Journal of Biotechnology 235 (2016) 162–170

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Review

Endemic plants harbour specific *Trichoderma* communities with an exceptional potential for biocontrol of phytopathogens

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ARTICLE INFO

Article history:
Received 27 November 2015
Received in revised form 26 March 2016
Accepted 29 March 2016
Available online 30 March 2016

Keywords:
Plant-associated *Trichoderma*
Endemic plants
Maize
Antagonist
Pathogens

ABSTRACT

Trichoderma strains exhibit enormous potential for applications in biotechnology, in particular as biocontrol agents against pathogens. However, little is known about the diversity of plant-associated *Trichoderma* communities at a global scale and their antagonistic spectrum. In order to gather information about structure and function, we compared *Trichoderma* biomes of endemic (*Aeonium, Diospyros, Hebe, Rhododendron*) and cosmopolitan plants (*Zea mays*) in a global study encompassing the area Northwest Africa to New Zealand via the European Alps and Madagascar. At the quantitative level we found no differences between cosmopolitan and endemic plants. Statistically significant differences were detected at the qualitative level: *Trichoderma* populations of endemic plants were highly specific and diverse with hot spots appearing in Madagascar and New Zealand. By contrast, maize plants from all sites shared the majority of *Trichoderma* species (65.5%). Interestingly, the high above ground biodiversity in ecosystems containing endemic plants was confirmed by a high below ground *Trichoderma* diversity. Despite the differences, we found a global *Trichoderma* core community shared by all analysed plants, which was dominated by *T. koningii* and *T. koningiopsis*. Amplicon-based network analyses revealed a high similarity between maize *Trichoderma* grown world-wide and distinct populations of endemic plants. Furthermore, *Trichoderma* strains from endemic plants showed a higher antagonistic activity against fungal pathogens compared to maize-associated strains. Our results showed that endemic plants are associated with a specific *Trichoderma* microbiome which possesses a high antagonistic activity indicating that it has potential to be used for biocontrol purposes.

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http://dx.doi.org/10.1016/j.jbiotec.2016.03.049
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1. Introduction

Fungi play an important role in addressing global challenges in agriculture (Lange, 2014). The mycotrophic fungal genus *Trichoderma* possesses a variety of biotrophic and saprotrophic lifestyles and can be found all over the world; whereas, Europe is the best-investigated continent with regard to the species diversity of *Trichoderma* (Jaklitsch and Voglmayr, 2015). To date, *Trichoderma* species are widely used for industrial purposes. Metabolic engineering and exploration of genome sequences has enabled the characterization and production of plant cell wall-degrading enzymes, which are second generation biofuels produced from waste products and are used to optimize brewing processes in the food industry (Schuster and Schmoll, 2010; Linke et al., 2015). The ability of *Trichoderma* to antagonize other fungi, based on antibiosis and the production of cell wall lytic enzymes, and its ability to produce secondary metabolites allows it to be used for plant protection against soilborne fungal diseases in agriculture as well as to enhance plant growth, development and yield (Woo et al., 2014). However, little is known about the diversity of plant-associated *Trichoderma* communities at a global scale and their antagonistic activities.

Earth's biota is unevenly distributed, and it is comprised of two opposing biogeographical patterns of species known as endemic and cosmopolitan distributions (Gaston, 2000). Endemism is the ecological state of being unique to a defined geographic region (e.g. island, mountain or habitat patch). Endemic plants are often indicator species for natural ecosystems with high diversity (Kier et al., 2009; Keesing et al., 2010). Because they are restricted to the habitat in biologically isolated areas they are at risk of becoming endangered or extinct if conditions change. Conversely, cosmopolitan plants are found all over the world and are adapted to a broad range of climatic and geological conditions. There are relatively few plant species that occur on all continents, however, the current ranges of cosmopolitan weeds and crops are mainly the result of anthropogenic introductions. They often grow in ecosystems that are highly influenced by anthropogenic activities. While the role of cosmopolitan and endemic plants as indicator for anthropogenic and native ecosystems has been well established (Kier et al., 2009), little is known about the distribution patterns of microbial communities and their impact at a global scale.

Plant-associated microbiomes are known for their specific compositions (rev. Berg and Smalla, 2009; Fierer et al., 2009; Bulgarelli et al., 2012; Lundberg et al., 2012). For example, invasive plants are supported by specific microorganisms (Morriën et al., 2010; Inderjit and van der Putten, 2010; Gladieux et al., 2015). Plants and microbes have developed different forms of mutualistic interactions as components of an evolutionary continuum during co-evolution (Germida and Siciliano, 2001; Cardinale et al., 2015). Due to the higher resolution of sequence data currently achievable, deeper insights into the structure and function of microbial communities is possible (Berg, 2015). One hypothesis for this study was that due to the long separation over time and space of rare and geographically isolated endemic plants they become associated with a unique plant-specific *Trichoderma* subset of the microbiome. Specificity of the plant microbiome was shown in pot experiments (Bulgarelli et al., 2012) and local field trials in Germany (Smalla et al., 2001; Berg et al., 2002, 2005) at cultivar and plant species level (Cardinale et al., 2015), respectively. We expected to observe this specificity at a global scale for selected endemic plants and for the cosmopolitan crop maize.

