

# Antagonistic activity of the phyllosphere fungal community

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Members of the phyllosphere fungal community possess a vast amount of unexplored antimicrobial substances. To detect and to quantify new antagonistic abilities, 241 fungal isolates were tested via confrontation tests. The 82 fungal species were isolated from the phyllosphere of different plants collected in Germany. Testing showed that 73 isolates belonging to 43 species had antagonistic effects against one or more of our test organisms: *Bacillus subtilis* (Bacteria, 39 active species), *Cryptococcus terreus* (Basidiomycota, 30 active species), *Candida tenuis* (Ascomycota, 17 active species) and *Mucor plumbeus* (Mucoromycotina, 3 active species). In most cases *B. subtilis* was suppressed, followed by *C. terreus*, *Ca. tenuis*, and *M. plumbeus*. Considerable antagonistic activity was shown by *Chaetosphaeronema hispidulum*, *Hypholoma capnoides* and *Penicillium biourgeianum*. These fungi exhibited antagonistic effects against all four test organisms. *Alternaria alternata*, *Anthostomella pinea*, *Cladosporium cladosporioides*, *Drechslera catenaria*, *Drechslera poae*, *Fusarium acuminatum*, *Fusarium avenaceum*, *Fusarium tricinctum*, *Holtermanniella takashimae*, *Penicillium brasilianum*, *Penicillium brevicompactum*, *Pseudoseptoria obscura* and an unidentified Sor-dariomycete also showed substantial activity, but not against *M. plumbeus*. Almost no activity was found in Pucciniomycotina, Ustilaginomycotina, Bulleribasidiaceae (Basidiomycota) and Glomerellales, Helotiales (Ascomycota). After two years of subculturing, changes in the antagonistic abilities could be shown for 45 of the 91 isolates where 7 species showed increasing activity.

Keywords: antagonism, endophytes, epiphytes, phyllosphere fungi.

The phyllosphere generally forms a large, multifaceted habitat for a vast diversity of living organisms (Petrini 1991). Driven by the differences in habitat conditions and microbial communities, the phyllosphere is divided into the epiphytic habitat (phylloplane) comprising the leaf surface and the endophytic habitat (endosphere) including the inner leaf tissues (Khawar et al. 2010). Solar radiation, limited resources, unstable temperature and moisture level as well as plant secondary compounds make the surface an extreme habitat compared to the inner parts of the leaf (Fonseca & Inácio 2006, Peñuelas et al. 2012). In comparison, endophytic organisms that colonize the inner leaf tissues face other difficulties like proliferation in limited space and avoidance of the plant's defence (Arnold 2007, Dupont et al. 2015). Microfungi like basidiomycetous yeasts and ascomycetous hyphomycetes have been found to fit best into these two connected habitats as they represent the majority of organisms here (Jumpponen & Jones 2009, Glushakova & Chernov 2010, Inácio et al. 2010, Botella & Diez 2011). Several species are found in both niches, attesting to a wide ecological range of these fungi (Santamaria & Bayman 2005). The epi- and endo-

phytic fungi developed several strategies to outpace competitors such as the ability to produce a vast amount of biocatalytic substances and secondary metabolites to survive in this tightly contested habitats (Suryanarayanan et al. 2012).

Several fungi and bacteria are able to produce secondary metabolites that are antagonistic to competing organisms in leaf habitats (Aktuganov 2008, Frisvad et al. 2008, Bills & Stadler 2014). Golubev (2006) invented the term 'mycocins' for (glycol-)peptides, which are secreted into the periphery of fungal colonies to strengthen their potential role in the phyllosphere. Several 'killer yeasts' are known to produce mycocins mostly at low pH values. They affect other fungi in tremendous ways by acting fungicidal or fungistatic (Golubev 2013, 2015, Yurkov & Golubev 2013). Isolates of *Ustilago maydis* (DC.) Corda, *Pseudozyma graminicola* Golubev, Sugita & N.W. Golubev, *P. flocculosa* (Traquair, L.A. Shaw & Jarvis) Boekhout & Traquair, *P. fusiformata* (Buha-giar) Boekhout, *Sympodiomyopsis paphiopedili* Sugiy., Tokuoka & Komag. and *Cryptococcus humicola* (Dasz.) Golubev secrete extracellular glycolipids, which possess antagonistic activity (Kulakovskaya et al. 2010).

Antagonistic activity in *Alternaria alternata* (Fr.) Keissl., *Aureobasidium pullulans* (de Bary) G. Arnaud, *Cladosporium cladosporioides* (Fresen.) G.A. de Vries, *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., *Curvularia lunata* (Wakker) Boedijn, *Flavodon flavus* (Klotzsch) Ryvardeen, *Fusarium oxysporum* Schltdl., *F. udum* E.J. Butler, *Microsporium gypseum* (E. Bodin) Guiart & Grigoraki, *Trichoderma harzianum* Rifai and *Trichophyton rubrum* (Castell.) Sabour. against pathogenic fungi was demonstrated without knowing the involved compounds in detail (Mounir et al. 2007, Khawar et al. 2010, Brum et al. 2012, Díaz et al. 2013). In total, the antagonistic strategies developed by epi- and endophytic fungi often seem to be based on secondary metabolites.

The purpose of our study is to investigate the antagonistic potential of phyllosphere fungi mainly isolated from broad bean (*Vicia faba*) and common bean (*Phaseolus vulgaris*) (Prior et al. 2017). To assess the antagonistic properties of these fungi, confrontation tests were performed with *Bacillus subtilis* (Ehrenberg) Cohn, *Cryptococcus terreus* Di Menna, *Candida tenuis* Diddens & Lodder and *Mucor plumbeus* Bonord. as tester strains. These were chosen to cover different phylogenetic groups and even bacteria. *B. subtilis* was chosen because growth rate and growth pattern are well characterized as this is a non-pathogenic model organism (Sender et al. 2004). Also the three fungal species show consistent growth patterns and are commonly known as ubiquitous and contain few defensive metabolites. Thus all of these four species are suitable for standardized tests and have already been applied here (Fonseca & Inacio 2006, Golubev 2006, Surup et al. 2014).

## Materials and methods

### Isolation of fungi

The 241 fungal strains were isolated between May and September in 2013 and 2014 following the protocol in Prior et al. (2017). A detailed description of the origin of isolates is provided in appendix I. Epiphytic isolates were washed off from the surface of defined leaf pieces (2.84 cm<sup>2</sup>) collected under sterile conditions to prevent contaminations and cultivated on diluted YM medium (YM10: 0.1 % (wt/vol) glucose, 0.03 % (wt/vol) yeast extract, 0.05 % (wt/vol) peptone, 0.03 % (wt/vol) malt extract and 0.2 % (wt/vol) agar filled to volume with deionized water; after autoclaving, 80 % lactic acid was added to a concentration of 0.1 % to prevent bacterial

growth; Prior et al. 2014, 2017). Endophytic isolates were obtained using the enzymatic digestion method introduced by Prior et al. (2014).

Identification of fungal isolates was conducted via ITS rDNA sequences following the procedure from Prior et al. (2014): Morphological pre-grouping followed by DNA fingerprinting resulted in rejection of genetically identical clones. The fungal barcode region ITS rDNA was sequenced for the finally selected strains (Schoch et al. 2012). Chromatograms were checked with Sequencher 5.0 (Gene Codes Corp., USA). Assignment of species names to nucleotide sequences is based on BLAST-searches in NCBI ([www.ncbi.nih.gov](http://www.ncbi.nih.gov); 05 April 2016) and Westerdijk Fungal Biodiversity Institute (<http://www.westerdijk.nl>; 05 April 2016) databases, respectively. Isolates with sequence identity higher than 98 % were considered as conspecific. Representative sequences were deposited in GenBank (NCBI; see Appendix I). Representative isolates showing antagonistic activity were deposited in the DSMZ (Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures; see Appendix I).

