Latvia
969	*T. viride*	Soil	Latvia
1011	*T. asperellum*	Wastwater sludge	Latvia
1012	*T. koningii*	Lake sapropel	Latvia
1024	*T. polysporum*	*Picea abies*, wood	Sweden
1025	*T. koningii*	*Picea abies*, wood	Latvia
1026	*T. viride*	*Alnus incana*, stem	Latvia
532	*H. annosum s.s.*	*Pinus sylvestris*, root	Latvia
980	*H. parviporum*	*Pinus sylvestris*, root	Latvia
981	*H. parviporum*	*Pinus sylvestris*	Latvia
1020	*H. annosum*	*Pinus sylvestris*	Latvia
1021	*H. annosum*	*Pinus sylvestris*	Latvia
1022	*H. parviporum*	*Picea abies*, stem	Latvia
1023	*H. parviporum*	*Picea abies*	Latvia

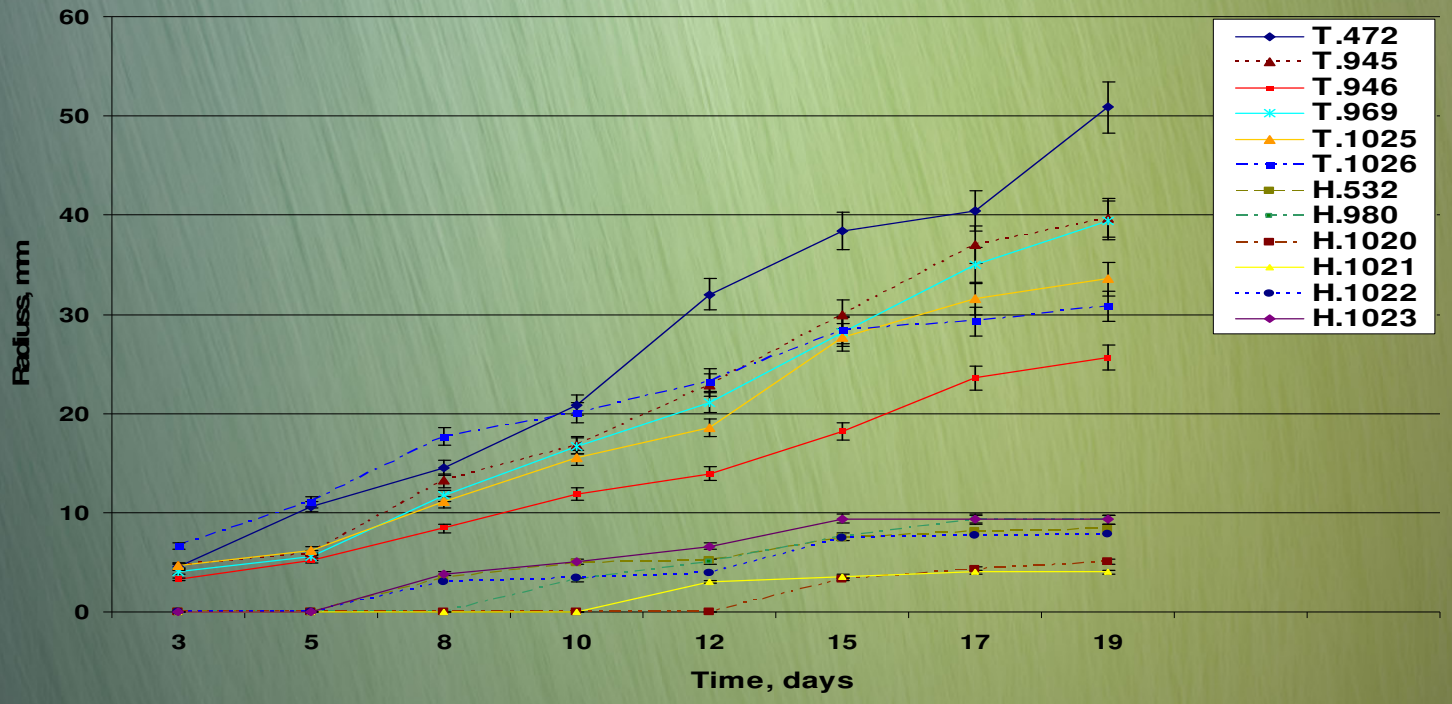
- Dual culture interaction between two fungi was studied by inoculating malt extract agar medium (MEA, Becton Dickinson) plates. The plates were incubated at 4 ℃, 15 ℃ and 21 ℃ for four weeks in darkness, the ability of one fungus to restrict the growth, or to overgrow the other was observed twice a week and mycelial extension was measured.