Specific plant microbiomes fulfil important functions for their host and can influence germination, growth and health (Berg et al., 2014). As part of the biome the genus *Trichoderma* is an indicator for plant and soil health (Meincke et al., 2009; Harman et al., 2012; Vandenkoornhuyse et al., 2002; Van der Heijden et al., 2008 Lange, 2014). The positive interaction with the plant host is related with the fungus' ability to parasitize pathogenic fungi (Harman et al., 2004; Zachow et al., 2009; Kubicek et al., 2011; Atanasova et al., 2013a) and direct effects on plant development and stress protection (Mastouri et al., 2012). In general, disease suppression is an important ecosystem function of the whole plant-associated microbiome because it ensures the overall stability of the ecosystem (Van Elsas et al., 2012; Mendes et al., 2011). Antagonists of pathogens are appropriate indicators for this function (Berg et al., 2002). Current evidence indicates that preserving intact ecosystems and their endemic plant biodiversity reduces the prevalence of pathogens and diseases (Keesing et al., 2010). In this study we hypothesize that endemic plants harbour a high proportion of specific *Trichoderma* strains as well as diverse antagonists to protect themselves against pathogens.

We studied endemic plants in comparison with the cosmopolitan and ubiquitous crop maize (*Zea mays* L.) at a global scale in order to evaluate all hypotheses. We monitored structural and functional parameters of fungal communities from the endemic plants *Aeonium cuneatum* Webb & Berthel. and *Aeonium urbicum* (C. Sm. ex Hornem.) Webb & Berthel. (Canary Island Tenerife), *Diospyros gracilipes* Hiern. and *Diospyros myriophylla* (H. Perrier) ined. (Madagascar), and *Hebe salicifolia* (G. Forst.) Pennell and *Hebe strictissima* (Kirk) L. B. Moore (New Zealand) and *Rhododendron ferrugineum* L. and *Rhododendron hirsutum* L. (Austria). The objectives of this study were i) to evaluate endemic plants as indicators for native *Trichoderma* diversity, ii) to analyse species specificity of *Trichoderma* communities on an intercontinental scale, and iii) to understand the relationship between diversity and pathogen suppression for *Trichoderma* plant-associated communities.

2. Material and methods

2.1. Experimental and statistical design

The rhizosphere samples were collected with minimal impact on the plants to reduce disruption of the environment. Rhizosphere (about 2 ml) and the surrounding soil were sampled at a depth of approximately 10 cm using surface-sterilized cork borers. Rhizospheres were sampled from each location in the environments of 1 m^2 around the plants in four independent samples. Samples were placed in sterile 1.5 ml-tubes and transported to the laboratory. In detail, two endemic plant species and maize were sampled (three plants per site) for each location of the four analysed regions (Austria—A, Canary Island Tenerife—C, Madagascar—M, New Zealand—NZ). Two independent replicates on distant locations were collected from each plant (Table 1). For each plant replication, four biological replicates were used for all analysis of the material

Table 1
Endemic plant species and maize plant origin and sampling site.

Sampling site (Abbreviation)	*Plant species*	*Coordinates*		*Altitude in m*[a]	*Sampling date*
Austria (A)	*Rhododendron ferrugineum*	47.43765	14.4308	1719	05–07-2009
	Rhododendron ferrugineum	46.809733	15.007167	1722	09–07-2009
	Rhododendron hirsutum	47.544567	14.513533	1576	05–07-2009
	Rhododendron hirsutum	47.544567	14.513533	1576	05–07-2009
	Zea mays	47.314983	14.95005	603	09–07-2009
	Zea mays	47.066367	16.006033	265	10–07-2009
Canary Island Tenerife (C)	*Aeonium cuneatum*	28.32945	−16.823267	877	04–01-2009
	Aeonium cuneatum	28.534917	−16.26365	881	06–01-2009
	Aeonium urbicum	28.292767	−16.402333	449	06–01-2009
	Aeonium urbicum	28.245733	−16.8011	711	04–01-2009
	Zea mays	28.497767	−16.30805	875	06–01-2009
	Zea mays	28.497767	−16.30805	612	06–01-2009
Madagascar (M)	*Diospyros gracilipes*	−21.3466	47.70363	471	03–12-2008
	Diospyros gracilipes	−21.3464	47.70361	480	03–12-2008
	Diospyros myriophylla	−21.3464	47.70361	40	27–11-2008
	Diospyros myriophylla	−20.1498	44.5054	35	27–11-2008
	Zea mays	−20.7672	47.323	1696	04–12-2008
	Zea mays	−20.7672	47.323	1474	04–12-2008
New Zealand (NZ)	*Hebe salicifolia*	−42.96156	171.57528	710	31–01-2009
	Hebe strictissima	−43.662528	172.767167	300	07–02-2010
	Hebe strictissima	−43.820833	172.8275	600	07–02-2010
	Zea mays	−43.62275	172.467667	12	19–01-2010
	Zea mays	−43.862	171.810556	91	19–01-2010

[a] *m—meter above sea level.*

and methods section if no other explanations were given. Sample assignment e.g. for Austria: endemic plant species 1–A1, A3, A5, A7 (replication 1); A41, A43, A45, A47 (replication 2); endemic plant species 2–A9, A11, A13, A15 (replication 1); A25, A27, A29 A31 (replication 2); maize – A17, A19, A21, A23 (replication 1); A33, A35, A37, A39 (replication 2).