### Test for antagonistic activity

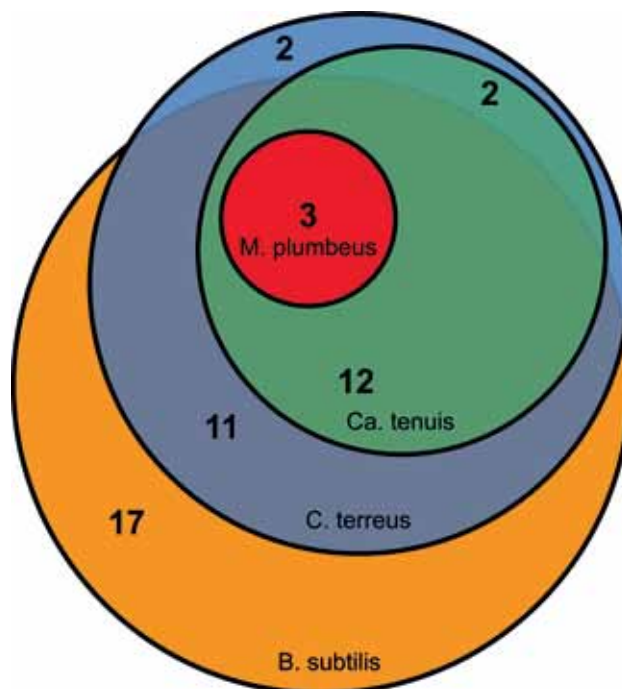
Tests for antagonistic activity of phyllosphere fungi were conducted using cultures of *Mucor plumbeus* (MUCL49355, Mucoromycotina), *Cryptococcus terreus* (MUCL30418, Basidiomycota), *Candida tenuis* (MUCL29892, Ascomycota) and *Bacillus subtilis* (DSM402), respectively. Pre-cultures of *M. plumbeus* were performed on solid YM medium and liquid pre-cultures from *Ca. tenuis*, *C. terreus* and *B. subtilis* in YM medium (YM: 1 % (wt/vol) glucose, 0.3 % (wt/vol) yeast extract, 0.5 % (wt/vol) peptone and 0.3 % (wt/vol) malt extract filled to volume with deionized water). For *Ca. tenuis* and *C. terreus* after autoclaving, 80 % lactic acid was added to a concentration of 0.1 % to support fungal growth. Liquid pre-cultures were used after two days. Spores of *M. plumbeus* were washed and collected from the 2–4 weeks old mycelium. To prepare Petri dishes to test for antagonistic activity, a standardized pre-culture of 1\*10<sup>6</sup> cells or spores per ml was prepared by diluting the suspension with YM liquid medium. Petri dishes previously filled with YM solid medium were inoculated with 100µl cell or spore suspension of one of the tester strains immediately before the fungal isolates were added. YM solid medium included 2 % agar added to liquid YM medium before autoclaving. For *C. terreus*, *Ca. tenuis* and *M. plumbeus* after autoclaving, 80 % lactic

acid was added to a concentration of 0.1 % to maintain optimal growth conditions for the fungi. For the test, a piece of agar (1 × 1 cm) containing the colony margin of the fungal isolates was cut out and placed in the middle of the prepared Petri dish (at least two replicates). The zones of inhibition were measured two days after inoculation as the gap between the growth margin of the fungal isolate and the growth margin of the respective tester strain. For 91 isolates, the antagonistic test was repeated after two years under starvation to reveal possible changes in performance due to two years of cultivation on solid medium with limited resources. Starvation was achieved through cultivation on YM10 medium and re-plating at intervals of ca. 5 months. Several isolates of the same species were tested when available to assess the variation within the respective species. Antagonistic effects of the various isolates on the four tester strains are presented in Tab.1 and in a Venn-diagram (Fig. 1). To evaluate the phylogenetic relevance of the detected effects, a taxonomic tree of the species was calculated (Taxonomy Common Tree, NCBI; <https://www.ncbi.nlm.nih.gov/Taxonomy/CommonTree/wwwcmt.cgi>) and the revealed antagonistic activity was plotted on the tree. As the tool can only handle species with sequence deposited at NCBI, unidentified species were not included. Detailed results regarding each isolate are listed in Appendix I.

### Results

We tested 241 fungal isolates belonging to 82 different species from the phyllosphere for their antagonistic activity against three fungal and one bacterial species (Tab 1). In general, antagonistic activity was detected in 73 of these isolates belonging to 43 species while 39 species (166 isolates) showed no activity (Appendix I). Activity against *B. subtilis* was detected in 39 species (66 isolates), against *C. terreus* in 30 species (44 isolates), against *Ca. tenuis* in 17 species (28 isolates) and against *M. plumbeus* in 3 species (4 isolates) (Tab. 1). Mostly the activity was linked hierarchically following the order *M. plumbeus* – *Ca. tenuis* – *C. terreus* – *B. subtilis*, if activity was detected against *M. plumbeus*, activity against *Ca. tenuis*, *C. terreus* and *B. subtilis* was also detected.

Ninety-one isolates were tested for a second time after two years (Appendix I). Of these, 43 isolates showed no activity during the first and second assays. However, 29 isolates showed a decrease in activity while an increase in activity was observed for 12 isolates. Only two isolates showed stable ac-



**Fig. 1.** Incidence of antagonistic activity among 43 active fungal species from 82 species tested. Most frequently antagonistic activity was shown against *B. subtilis* (39, orange) followed by *C. terreus* (30, blue), *Ca. tenuis* (17, green) and *M. plumbeus* (3, red).

tivity in both runs while the performance of five isolates was variable (Tab. 1, Appendix I). A decrease in activity was indicated primarily if the activity against at least one tester strain got lost or secondarily if the ZOI for at least one tester strain decreased by more than 1 mm. In contrast an increase was indicated if activity against at least one more tester strain emerged or secondarily if the ZOI for at least one tester strain increased by more than 1 mm. A stable reaction was indicated by no changes in affected tester strains and no decrease or increase of the ZOI by 1 mm or less. Variable performance was indicated if activity was decreased or lost against at least one tester strain while it emerged or was increased against at least one other tester strain.

*Chaetosphaeronema hispidulum* (Corda) Moesz, *Hypholoma capnoides* (Fr.) P. Kumm. and *Penicillium biourgeianum* K.M. Zalesky showed substantial high antagonistic activity, because they inhibit growth of all four tester strains. Additionally isolates of *Alternaria alternata*, *Anthostomella pinea* Crous, *Cladosporium cladosporioides*, *Drechslera catenaria* (Drechsler) S. Ito, *D. poae* (Baudyš) Shoemaker, *Fusarium acuminatum* Ellis & Everh., *F. ave-*

**Tab. 1.** Detected antagonistic activity itemised by species. The antagonistic activity against the respective test strain (B.t. = *Bacillus subtilis*, C.t. = *Cryptococcus terreus*, Ca.t. = *Candida tenuis*, M.p. = *Mucor plumbeus*) is shown as active isolates against all fresh isolates from that species. If the test was repeated after two years, the shift of antagonistic activity is shown in the last column. Changes in performance are itemised as stable, inconsistent, decreasing and increasing (stable = no changes in activity, inconsistent = some isolates show decreasing, others increasing or stable activity, decreasing = at least one isolate shows decreasing activity, increasing = at least one isolate shows increasing activity). Blank values indicate no observed activity of fresh isolates.