Results and discussion

- Almost all of the investigated fungi were growing at all tested temperatures (4 ℃, 15 ℃ and 21 ℃), but the growth was comparatively slow at 4 ℃. Moreover, the growth rate of *Heterobasidion* strains was slower than most of investigated *Trichoderma* strains (Fig. 1).

Fig. 1 . Mycelial radii of *Trichoderma* (T.) and *Heterobasidion* (H.) colonies during incubation on MEA at 4 oC.

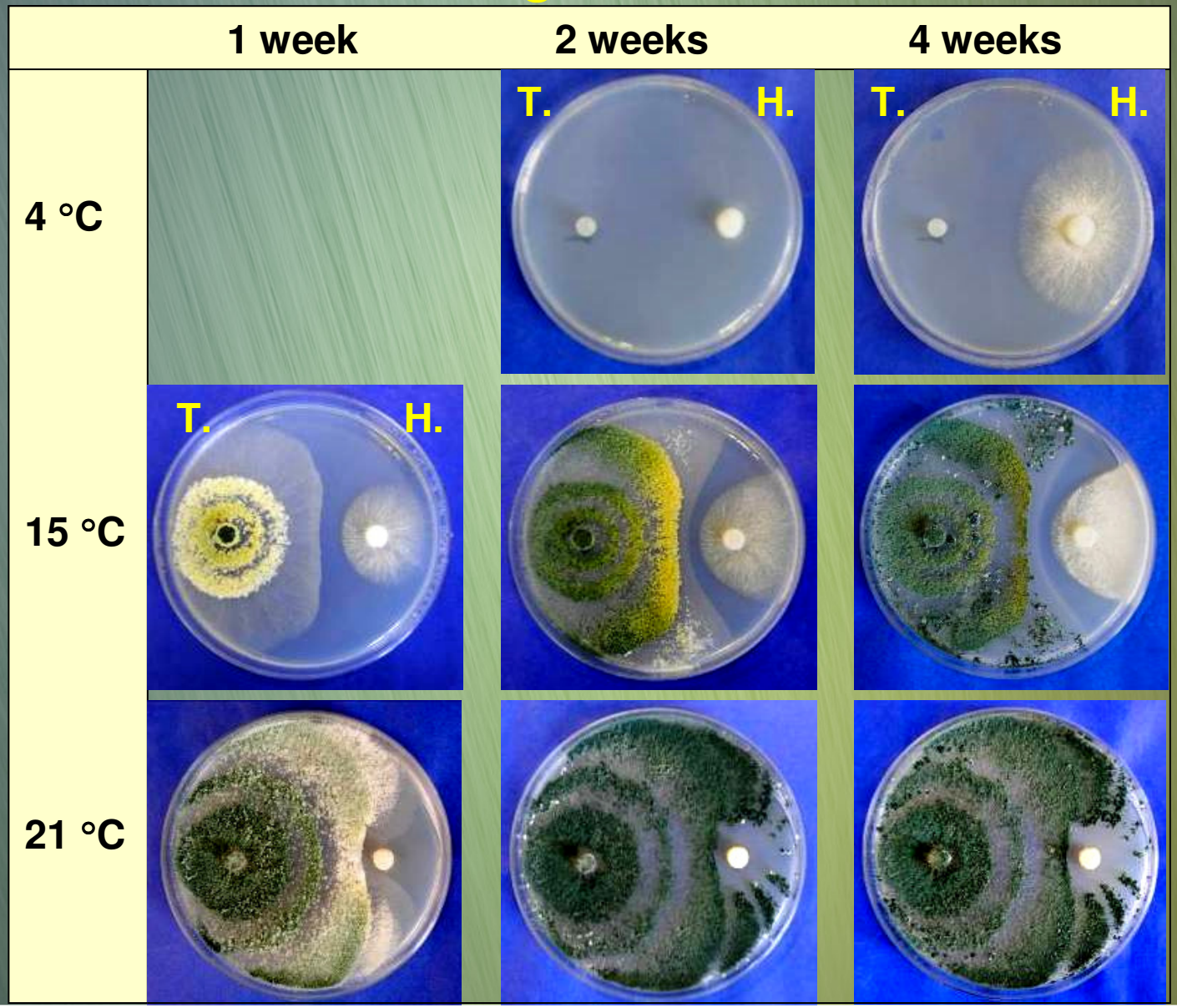