2.2. Isolation and characterization of Trichoderma

The soil dilution plate method was used for isolation of *Trichoderma* according to Zachow et al. (2009). In brief, 100 mg of rhizosphere were mixed with 0.05% water agar. Serial dilutions were spread on 90-mm diameter petri plates containing *Trichoderma* selective medium (TSM; Smith et al., 1990), TSMC (Elad and Chet, 1983), potato dextrose agar (PDA; Merck & Co., Whitehouse Station, NJ, USA) amended with 100 mg ml^{-1} streptomycin and tetracycline, respectively, and synthetic nutrient agar (SNA) (Berg et al., 2005). Plates were incubated at 25 °C. Two plates were prepared per suspension, yielding six plates per substrate (TSM, TSMC, PDA, or SNA). After seven days, colony forming fungi and *Trichoderma* were counted to calculate the abundances (log_{10} colony forming unit (CFU)) based on fresh weight. In total, 576 *Trichoderma* isolates per sampling site were randomly selected. *Trichoderma* colonies were subsequently cultured using single-spore technique and stored at 4 °C until DNA extraction. All isolates described in this study were conserved as plugs in liquid storage medium containing glycerol (final concentration 15%) in the strain collection SCAM (strain collection for antagonistic microorganisms) at the Graz University of Technology (Austria).

2.3. Quantitative real time PCR

Quantitative analyses and DNA extraction were conducted according to Zachow et al. (2009) with a few additional steps. Briefly, standards for absolute quantification of environmental *Trichoderma* DNA were prepared from targeted ITS regions (ITS1-ITS4) of *Trichoderma velutinum* G1/8 (Grosch et al., 2006) by cloning into the pGEM-T Easy vector (Promega). Reaction components per 10 μl included 1 × SYBR™ Green PCR Master Mix (Invitrogen, Carlsbad, CA), 0.01 pmol of each primer (ITS1Trf/ITS4Trr), and 1 μl RNase-treated template DNA. The cycler conditions were as follows: initial 95 °C for 5 min followed by 35 cycles of 95 °C for 30 s, 53 °C for 35 s, 72 °C for 2 min, and for fungi: initial 95 °C for 5 min followed by 35 cycles of 95 °C for 45 s, 62 °C for 35 s, 72 °C for 30 s and 72 °C for 10 min.

Concentrations determined by absolute quantification were calculated to copy number per g fresh weight. Each sample was prepared in triplicates and in serial dilutions for the soil. Cycling, data collection, and calculation were done by the Rotor-Gene™ 6000 software according to the manufacturer's instructions. After quantification, the samples were analysed by melting curve calculation and agarose gel electrophoresis for quality control of the PCR. Significant differences of rhizosphere abundances were compared with each other after using ANOVA and the Scheffé test with P = 0.05 (PASW Statistics 18, SPSS Inc., Chicago, USA).

2.4. Amplicon sequencing and bioinformatic analysis

Diversity of *Trichoderma* associated with endemic plant species and maize was studied using a barcoded amplicon pyrosequencing approach. Total community DNA was amplified with the set of *Trichoderma*-specific primers, (ITS1Trf/ITS4Trr; Meincke et al., 2009) in the nested PCR approach using Taq-&Go™Ready-to-use PCR Mix (MP Biomedicals). Duplicate PCR products from all templates (eight per plant) were purified with Wizard® SV Gel and the PCR Clean-Up System (Promega). Amplicons of each endemic- and maize plant-associated *Trichoderma*, respectively, were pooled together and pyrosequenced using the Roche/454 GS FLX+ Titanium platform executed by GATC Biotech (Konstanz, Germany). Raw sequencing reads were subjected to demultiplexing, denoising (Reeder and Knight 2010) and de novo chimera removal (Edgar, 2010) prior to quality (score > 25) and length filtering (150–300 bp) using scripts provided by the open source software package QIIME v. 1.6.0 (Caporaso et al., 2010). Remaining reads were *de novo* clustered to OTUs at a similarity level of 98% using a uclust algorithm (Edgar, 2010). The study is registered as a NCBI BioProject 239662, the metadata for each sample are available at NCBI in the BioSample database (accession numbers SAMN04202633–SAMN04202644).