Species	B.t.	C.t.	Ca.t.	M.p.	After 2 years
<i>Alternaria alstroemeriae</i>	1/1	1/1			stable
<i>Alternaria alternata</i>	9/33	8/33	1/33		inconsistent
<i>Alternaria infectoria</i>					
<i>Alternaria</i> sp.	1/2				
<i>Anthostomella pinea</i>	1/1	1/1	1/1		decreasing
<i>Apiognomonium</i> sp.					
<i>Ascochyta skagwayensis</i>	1/1				decreasing
<i>Aureobasidium pullulans</i>	5/24	1/24			decreasing
<i>Biscogniauxia nummularia</i>					
<i>Botrytis cinerea</i>					
<i>Botrytis</i> sp.					
<i>Cadophora fastigiata</i>					
<i>Cadophora luteo-olivacea</i>					
<i>Ceratocystis paradoxa</i>					
<i>Chaetosphaeronema hispidulum</i>	1/1	1/1	1/1	1/1	decreasing
<i>Chalara</i> sp.	1/1				decreasing
<i>Cladosporium allii</i>					
<i>Cladosporium cladosporioides</i>	2/9	2/9	2/9		increasing
<i>Cladosporium cucumerinum</i>					
<i>Cladosporium macrocarpum</i>					
<i>Colletotrichum gloeosporioides</i>	1/2				decreasing
<i>Diaporthe eres</i>					
<i>Dioszegia fristingensis</i>					
<i>Dioszegia hungarica</i>					
<i>Discosia</i> sp.		1/1	1/1		decreasing
<i>Drechslera catenaria</i>	1/1	1/1	1/1		inconsistent
<i>Drechslera erythrospila</i>	1/2	1/2	1/2		inconsistent
<i>Drechslera poae</i>	1/1	1/1	1/1		decreasing
<i>Epicoccum nigrum</i>	3/9	1/9			decreasing
<i>Exophiala</i> sp.					
<i>Exophiala xenobiotica</i>					
<i>Fusarium acuminatum</i>	6/6	5/6	6/6		decreasing
<i>Fusarium avenaceum</i>	2/2	2/2	2/2		decreasing
<i>Fusarium graminearum</i>					
<i>Fusarium tricinctum</i>	4/4	3/4	4/4		decreasing
<i>Gibellulopsis nigrescens</i>					
<i>Glonium pusillum</i>	3/3	1/3			decreasing
<i>Golubevia pallescens</i>		1/1			
<i>Holtermanniella festucosa</i>	1/1				
<i>Holtermanniella takashimae</i>	1/1	1/1			increasing
<i>Hyaloscypha</i> sp.	1/1				
<i>Hypholoma capnoides</i>	1/1	1/1	1/1	1/1	decreasing
<i>Hypholoma fasciculare</i>	1/1	1/1			decreasing
<i>Lemonniera</i> sp.	1/1	1/1			increasing
<i>Leptosphaeria</i> sp.					
<i>Leucosporidium golubevii</i>					
<i>Microbotryum lychnidis-dioicae</i>					
<i>Microdochium bolleyi</i>	2/3				decreasing
<i>Mucor hiemalis</i>					
<i>Mucor piriformis</i>					
<i>Mycosphaerella coacervata</i>	1/1				increasing
<i>Nodulisporium</i> sp.					
<i>Occultifur aff. externus</i>					
<i>Paraconiothyrium sporulosum</i>	1/1				decreasing
<i>Paraphoma chrysanthemicola</i>					
<i>Penicillium biourgeianum</i>	2/2	2/2	2/2	2/2	

Species	B.t.	C.t.	Ca.t.	M.p.	After 2 years
<i>Penicillium brasilianum</i>	1/1	1/1	1/1		decreasing
<i>Penicillium brevicompactum</i>	1/1	1/1	1/1		inconsistent
<i>Phaeosphaeria pontiformis</i>					
<i>Phialocephala fortinii</i>	1/1				stable
<i>Plectosphaerella cucumerina</i>					
<i>Plectosphaerella</i> sp.					
<i>Pleospora herbarum</i>					
<i>Pleosporales</i> sp. A		1/1	1/1		increasing
<i>Pleosporales</i> sp. B	1/1	1/1			increasing
<i>Pseudoseptoria obscura</i>	1/1	1/1	1/1		inconsistent
<i>Ramularia endophylla</i>		1/1			increasing
<i>Ramularia grevilleana</i>					
<i>Septoria arundinacea</i>	1/1				decreasing
<i>Septoria convolvuli</i>					
<i>Sordariomyces</i> sp. A	1/1	1/1			decreasing
<i>Sordariomyces</i> sp. B	1/1	1/1			decreasing
<i>Stemphylium</i> sp.					
<i>Stereum hirsutum</i>					
<i>Tetracladium</i> sp.					
<i>Tilletiopsis washingtonensis</i>					
<i>Ustilago maydis</i>					
<i>Vishniacozyma dimennae</i>	1/1				
<i>Vishniacozyma victoriae</i>					
<i>Xylaria castorea</i>					
<i>Xylaria hypoxylon</i>	2/2				decreasing
<i>Xylariaceae</i> sp.	1/2	2/2			decreasing

naceum (Fr.) Sacc., *F. tricinctum* (Corda) Sacc., *Holtermanniella takashimae* Wuczkowski, Passoth, Andersson, Turchetti, Prillinger, Boekhout, *Penicillium brasilianum* Bat., *P. brevicompactum* Dierckx, *Pseudoseptoria obscura* Quaedvlieg, Verkley & Crous and an unidentified *Sordariomyces* showed considerable high activity resulting in large inhibition zones (Appendix I).