- In accordance with growth rate, it is possible to divide investigated *Trichoderma* species in three groups. *T.viride* strains formed the fast growing group. *T. asperellum* represented the slow growing group but *T. koningii* and *T.viridescens* included in fastest group growing at low temperature and in the slowest group when growing at the moderate temperature.

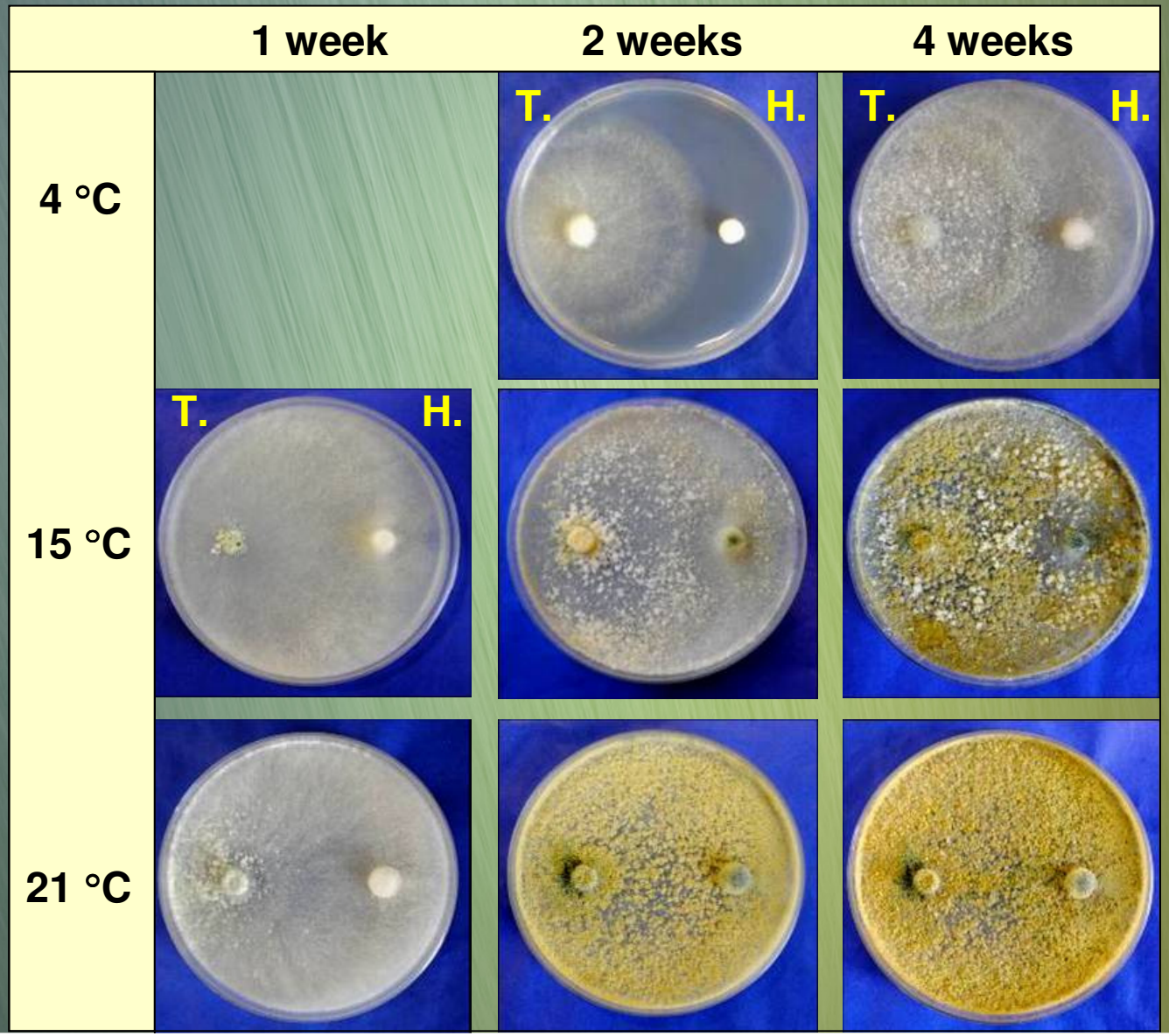
Table 2. Radial growth rate (mm h^{-1}) of colonies of *Trichoderma* and *Heterobasidion* species on MEA at different temperatures