The taxonomy-based gene amplicon libraries specific for *Trichoderma* were analysed with *Trich*OKEY (http://www.isth.info/tools/molkey/index.php). Rarefaction analysis was performed for phylotype clusters at 98% similarity by using the tools of the

RDP's Pyrosequencing Pipeline (Cole et al., 2009). Due to differing numbers of sequences among samples, datasets were randomly normalized to the lowest common number. The open source software package QIIME was used to calculate the richness and diversity indices (Caporaso et al., 2010). Species identification of particular OTUs was carried out according to the method described by Druzhinina et al. (2006). For the calculation of the core microbiome, OTUs were only considered as core when they occurred in ten of all 12 rhizosphere samples.

A network-based analysis with Cytoscape (Shannon et al., 2003) was applied to examine the co-occurrence of OTUs in the rhizosphere samples. The network allows the visualization of OTUs that are either specific for plants or sampling sites, or that are shared by specific groups. The network diagram additionally visualizes connections between rhizosphere samples and OTUs. In order to cluster the OTUs in the network, a default-directed algorithm layout with an applied read change of five was used with edge and node widths that are defined as the abundances of sequences and the mean from each OTU that occurred within the sample, respectively.

2.5. Screening for antagonistic potential to plant pathogens

The *in vitro* inhibition of *Botrytis cinerea* Pers. P3, *Rhizoctonia solani* Kühn AG2-2IIIB and AG4, *Sclerotium rolfsii* Sacc. 398, and *Verticillium dahliae* Kleb. V25 were determined by a dual-culture assay on Waksman agar according to Berg et al. (2005). Zones of hyperparasitism or inhibition were measured after three to seven days of incubation at 25 °C. Assays were done for each plant in four replications sampled from two sites. Significant differences in antagonistic percentages and abundances (see below) of fungi and *Trichoderma* isolates were calculated using ANOVA with the post hoc test Scheffé with SPSS PASW Statistics v.18.

3. Results

3.1. Analysis Trichoderma abundances

Using different media for cultivation combined with a cultivation-independent approach (total *Trichoderma* gene copy numbers by quantitative real time PCR) in all samples *Trichoderma* strains were detected. Colony forming units (CFU) of fungi ranged from $\log_{10}$ $5.7 \pm 0.2\,g^{-1}$ to $\log_{10}$ $6.6 \pm 0.1\,g^{-1}$, and from $\log_{10}$ $3.9 \pm 0.2\,g^{-1}$ to $\log_{10}$ $5.3 \pm 0.6\,g^{-1}$ for cultivated *Trichoderma* species (Table S1). There were no statistically significant differences between fungal and *Trichoderma* abundances calculated for the endemic plants and maize. Abundances of *Trichoderma* determined by qPCR resulted in generally higher abundances for *Trichoderma* (from $\log_{10}$ $5.4 \pm 0.2\,g^{-1}$ to $\log_{10}$ $8.5 \pm 0.2\,g^{-1}$) in comparison with the CFU obtained by cultivation, however, there were no statistically significant differences between both plant groups.

3.2. Deep insight into the Trichoderma community by amplicon sequencing

For a more complex understanding of *Trichoderma* diversity, samples of endemic and the crop plant maize from all sites were analysed and compared with ITS1 and 2 amplicon libraries obtained by 454-pyrosequencing. From the 12 rhizosphere samples from endemic and maize plants collected worldwide, a total of 16,596 quality sequences with a read length of 150–300 bp were recovered and comprised 74 operational taxonomic units (OTUs). From representative reads of all OTUs, 62 were able to be classified by *Trich*OKEY. Altogether 12 were assigned to an unidentified species of *Trichoderma* and were additionally compared with *Trich*oBLAST. Rarefaction curves of observed OTUs continued to rise with increasing numbers of sequences (Fig. S1).

Diversity of *Trichoderma* species explored by the Shannon diversity index (H') and calculated at the genetic distance of 3%, indicated different diversities for different plant species. Maize plants (average 5.5 ± 0.6) had a generally high diversity independent of the site, while the diversity on endemic plants was quite different (average 4.8 ± 0.4) (Table S2). The highest diversity was found for *Diospyros* in Madagascar (average 5.9 ± 0.2) and *Hebe* in New Zealand (average 5.2 ± 0.0), while endemic plants of Canary Island (average 4.2 ± 0.2) and the Austrian Alps (average 3.8 ± 0.1) had a lower *Trichoderma* diversity.

The *Trichoderma* community structures of all plants were compared to identify overlapping OTUs among all sampling sites and plants. The core was represented by three OTUs, which represented 26.0% (4314) of the total sequences and were shared by all plants. The shared OTUs were dominated by *T. koningii* (54.0%) and *T. koningiopsis* (45.0%) with a small percentage (1.3%) from *T. paucisporum*. The highest proportion of the shared sequences were found in associations with plants from Canary Island (34.5%) and Madagascar (33.5%), followed by Austria (20.6%) and New Zealand (15.9%).