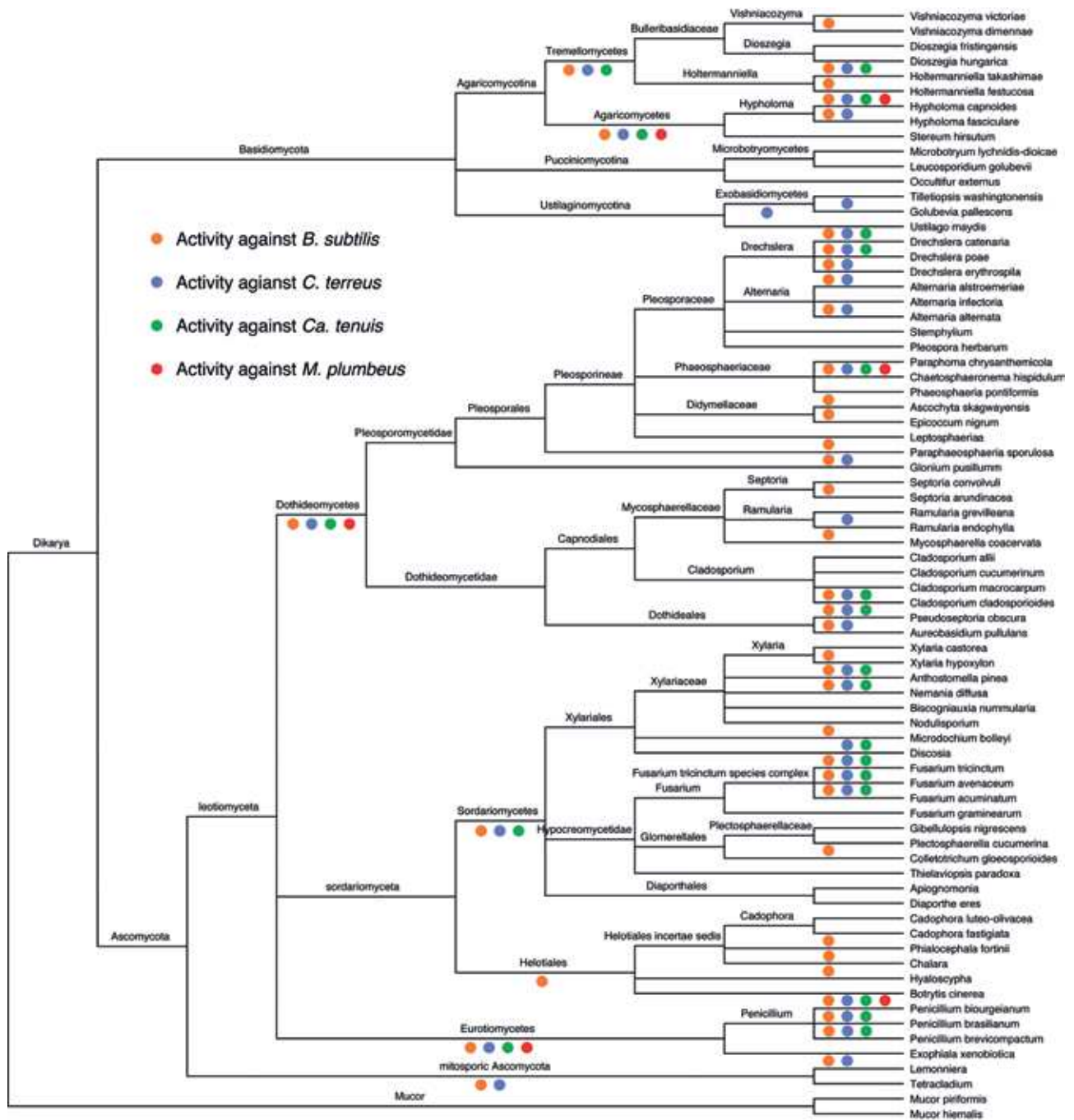
### Discussion

While sampling from the phyllosphere habitat, 241 fungal isolates could be obtained for a screening of antagonistic activity against the four test organisms *Bacillus subtilis*, *Cryptococcus terreus*, *Candida tenuis* and *Mucor plumbeus*, representing bacteria and fungi of three different phylogenetic lineages to account for different physiological capabilities. Our results show that antagonistic capabilities are widespread among members of the fungal phyllosphere community. In most cases the defence mechanisms were directed against bacterial growth followed by the basidiomycetous and ascomycetous yeast and the hyphal growing Mucoromycete (Fig. 1). Additionally, the average inhibition zone formed around the phyllosphere fungus was largest when *B. subtilis* was the tester strain followed by *C. terreus*, *Ca. tenuis* and *M. plumbeus*, which had the narrowest inhibition zone. From 82

species, 52 % exhibited antagonistic activity or 30 % of the 241 isolates, respectively. Kettering (2004) estimated a slightly higher proportion with nearly 40 % of Ascomycota and 50 % of Basidiomycota producing antibiotics and other biologically active compounds. Thus, there is a bias in the assemblage of our tested isolates because all isolates originated from phyllosphere and are therefore not representative for the broad ecology of fungi. However, the differences between Kettering (2004) and our data do not seem to be significant. Although our sampling includes much more Ascomycota than Basidiomycota, a larger proportion was revealed to produce biologically active compounds, supporting the relevance of secondary metabolites for phyllosphere fungi. The results depicted in Fig. 1 also provide the assumption that activity against bacteria is most important for phyllosphere fungi and that bacteria are most susceptible for fungal antagonistic substances, respectively.

Manifestation of antagonistic activity in different lineages

The production of secondary metabolites is often linked to phylogenetic clades, such as Xylariaceae or Sordariaceae, which are known to produce a very diverse set of bioactive substances (Surup et al. 2014, Busby et al. 2016). This connection was tested with



**Fig. 2.** Antagonistic activity against *B. subtilis*, *C. terreus*, *Ca. tenuis* and *M. plumbeus* mapped on a taxonomic tree (Taxonomy Common Tree, NCBI). The activity is indicated for a species if it emerged in at least one isolate. Eight species could not be shown here because of insufficient identification.

isolates from the same ecological niche, but representing different lineages of the fungal tree. The taxonomic tree (Fig. 2) reveals some clades, which clearly differ from others with respect to the antagonistic activity, but this appears only to terminal clades. Most often activity was found in the genera of *Alter-*

*naria*, *Drechslera*, *Fusarium*, *Penicillium* (Ascomycota) and *Hypholoma* (Basidiomycota) and some species where no second species could be tested from the same genus. In contrast the subphyla Pucciniomycotina, Ustilaginomycotina and the family Bulweribasidiaceae from Basidiomycota and the orders

Glomerellales and Helotiales from Ascomycota were almost free from antagonistic activity. Unexpected results were revealed from within some genera. While three *Fusarium* species were very active, *F. graminearum* Schwabe exhibited no antagonistic activity against our tester strains. However, the result is in concordance with the taxonomic grouping where *F. graminearum* shows a remote affiliation to the other three species (Yli-Mattila et al. 2002). The opposite pattern is realised in *Cladosporium*, only *Cl. cladosporioides* showed considerable activity while three additional species of *Cladosporium* did not. It is likely to link these differences between closely related species to genes which are present or active in one species while they are not present or dormant in other species. When the test was repeated for a subset of isolates after two years, seven from nine isolates showing increased antagonistic activity are Dothideomycetes. Also most of the stable and variable isolates, which did not reduce their activity, belong to this class. This leads to the hypothesis that the Dothideomycetes exhibit some mechanism to maintain and/or extend those capabilities. However, there remains great uncertainty regarding the extent of phylogenetic relevance and often the degree of activity seems to be strain dependent (Tab. 1, Appendix I, Bills & Stadler 2014). In fact, only one third of the isolates from *Alternaria alternata* showed antagonistic activity at all and only one isolate from 33 showed activity against *Ca. tenuis*. In contrast, the application of chemotaxonomy for phylogenetic purposes based on secondary metabolites shows that the production of such antagonistic substances is often linked to closely related species (Kuhnert et al. 2015, Richter et al. 2015).

In many cases the results reflect previous observations and several of the detected inhibitory activity might be attributed to substances already described for the respective species, e.g. Cladosporin, Cladospolide A and B from *Cladosporium cladosporioides* (Hirota et al. 1985, Scott et al. 1971), Hispidospermidin from *Chaetosphaeronema hispidulum* (Ohtzuka et al. 1994), p-Anisaldehyde and 3-chloro-p-Anisaldehyde from *Hypholoma capnoides* or 3,5-dichloro-4-metoxi-benzyl Alcohol and Naematolin from *Hypholoma fasciculare* (Huds.) P. Kumm. (Aqueveque et al. 2006). Furthermore, Kiss (2003) could show that *Golubevia pallescens* (Gokhale) Q.M. Wang, F.Y. Bai, Begerow & Boekhout had antagonistic activity against powdery mildew on grasses, roses and cucumber. *Fusarium tricinctum* performed best in suppressing the oak pathogen *Diplodia corticola* A.J.L. Phillips, A. Alves & J. Luque (Campanile et al. 2007). Thus, while antibiotic and

antagonistic properties of some fungi frequently found as epi- and endophytes are already characterized, this is not true for several other members of the phyllosphere community. However, antagonistic activity for *Pseudoseptoria obscura* could be identified for the first time. Additionally, ten of the unassigned strains showed antagonistic activity against at least one of the tester strains. As no close relatives could be identified by comparing the ITS rDNA sequence with public databases, this could be lineages not intensively studied so far.

#### Ecological principles for antagonistic activity

For species with several strains like *Alternaria alternata*, *Aureobasidium pullulans* and *Cladosporium cladosporioides* roughly, only a half of the isolates showed antagonistic activity under our test conditions (Tab. 1). Bills & Stadler (2014) already described this phenomenon, but detailed studies to address this observation are lacking. Genotypic variation within a species or phenotypic plasticity could be possible explanations. So far frequent subculturing is only associated with reduction of antagonistic activity (Bills et al. 2009, Abd-Elsalam 2010), assuming silencing of relevant genes due to the favourable conditions in culture. Therefore, a shortage of nutrients was generated to provoke starvation and simulate a competitive situation like in the natural habitat. As expected 29 isolates of the tested subset of 91 strains showed an overall decrease in antagonistic activity, but 12 isolates revealed increased activity after two years of subculturing (six times during two years). Thus, either the antagonistic activity of these isolates results from *de novo* generated pathways or, more likely, from phenotypic plasticity of the strains and species. In conclusion, we assume that the various strains need different triggers to activate their respective pathways and starvation could be a relevant signal for some fungi.

Especially the closely related but still unidentified Pleosporales sp. A and B originating from the epiphytic and the endophytic habitat, respectively, raise attention in this context. Both species did not exhibit antagonistic activity at the beginning, but were active after two years of subculturing. Thus, detailed more studies might reveal the signal that finally triggers the production of secondary metabolites under laboratory conditions.