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

Dual growth of *T.asperellum* 1011 (T.) and *H. parviporum* 981 (H.), incubated in different temperature regimes



Dual growth of *T. viride* 946 (T.) and *H. parviporum* 980 (H.), incubated in different temperature regimes



- *Heterobasidion* spp. strains were overgrown by 63% of *Trichoderma* spp. strains after two weeks at 21 ℃ and by 33% strains at 15 ℃. 25% from all tested *Trichoderma* strains did not grow and only one strain (*T. viride* 969) could overgrow *Heterobasidion* spp. after two week incubation at 4 ℃. However *T. viridescens* 472, *T. viride* 585, *T. viride* 945 and *T. viride* 946 showed significant level of antagonism at all of the investigated temperatures.

- Gradually *Trichoderma* started overgrowing *H. annosum* s.l. Only few strains formed antagonism – a sterile zone between antagonistic colonies (Fig.3A) or no zone of inhibition formed between colonies (Fig. 3B).

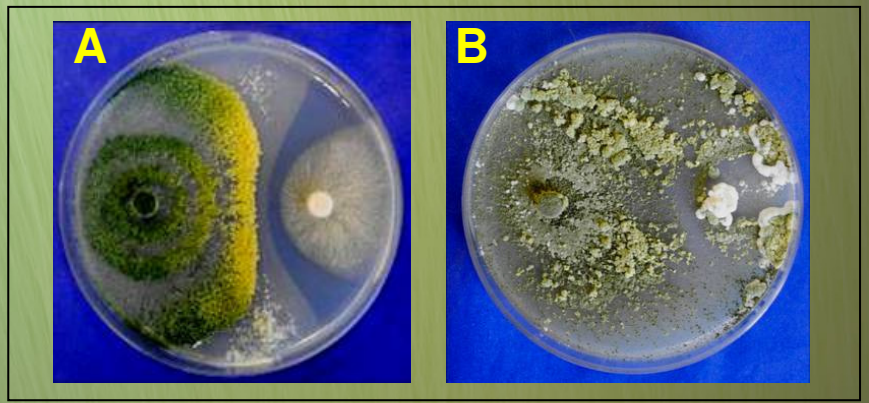


Fig. 3. Dual growth after 4 weeks at 15 ℃ (A) of *T. asperellum* 1011 (on the left) and *H. parviporum* 981 (on the right), (B) *Trichoderma* sp. 584 (on the left) and *H. annosum* s.s. 532 (on the right)

Conclusions

- The growth of investigated *Trichoderma* strains was faster in comparison with the growth of all seven *Heterobasidion* strains. It was temperature depending.
- All *Trichoderma* strains showed antagonistic activity against *Heterobasidion.*
- Selected psychrotrophic fast growing *T. viride* and *T. viridescens* strains could be recommended for further investigations of antagonist agents for the control of *H. annosum* and *H. parviporum* within a wide range of temperatures what is essential for temperate climate zone where fluctuating temperatures are common.

Thank you for your attention!

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