Comparison of the classified reads revealed that the *Trichoderma* populations in rhizosphere of endemic plants were specific (Fig. 1). The highest proportion of specific OTUs were found among the endemic plants in Madagascar (23.6%) and New Zealand (12.2%), followed by Canary Island (3.7%) and Austria (0.5%). Sequence comparisons revealed one of the dominant and specific species of *Diospyros* sp. (M) was *T. spirale* with 63.4% or of *Hebe* sp. (NZ) *T. stromaticum* with 40.6%. All *Trichoderma* rhizosphere populations in maize shared 5294 reads (average 65.6%) comprising six OTUs containing four species. These common maize OTUs were found independently from their origin with the highest proportion in New Zealand (82.7%) with *T. hamatum* as the dominant species (59.6%).

Principal component analysis (PCA) based on *Trichoderma* OTUs of endemic plants and maize showed the close similarity of plant-associated communities depending on the sampling site (Fig. 2). The crowding points of the PCA showed a specific cloud comprising all OTUs from one location. Conversely, OTUs of maize plants occurred worldwide. The crowding points of the PCA showed an overlap of the dominant *Trichoderma* with the worldwide occurring plant OTUs, thus *Trichoderma* communities obtained from all maize plants shared OTUs from all sampling sites.

3.3. Network analyses to connect Trichoderma diversity

According to Yodzis (1980) the taxonomic data was summarized in a default-directed network to visualize the connectivity between pairs of host plants and *Trichoderma* OTUs as well as to understand how the OTUs are partitioned between different endemic or maize plants (Fig. 3). The node and edge sizes represent the abundance and average of sequences respectively (per plant/site). The *Trichoderma* network of the endemic plants consisted of 58 nodes and 94 edges, while the maize plants contained 48 nodes and 94 edges (Fig. 3a). The edges clearly showed a *Trichoderma* hotspot of specific OTUs from 12 specific nodes on *Diospyros* (M) and *Hebe* species (NZ), whereas only a few specific OTUs were detected on *Aeonium* (C) and *Rhododendron* (A). Remarkably, the greatest disparity in OTUs (9) was shared by *Rhododendron* (A) and *Hebe* (NZ) species. Conversely, the OTUs from the *Trichoderma* community on maize worldwide were highly common (Fig. 3b). Many OTUs were shared between Austria, Madagascar and New Zealand (6), followed by Madagascar and New Zealand (5), and lastly by Austria and New Zealand (4). However, with an applied read change of five, which considered only the dominant species in a comparison of two, no connectivity was visualized for all sites, even though core species were found in all sites. Note that the connectivity supports the visualization of the most dominant species in all sites. All possible shared com-