An explanation for the low activity in some lineages (Fig. 2) could be the ecology of the respective species, which might be adapted to fast colonisation, growth and proliferation and do not need to outcompete slower growing fungi. However, as discussed

**Appendix I:** Raw data, accession numbers and source.

Detailed test results for each single isolate. Results for isolates tested repeatedly after two years are given in the second group of columns. Zones of inhibition [mm] between the phyllosphere fungus and the tester strain (B.t.: *Bacillus subtilis*, C.t.: *Cryptococcus terreus*, Ca.t.: *Candida tenuis*, M.p.: *Mucor plumbeus*) were measured two days after inoculation. Source of all isolates, GenBank accession numbers (NCBI) and DSMZ collection numbers (Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures) for representative isolates are indicated.

Species	Isolate	Fresh isolates				Isolates after 2 years				Change after two years	GenBank accession number	DSMZ collection number	Source organism	
		B.t.	C.t.	Ca.t.	M.p.	B.t.	C.t.	Ca.t.	M.p.					
<i>Alternaria alstroemeriae</i>	129-4-k	3	4	0	0	4	3	0	0	stable	KX096654	DSM 101969	<i>Vicia faba</i>	endophyte
<i>Alternaria alternata</i>	120-2-5	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Alternaria alternata</i>	120-2-6	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Alternaria alternata</i>	121-2-10	3	0	0	0	5	0	1	0	increasing	KX067791	DSM 101694	<i>Phaseolus vulgaris</i>	endophyte
<i>Alternaria alternata</i>	122-2-3	7	7	3	0	15	12	2	0	increasing			<i>Phaseolus vulgaris</i>	endophyte
<i>Alternaria alternata</i>	122-2-5	3	6	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Alternaria alternata</i>	122-5-2	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Alternaria alternata</i>	125-1-10	10	10	0	0	8	0	0	0	decreasing			<i>Vicia faba</i>	endophyte
<i>Alternaria alternata</i>	125-1-11	10	7	0	0	8	0	0	0	decreasing			<i>Vicia faba</i>	endophyte
<i>Alternaria alternata</i>	125-2-2	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Alternaria alternata</i>	125-4-9	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Alternaria alternata</i>	126-2-3	0	0	0	0	0	0	0	0				<i>Vicia faba</i>	endophyte
<i>Alternaria alternata</i>	126-3-4	0	0	0	0	0	0	0	0				<i>Vicia faba</i>	endophyte
<i>Alternaria alternata</i>	126-4-2	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Alternaria alternata</i>	126-4-5	0	0	0	0	0	0	0	0				<i>Vicia faba</i>	endophyte
<i>Alternaria alternata</i>	127-1-4	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Alternaria alternata</i>	128-4-5	0	0	0	0	0	0	0	0				<i>Vicia faba</i>	endophyte
<i>Alternaria alternata</i>	129-1-2	0	5	0	0	1	8	0	0	increasing			<i>Vicia faba</i>	endophyte
<i>Alternaria alternata</i>	129-1-3	2	10	0	0	7	0	0	0	decreasing			<i>Vicia faba</i> ,	endophyte
<i>Alternaria alternata</i>	129-3-2	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Alternaria alternata</i>	129-3-6	7	7	0	0	4	3	0	0	decreasing			<i>Vicia faba</i>	endophyte
<i>Alternaria alternata</i>	129-4-3	0	0	0	0	0	0	0	0				<i>Vicia faba</i>	endophyte
<i>Alternaria alternata</i>	131-1-7	0	0	0	0	0	0	0	0				<i>Pisum sativum</i>	endophyte
<i>Alternaria alternata</i>	131-1-8	0	0	0	0	0	0	0	0				<i>Pisum sativum</i>	endophyte
<i>Alternaria alternata</i>	131-2-7	0	0	0	0								<i>Pisum sativum</i>	endophyte
<i>Alternaria alternata</i>	131-4-6	0	0	0	0								<i>Pisum sativum</i>	endophyte
<i>Alternaria alternata</i>	131-4-7	1	0	0	0	0	3	0	0	variable			<i>Pisum sativum</i>	endophyte
<i>Alternaria alternata</i>	132-2-5	0	0	0	0								<i>Pisum sativum</i>	endophyte
<i>Alternaria alternata</i>	132-2-8	0	0	0	0								<i>Pisum sativum</i>	endophyte
<i>Alternaria alternata</i>	132-4-4	0	0	0	0	0	0	0	0				<i>Pisum sativum</i>	endophyte
<i>Alternaria alternata</i>	138-19	0	0	0	0								<i>Vicia faba</i>	epiphyte
<i>Alternaria alternata</i>	169-32	0	10	0	0	0	6	0	0	decreasing			<i>Taraxacum officinale</i>	endophyte



Species	Isolate	Fresh isolates				Isolates after 2 years				Change after two years	GenBank accession number	DSMZ collection number	Source organism	
		B.t.	C.t.	Ca.t.	M.p.	B.t.	C.t.	Ca.t.	M.p.					
<i>Alternaria alternata</i>	433-3	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Alternaria alternata</i>	434-1	6	0	0	0								<i>Vicia faba</i>	endophyte
<i>Alternaria infectoria</i>	121-2-7	0	0	0	0	0	0	0	0				<i>Phaseolus vulgaris</i>	endophyte
<i>Alternaria infectoria</i>	128-4-6	0	0	0	0	0	0	0	0				<i>Vicia faba</i>	endophyte
<i>Alternaria infectoria</i>	129-3-5	0	0	0	0	0	0	0	0				<i>Vicia faba</i>	endophyte
<i>Alternaria infectoria</i>	131-1-6	0	0	0	0	0	0	0	0				<i>Pisum sativum</i>	endophyte
<i>Alternaria infectoria</i>	143-3-2	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Alternaria infectoria</i>	143-3-3	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Alternaria infectoria</i>	148-1-1	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Alternaria infectoria</i>	160-1-1	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Alternaria</i> sp.	152-1-1	2	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Alternaria</i> sp.	160-1-2	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Anthostomella pinea</i>	139-2-1	7	7	5	0	7	0	0	0	decreasing	KX096656	DSM 101971	<i>Phaseolus vulgaris</i>	endophyte
<i>Apiognomonium</i> sp.	127-3-6	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Apiognomonium</i> sp.	127-3-8	0	0	0	0	0	0	0	0				<i>Vicia faba</i>	endophyte
<i>Ascochyta skagwayensis</i>	121-1-1	7	0	0	0	0	0	0	0	decreasing	KX096657	DSM 101962	<i>Phaseolus vulgaris</i>	endophyte
<i>Aureobasidium pullulans</i>	121-2-9	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Aureobasidium pullulans</i>	125-3-11	2	0	0	0	0	0	0	0	decreasing			<i>Vicia faba</i>	endophyte
<i>Aureobasidium pullulans</i>	125-3-12	3	0	0	0	0	0	0	0	decreasing			<i>Vicia faba</i>	endophyte
<i>Aureobasidium pullulans</i>	126-4-1	0	0	0	0	0	0	0	0				<i>Vicia faba</i>	endophyte
<i>Aureobasidium pullulans</i>	128-4-7	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Aureobasidium pullulans</i>	129-1-5	0	0	0	0	0	0	0	0				<i>Vicia faba</i>	endophyte
<i>Aureobasidium pullulans</i>	135-31	0	0	0	0								<i>Vicia faba</i>	epiphyte
<i>Aureobasidium pullulans</i>	135-32	0	0	0	0	0	0	0	0				<i>Vicia faba</i>	epiphyte
<i>Aureobasidium pullulans</i>	138-20	0	0	0	0								<i>Vicia faba</i>	epiphyte
<i>Aureobasidium pullulans</i>	218-1	0	0	0	0								<i>Vicia faba</i>	epiphyte
<i>Aureobasidium pullulans</i>	268-10	4	7	0	0						KX067792		<i>Vicia faba</i>	endophyte
<i>Aureobasidium pullulans</i>	269-8	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Aureobasidium pullulans</i>	276-12	3	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Aureobasidium pullulans</i>	279-10	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Aureobasidium pullulans</i>	280-7	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Aureobasidium pullulans</i>	282-3	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Aureobasidium pullulans</i>	285-10	0	0	0	0	0	0	0	0				<i>Vicia faba</i>	endophyte
<i>Aureobasidium pullulans</i>	334-9	0	0	0	0								<i>Pisum sativum</i>	endophyte
<i>Aureobasidium pullulans</i>	342-1	0	0	0	0								<i>Pisum sativum</i>	endophyte
<i>Aureobasidium pullulans</i>	347-11	0	0	0	0								<i>Vicia faba</i>	endophyte

Species	Isolate	Fresh isolates				Isolates after 2 years				Change after two years	GenBank accession number	DSMZ collection number	Source organism	
		B.t.	C.t.	Ca.t.	M.p.	B.t.	C.t.	Ca.t.	M.p.					
<i>Aureobasidium pullulans</i>	354-1	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Aureobasidium pullulans</i>	355-14	6	0	0	0								<i>Vicia faba</i>	endophyte
<i>Aureobasidium pullulans</i>	419-16	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Aureobasidium pullulans</i>	424-11	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Biscogniauxia nummularia</i>	431-4	0	0	0	0						KX096660	DSM 101980	<i>Vicia faba</i>	endophyte
<i>Biscogniauxia nummularia</i>	432-3	0	0	0	0	0	0	0	0				<i>Phaseolus vulgaris</i>	endophyte
<i>Botrytis cinerea</i>	116-23	0	0	0	0	0	0	0	0				<i>Phaseolus vulgaris</i>	epiphyte
<i>Botrytis cinerea</i>	122-1-3	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Botrytis cinerea</i>	127-4-3	0	0	0	0	0	0	0	0				<i>Vicia faba</i>	endophyte
<i>Botrytis cinerea</i>	135-30	0	0	0	0								<i>Vicia faba</i>	epiphyte
<i>Botrytis cinerea</i>	434-2	0	0	0	0						KX096661	DSM 101983	<i>Vicia faba</i>	endophyte
<i>Botrytis</i> sp.	127-4-1	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Botrytis</i> sp.	127-4-4	0	0	0	0	0	0	0	0				<i>Vicia faba</i>	endophyte
<i>Cadophora fastigiata</i>	430-1	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Cadophora fastigiata</i>	431-2	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Cadophora fastigiata</i>	431-7	0	0	0	0						KX096678	DSM 101981	<i>Vicia faba</i>	endophyte
<i>Cadophora fastigiata</i>	433-7	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Cadophora fastigiata</i>	434-4	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Cadophora fastigiata</i>	435-1	0	0	0	0								<i>Phaseolus vulgaris</i>	epiphyte
<i>Cadophora fastigiata</i>	436-2	0	0	0	0								<i>Malva sylvestris</i>	endophyte
<i>Cadophora luteo-olivacea</i>	122-1-2	0	0	0	0	0	0	0	0				<i>Phaseolus vulgaris</i>	endophyte
<i>Cadophora luteo-olivacea</i>	257-4	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Cadophora luteo-olivacea</i>	264-5	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Cadophora luteo-olivacea</i>	272-6	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Ceratocystis paradoxa</i>	144-4-1	0	0	0	0							DSM 27666	<i>Phaseolus vulgaris</i>	endophyte
<i>Ceratocystis paradoxa</i>	144-4-2	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Chaetosphaeronema hispidulum</i>	122-5-1	13	15	2	4	12	15	0	0	decreasing	KX096655	DSM 101966	<i>Phaseolus vulgaris</i>	endophyte
<i>Chalara</i> sp.	203-21	4	0	0	0	0	0	0	0	decreasing	KX096663	DSM 102896	<i>Oxalis acetosella</i>	endophyte
<i>Cladosporium allii</i>	141-2-3	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Cladosporium allii</i>	160-3-1	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Cladosporium cladosporioides</i>	125-2-4	0	0	0	0	0	0	0	0				<i>Vicia faba</i>	endophyte
<i>Cladosporium cladosporioides</i>	125-4-13	0	0	0	0	0	0	0	0				<i>Vicia faba</i>	endophyte
<i>Cladosporium cladosporioides</i>	126-1-x	0	2	2	0								<i>Vicia faba</i>	endophyte
<i>Cladosporium cladosporioides</i>	126-3-2	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Cladosporium cladosporioides</i>	127-4-5	2	0	0	0	12	3	2	0	increasing			<i>Vicia faba</i>	endophyte
<i>Cladosporium cladosporioides</i>	128-2-4	4	8	4	0	15	8	3	0	increasing	KX067796	DSM 101967	<i>Vicia faba</i>	endophyte

Species	Isolate	Fresh isolates				Isolates after 2 years				Change after two years	GenBank accession number	DSMZ collection number	Source organism	
		B.t.	C.t.	Ca.t.	M.p.	B.t.	C.t.	Ca.t.	M.p.					
<i>Cladosporium cladosporioides</i>	130-3-9	0	0	0	0	0	1	3	0	increasing			<i>Pisum sativum</i>	endophyte
<i>Cladosporium cladosporioides</i>	424-12	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Cladosporium cladosporioides</i>	433-1	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Cladosporium cucumerinum</i>	126-2-2	0	0	0	0	0	0	0	0				<i>Vicia faba</i>	endophyte
<i>Cladosporium cucumerinum</i>	126-4-4	0	0	0	0	0	0	0	0				<i>Vicia faba</i>	endophyte
<i>Cladosporium cucumerinum</i>	131-3-3	0	0	0	0	0	0	0	0				<i>Pisum sativum</i>	endophyte
<i>Cladosporium macrocarpum</i>	122-5-4	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Cladosporium macrocarpum</i>	127-3-4	0	0	0	0	0	0	0	0				<i>Vicia faba</i>	endophyte
<i>Cladosporium macrocarpum</i>	127-3-5	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Cladosporium macrocarpum</i>	132-2-6	0	0	0	0								<i>Pisum sativum</i>	endophyte
<i>Colletotrichum gloeosporioides</i>	125-1-12	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Colletotrichum gloeosporioides</i>	257-2	3	0	0	0	0	0	0	0	decreasing			<i>Phaseolus vulgaris</i>	endophyte
<i>Diaporthe eres</i>	127-1-5	0	0	0	0	0	0	0	0				<i>Vicia faba</i>	endophyte
<i>Dioszegia fristingensis</i>	117-19	0	0	0	0								<i>Pisum sativum</i>	epiphyte
<i>Dioszegia fristingensis</i>	131-1-2	0	0	0	0								<i>Pisum sativum</i>	endophyte
<i>Dioszegia hungarica</i>	131-3-1	0	0	0	0								<i>Pisum sativum</i>	endophyte
<i>Discosia</i> sp.	150-4-2	0	15	5	0	0	3	0	0	decreasing		DSM 27673	<i>Vicia faba</i>	endophyte
<i>Drechslera catenaria</i>	120-4-I	15	5	1	0	12	10	5	0	variable	KX0 96672	DSM 101961	<i>Phaseolus vulgaris</i>	endophyte
<i>Drechslera erythrospila</i>	122-3-3	4	12	2	0	10	3	0	0	variable		DSM 101965	<i>Phaseolus vulgaris</i>	endophyte
<i>Drechslera erythrospila</i>	131-4-5	0	0	0	0								<i>Pisum sativum</i>	endophyte
<i>Drechslera poae</i>	121-2	20	5	2	0	3	3	0	0	decreasing		DSM 102906	<i>Phaseolus vulgaris</i>	endophyte
<i>Epicoccum nigrum</i>	120-1-4	0	0	0	0	0	0	0	0				<i>Phaseolus vulgaris</i>	endophyte
<i>Epicoccum nigrum</i>	122-2-4	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Epicoccum nigrum</i>	122-5-5	7	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Epicoccum nigrum</i>	125-3-13	4	4	0	0	0	0	0	0	decreasing			<i>Vicia faba</i>	endophyte
<i>Epicoccum nigrum</i>	125-4-10	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Epicoccum nigrum</i>	125-4-11	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Epicoccum nigrum</i>	125-4-12	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Epicoccum nigrum</i>	126-4-3	7	0	0	0	3	0	0	0	decreasing			<i>Vicia faba</i>	endophyte
<i>Epicoccum nigrum</i>	127-3-7	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Exophiala</i> sp.	141-4-1	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Exophiala</i> sp.	146-5-2	0	0	0	0							DSM 27486	<i>Vicia faba</i>	endophyte
<i>Exophiala</i> sp.	149-4-4	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Exophiala</i> sp.	160 -2-2	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Exophiala xenobiotica</i>	141-5-2	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Exophiala xenobiotica</i>	154-2-1	0	0	0	0							DSM 27488	<i>Phaseolus vulgaris</i>	endophyte