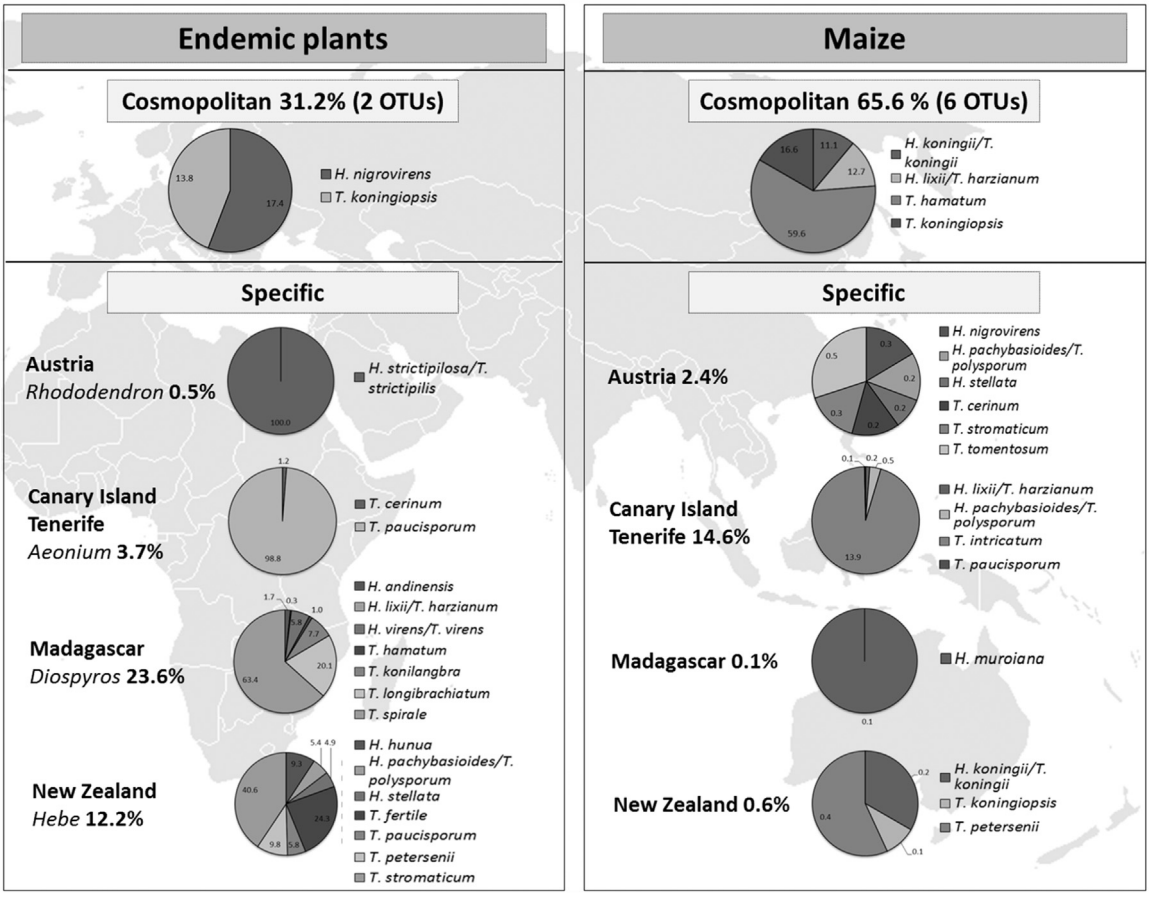


Fig. 1. Distribution of cosmopolitan and specific *Trichoderma* operational taxonomic units associated with endemic plants and maize.

Table 2a
Proportion (%) of antagonistic *Trichoderma* isolates towards different phytopathogens obtained by dual culture *in vitro* experiments. Different letters indicate statistically different values (Tukey's post hoc test: $P < 0.05$).

Pathogen	*%*	
B. cinerea	61.7 ± 5.1	b
R. solani AG2	52.9 ± 5.3	ab
R. solani AG4	52.8 ± 5.4	ab
S. rolfsii	50.1 ± 5.7	a
V. dahliae	56.4 ± 5.3	ab
Average	*54.8 ± 2.4*	

Table 2b
Proportion (%) of antagonistic *Trichoderma* isolates of endemic and maize plants obtained by dual culture *in vitro* experiments. Different letters indicate statistically different values (Tukey's post hoc test: $P < 0.05$).

Sampling site	*Plant species*	*%*	
Austria	*Rhododendron ferrugineum*	43.9 ± 9.8	ab
(A)	*Rhododendron hirsutum*	29.3 ± 7.0	a
	Zea mays	50.2 ± 8.6	abc
Canary	*Aeonium cuneatum*	58.0 ± 7.0	bcd
Island	*Aeonium urbicum*	50.8 ± 8.9	abc
(C)	*Zea mays*	42.7 ± 8.3	ab
Madagascar	*Diospyros gracilipes*	73.7 ± 7.2	cd
(M)	*Diospyros myriophylla*	62.6 ± 7.4	bcd
	Zea mays	57.0 ± 6.9	bcd
New	*Hebe salicifolia*	38.3 ± 6.1	ab
Zealand	*Hebe strictissima*	59.1 ± 8.1	bcd
(NZ)	*Zea mays*	77.6 ± 6.1	d

binations were found with the exception of Austria/Canary Island Tenerife/Madagascar and Canary Island Tenerife/Madagascar.

3.4. *Antagonistic potential of the* Trichoderma *community*

To assess the indigenous antifungal potential, the antagonistic activity against five major phytopathogenic fungi (*Botrytis cinerea*, *Rhizoctonia solani* AG2-2IIIB and AG4, *Sclerotium rolfsii*, and *Verticillium dahliae)* was determined. In total, 9020 randomly selected *Trichoderma* isolates were tested. On average, the highest proportion of *Trichoderma* antagonists were found against *B. cinerea*, followed by *V. dahliae*, *R. solani* AG2, *R. solani* AG4, and *S. rolfsii* (Table 2a). With regards to the sampling site, *Trichoderma* isolates from Madagascar and New Zealand (average: M: 64.5% ± 4.2, NZ: 62.3% ± 4.7) demonstrated the highest *in vitro* antagonism against all five pathogens. All *Trichoderma* isolates associated with endemic plants showed a higher antifungal potential than maize-associated strains, with the exception of maize grown in Austria and New Zealand (Table 2b).

4. Discussion

Our study contributes valuable information concerning *Trichoderma* biodiversity, community structure and antagonistic potential at a global scale to the current knowledge (Jaklitsch and Voglmayr, 2015). The specificity of the *Trichoderma* populations for endemic plants and additionally maize was high. Moreover, endemic plants functioning as indicators for natural ecosystems are characterized by a distinct fungal microbiome containing a high proportion of antagonistic *Trichoderma* strains. As a result of our thorough investigation of the genus *Trichoderma* at a global scale, we made three observations summarizing our hypothesis: i) Native *Trichoderma* diversity was highest for endemic plants with highly specific OTUs (up to 24%); whereas common maize OTUs (65.5%)