Species	Isolate	Fresh isolates				Isolates after 2 years				Change after two years	GenBank accession number	DSMZ collection number	Source organism	
		B.t.	C.t.	Ca.t.	M.p.	B.t.	C.t.	Ca.t.	M.p.					
<i>Fusarium acuminatum</i>	120-2-3	6	10	4	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Fusarium acuminatum</i>	120-2-4	7	1	5	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Fusarium acuminatum</i>	128-2-5	12	5	10	0	7	2	5	0	decreasing	KX067809	DSM 101968	<i>Vicia faba</i>	endophyte
<i>Fusarium acuminatum</i>	411-2	10	5	9	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Fusarium acuminatum</i>	418-1	7	5	7	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Fusarium acuminatum</i>	418-2	10	0	8	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Fusarium avenaceum</i>	168-22	8	10	5	0	0	5	3	0	decreasing	KX096673	DSM 101957	<i>Phaseolus vulgaris</i>	epiphyte
<i>Fusarium avenaceum</i>	168-7	7	10	4	0								<i>Phaseolus vulgaris</i>	epiphyte
<i>Fusarium graminearum</i>	115-57	0	0	0	0	0	0	0	0				<i>Phaseolus vulgaris</i>	epiphyte
<i>Fusarium tricinctum</i>	125-4a	7	7	8	0								<i>Vicia faba</i>	endophyte
<i>Fusarium tricinctum</i>	126-2	4	5	3	0								<i>Vicia faba</i>	endophyte
<i>Fusarium tricinctum</i>	164-41	3	0	4	0	0	0	0	0	decreasing	KX096674	DSM 102909	<i>Vicia faba</i>	endophyte
<i>Fusarium tricinctum</i>	172-32	12	12	7	0								<i>Medicago lupulina</i>	endophyte
<i>Gibellulopsis nigrescens</i>	158-5-2a	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Gibellulopsis nigrescens</i>	252-1	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Gibellulopsis nigrescens</i>	267-1	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Gibellulopsis nigrescens</i>	267-9	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Gibellulopsis nigrescens</i>	374-10	0	0	0	0								<i>Pisum sativum</i>	epiphyte
<i>Glonium pusillum</i>	128-3	5	6	0	0	0	0	0	0	decreasing			<i>Vicia faba</i>	endophyte
<i>Glonium pusillum</i>	390-11	7	0	0	0						KX067812		<i>Phaseolus vulgaris</i>	epiphyte
<i>Glonium pusillum</i>	433-2	5	0	0	0								<i>Vicia faba</i>	endophyte
<i>Golubevia pallescens</i>	118-14	0	2	0	0						KX096694	DSM 101960	<i>Pisum sativum</i>	epiphyte
<i>Holtermanniella festucosa</i>	121-5-3	2	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Holtermanniella takashimae</i>	217-2	7	3	1	0	8	15	3	0	increasing			<i>Vicia faba</i>	epiphyte
<i>Hyaloscypha</i> sp.	152-3-2	2	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Hypholoma capnoides</i>	432-5	20	20	10	5	0	7	0	0	decreasing	KX096676	DSM 101982	<i>Phaseolus vulgaris</i>	endophyte
<i>Hypholoma fasciculare</i>	165-63	7	4	0	0	7	0	0	0	decreasing	KX096677	DSM 101954	<i>Oenothera biennis</i>	epiphyte
<i>Lemonniera</i> sp.	119-1	0	0	0	0	4	8	0	0	increasing	KX096679	DSM 102907	<i>Pisum sativum</i>	epiphyte
<i>Leptosphaeria</i> sp.	149-1-1	0	0	0	0							DSM 27670	<i>Vicia faba</i>	endophyte
<i>Leptosphaeria</i> sp.	149-1-2	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Leucosporidium golubevii</i>	132-4-3	0	0	0	0	0	0	0	0				<i>Pisum sativum</i>	endophyte
<i>Microbotryum lychnidis dioicae</i>	126-3-6	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Microdochium bolleyi</i>	271-1	9	0	0	0	3	0	0	0	decreasing			<i>Phaseolus vulgaris</i>	endophyte
<i>Microdochium bolleyi</i>	271-2	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Microdochium bolleyi</i>	271-3	1	0	0	0						KX067817	DSM 102905	<i>Phaseolus vulgaris</i>	endophyte
<i>Mucor hiemalis</i>	126-4-6	0	0	0	0	0	0	0	0				<i>Vicia faba</i>	endophyte

Species	Isolate	Fresh isolates				Isolates after 2 years				Change after two years	GenBank accession number	DSMZ collection number	Source organism	
		B.t.	C.t.	Ca.t.	M.p.	B.t.	C.t.	Ca.t.	M.p.					
<i>Mucor hiemalis</i>	242-4a	0	0	0	0						KX067818	DSM 101974	<i>Phaseolus vulgaris</i>	epiphyte
<i>Mucor piriformis</i>	317-1	0	0	0	0	0	0	0	0		KX096681	DSM 101978	<i>Pisum sativum</i>	epiphyte
<i>Mycosphaerella coacervata</i>	138-22	3	0	0	0	7	0	0	0	increasing	KX096658		<i>Vicia faba</i>	epiphyte
<i>Nodulisporium</i> sp.	144-5-2	0	0	0	0							DSM 27668	<i>Phaseolus vulgaris</i>	endophyte
<i>Occultifur</i> aff. <i>externus</i>	158-3-1	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Paraconiothyrium sporulosum</i>	213-2	10	0	0	0	0	0	0	0	decreasing			<i>Oxalis acetosella</i>	endophyte
<i>Paraphoma chrysanthemicola</i>	121-2-8	0	0	0	0	0	0	0	0				<i>Phaseolus vulgaris</i>	endophyte
<i>Penicillium biourgeianum</i>	114-36	7	4	3	7						KX067821	DSM 101959	<i>Phaseolus vulgaris</i>	epiphyte
<i>Penicillium biourgeianum</i>	375-5	12	5	3	5								<i>Phaseolus vulgaris</i>	epiphyte
<i>Penicillium brasilianum</i>	168-10	7	3	3	0	7	0	0	0	decreasing	KX096683	DSM 101956	<i>Phaseolus vulgaris</i>	epiphyte
<i>Penicillium brevicompactum</i>	288-15	10	10	2	0	10	12	5	0	variable	KX067822	DSM 102900	<i>Phaseolus vulgaris</i>	epiphyte
<i>Phaeosphaeria pontiformis</i>	141-1-1	0	0	0	0							DSM 27660	<i>Vicia faba</i>	endophyte
<i>Phaeosphaeria pontiformis</i>	141-1-2	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Phaeosphaeria pontiformis</i>	141-1-3	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Phialocephala fortinii</i>	138-21	8	0	0	0	9	0	0	0	stable	KX096685	DSM 101970	<i>Vicia faba</i>	epiphyte
<i>Plectosphaerella cucumerina</i>	158-2-1	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Plectosphaerella cucumerina</i>	158-2-2	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Plectosphaerella cucumerina</i>	158-3-2	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Plectosphaerella cucumerina</i>	158-3-3	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Plectosphaerella cucumerina</i>	158-4-1	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Plectosphaerella cucumerina</i>	158-4-2	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Plectosphaerella cucumerina</i>	158-4-3	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Plectosphaerella cucumerina</i>	158-4-4	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Plectosphaerella cucumerina</i>	159-5-1	0	0	0	0	0	0	0	0				<i>Phaseolus vulgaris</i>	endophyte
<i>Plectosphaerella cucumerina</i>	267-10	0	0	0	0						KX067823		<i>Phaseolus vulgaris</i>	endophyte
<i>Plectosphaerella cucumerina</i>	267-7	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Plectosphaerella cucumerina</i>	278-5	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Plectosphaerella cucumerina</i>	411-1	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Plectosphaerella cucumerina</i>	411-4	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Plectosphaerella cucumerina</i>	411-5	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Plectosphaerella</i> sp.	132-4-5	0	0	0	0	0	0	0	0				<i>Pisum sativum</i>	endophyte
<i>Pleospora herbarum</i>	125-2-1	0	0	0	0	0	0	0	0				<i>Vicia faba</i>	endophyte
<i>Pleosporales</i> sp. A	133-16	0	0	0	0	0	2	3	0	increasing	KX096687	DSM 102910	<i>Vicia faba</i>	epiphyte
<i>Pleosporales</i> sp. B	121-2-6	0	0	0	0	2	3	0	0	increasing	KX096688	DSM 101963	<i>Phaseolus vulgaris</i>	endophyte
<i>Pseudoseptoria obscura</i>	114-35	10	3	5	0	3	4	8	0	variable	KX096689	DSM 101958	<i>Phaseolus vulgaris</i>	epiphyte
<i>Ramularia endophylla</i>	115-56	0	4	0	0						KX096690	DSM 102908	<i>Phaseolus vulgaris</i>	epiphyte