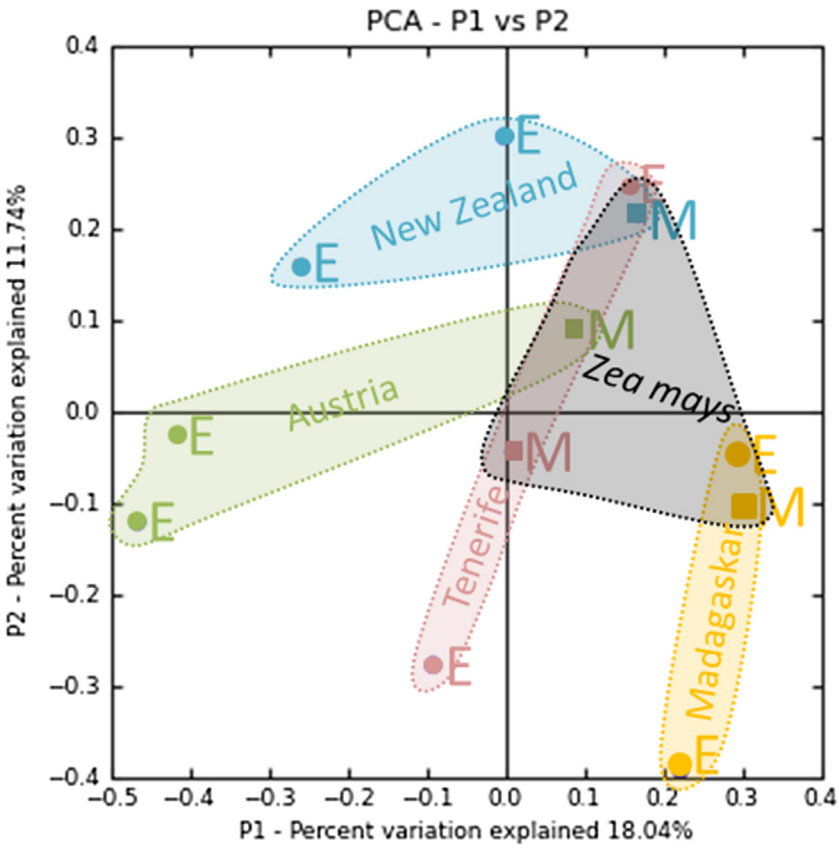


Fig. 2. Principal component analysis biplot of operational taxonomic units identified by *Trichoderma* amplicon sequencing. Legend: E—endemic plants, M—maize plants, colour code: green—Austria, pink—Canary Island Tenerife, yellow—Madagascar, blue—New Zealand, black—maize-associated reads.

exist at all locations. On an intercontinental scale ii) a global core community, which is shared by all analysed plants (26% of genus-specific sequences, three OTUs), was found and iii) endemic plants harbour a higher proportion of antagonistic *Trichoderma* isolates than maize-associated strains for the hotspots Madagascar and New Zealand.

Specific OTUs for endemic plants reaching up to 24% were found in the *Trichoderma* community structure in the hotspots Madagascar and New Zealand. The endemic plants *Diospyros* (Madagascar) and *Hebe* (New Zealand) were associated with the highest number of specific OTUs and revealed the highest diversity indices in comparison with the other endemic plants (Fig. 1). For the fungal community, diverse ecological and evolutionary strategies were presented. In comparison with the whole fungal community *Trichoderma*-specific communities showed a low diversity and no correlation to plant communities or abiotic factors (Zachow et al., 2009). In fact, a higher proportion of specificity was found for the entire plant-associated fungal community of forest trees, where 35–37% of fungal OTUs were found to be tree-specific (Urbanová et al., 2015). Only few cases of correlation of species occurrence with abiotic factors for soil *Trichoderma* from the Island of Sardinia were recorded (Migheli et al., 2009). The increasing resolution of sequence data and the sampling of endemic plant rhizospheres allowed us to detect a relatively high (up to 24%) specificity of *Trichoderma*. Here, a follow-up study is conceivable analysing the fungal community of the endemic plants and maize at a global scale. For the endemic plant-*Trichoderma* systems a higher proportion of specific OTUs were found compared to the interactions of *Trichoderma* and maize crops.

Moreover, in our study dealing with worldwide samples, 65.5% of maize *Trichoderma* OTUs was shared in all locations, regardless of maize cultivar and geographical region. Both factors are known drivers of microbial diversity (Green and Bohannan, 2006; Rousk et al., 2010; Bulgarelli et al., 2012). The low specificity level and opportunistic character as a result of extremely high antagonistic activity against fungi support that *Trichoderma* have become ubiquitous cosmopolitans with a highly invasive potential into existing agricultural systems. Fungi have been moved across continents, even though their impact on ecosystem structure and function has rarely been quantified (Gladieux et al., 2015). Possible mechanisms for species transfer over long distances include transmission by strong winds; fungal spores have been detected in the stratosphere (Muñoz et al., 2004; Wilkinson et al., 2012). Soil pH, moisture content and geographic patterns likely interact to shape the spatial scaling of the rhizosphere microbiota of the bacterial microbiome from the maize rhizosphere Peiffer et al. (2013). A core of 49.2% common bacterial OTUs were found in a comparison of four main *Lactuca sativa* cultivar groups and the wild ancestor *Lactuca serriola* grown in the same environment (Cardinale et al., 2015). What our findings add is that a high proportion of the rhizosphere *Trichoderma* can assemble quite similarly across environments. The influence of domestication exceeded that of environment, which suggests that the host genotype is of main influence on the root microbiota, demonstrating that a part of the microbiota remains responsible for the core community.