Species	Isolate	Fresh isolates				Isolates after 2 years				Change after two years	GenBank accession number	DSMZ collection number	Source organism	
		B.t.	C.t.	Ca.t.	M.p.	B.t.	C.t.	Ca.t.	M.p.					
<i>Ramularia endophylla</i>	122-2-6	0	0	0	0	0	12	0	0	increasing			<i>Phaseolus vulgaris</i>	endophyte
<i>Ramularia grevilleana</i>	276-11	0	0	0	0	0	0	0	0		KX067790	DSM 101977	<i>Phaseolus vulgaris</i>	endophyte
<i>Septoria arundinacea</i>	271-6	13	0	0	0	0	0	0	0	decreasing		DSM 102902	<i>Phaseolus vulgaris</i>	endophyte
<i>Septoria convolvuli</i>	122-2-H	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Sordariomycetes</i> sp. A	181-1	3	3	0	0	0	4	0	0	decreasing			<i>Oenothera biennis</i>	endophyte
<i>Sordariomycetes</i> sp. B	433-8	1	3	0	0						KX096692	DSM 102897	<i>Vicia faba</i>	endophyte
<i>Stemphylium</i> sp.	150-4-3	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Stereum hirsutum</i>	142-1-1	0	0	0	0							DSM 27663	<i>Vicia faba</i>	endophyte
<i>Tetracladium</i> sp.	122-4-2	0	0	0	0	0	0	0	0				<i>Phaseolus vulgaris</i>	endophyte
<i>Tilletiopsis washingtonensis</i>	277-1	0	0	0	0								<i>Pisum sativum</i>	endophyte
<i>Tilletiopsis washingtonensis</i>	370-2	0	0	0	0	0	0	0	0				<i>Pisum sativum</i>	epiphyte
<i>Ustilago maydis</i>	436-3	0	0	0	0						KX096695	DSM 102895	<i>Malva sylvestris</i>	endophyte
<i>Vishniacozyma dimennae</i>	403-9	2	0	0	0								<i>Vicia faba</i>	endophyte
<i>Vishniacozyma victoriae</i>	217-1	0	0	0	0	0	0	0	0				<i>Vicia faba</i>	epiphyte
<i>Xylaria castorea</i>	142-3-2	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Xylaria castorea</i>	142-5-1	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Xylaria castorea</i>	143-2-5	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Xylaria castorea</i>	144-3-1	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Xylaria castorea</i>	146-5-1	0	0	0	0							DSM 27669	<i>Vicia faba</i>	endophyte
<i>Xylaria castorea</i>	147-3-1	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Xylaria castorea</i>	150-4-1	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Xylaria castorea</i>	153-2-1	0	0	0	0								<i>Vicia faba</i>	endophyte
<i>Xylaria castorea</i>	154-1-1	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Xylaria castorea</i>	157-2-1	0	0	0	0								<i>Phaseolus vulgaris</i>	endophyte
<i>Xylaria castorea</i>	159-4-1	0	0	0	0	0	0	0	0				<i>Phaseolus vulgaris</i>	endophyte
<i>Xylaria hypoxyton</i>	431-5	1	0	0	0								<i>Vicia faba</i>	endophyte
<i>Xylaria hypoxyton</i>	432-6	9	0	0	0	0	0	0	0	decreasing	KX096696	DSM 102899	<i>Phaseolus vulgaris</i>	endophyte
<i>Xylariaceae</i> sp.	127-2-6	6	15	0	0	0	0	0	0	decreasing		DSM 102901	<i>Vicia faba</i>	endophyte
<i>Xylariaceae</i> sp.	431-6	0	10	0	0								<i>Vicia faba</i>	endophyte

above, it seems epi- and endophytic fungi show elevated antagonistic activity compared to the average of all Asco- and Basidiomycota. This result could be linked to specific conditions of the phyllosphere. Bacteria are known to be the first colonizers of the phylloplane (Fonseca & Inacio 2006). As a consequence, the fungal species have to develop strategies to outcompete and replace the bacteria such as producing antibiotics or other poisonous substances. Still notable activity could be found against the two test yeast species, although *C. terreus* seems to be more susceptible to the secreted substances.

While epiphytes and endophytes share the leaves as one habitat on the macroscopic perspective, these leaves are divided into two largely different habitats. Yeasts are very common in the epiphytic community; the endophytic assemblages are dominated by hyphal fungi (Fonseca & Inacio 2006, Glushakova & Chernov 2010, Jumpponen & Jones 2009, Botella & Diez 2011). In contrast to epiphytic fungi, which have to establish in competition with bacteria and other yeasts, endophytic fungi have to survive this competition only shortly, while entering the plant tissue. Consequently, it may be not necessary to evolve pronounced strategies against epiphytic yeasts. A similar situation is assumed for *M. plumbeus*. Species of this lineage are found as members of the phyllosphere community, but appear infrequently and rare in this habitat because they are not adapted to it (Newton et al. 2010), but are common soil fungi. In addition, Zygomycota are extremely effective in biotransforming the compounds produced by other fungi (Frisvad et al. 2008). Therefore, an antagonistic effect of *Chaetosphaeronema hispidulum*, *Hypholoma capnoides* and *Penicillium biourgeianum* against *M. plumbeus* might be related to a very broad combination of toxins, which is also assured by the strong effect on the other three tester strains. In contrast to the closely related *Hypholoma fasciculare*, *H. capnoides* may be a promising candidate as biocontrol agent in living plants, because it is non-poisonous to humans while exhibiting a profound antagonistic pattern in our study.

In conclusion, properties and requirements accompanied with the colonisation of the phyllosphere habitat resulted in multifaceted strategies of competition between the inhabiting fungi, especially the production of antagonistic secondary metabolites seems higher than average. Members of the Dothideomycetes seem to possess very effective antagonistic mechanisms. The different bioactive substances of phyllosphere fungi yield a serious source for plant protection as well as for antibiotics and fungicides for medicinal use.

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