We found a global core community for *Trichoderma* that was shared by all analysed plants (26% of genus-specific sequences, three OTUs). Several previous publications have already shown the opportunistic character of cosmopolitan *Trichoderma* species, e.g. *T. koningii, T. koningiopsis* and *T. paucisporum*, which were described as pan-Europe and/or pan-global *Trichoderma* species (Atanasova et al., 2013b; Friedl and Druzhinina, 2012; Migheli

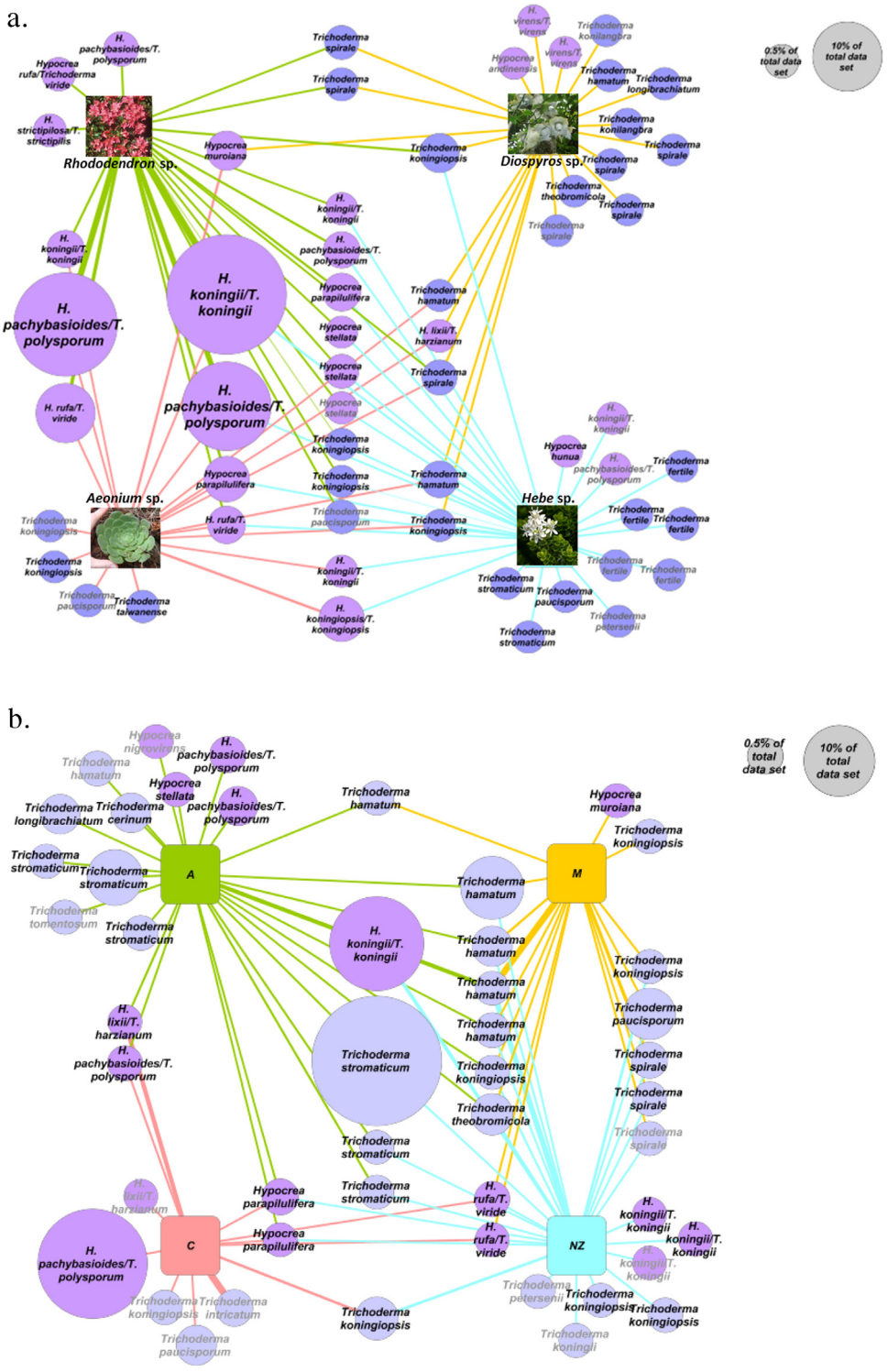


Fig. 3. Profile clustering network analyses of (a, b) *Trichoderma* associated with plants worldwide. OTUs at a dissimilarity level of 3% (black node labels indicate a taxonomic similarity ≥97%) obtained by an edged-weighted force-directed algorithm were visualized. The relative abundance values for operational taxonomic units with a cumulative read change ≥ 5 were used. Node sizes of OTUs correspond to the mean relative abundance change between the (b) endemic plants and (c) maize plants and a node matching to 0.5% and 10% of the total data set were added as reference points. A—Austria, C—Canary Island Tenerife, M—Madagascar, NZ—New Zealand. Node label colour indicate the taxonomic similarity black—≥97%, grey—≤97%, purple—sequences were locked by the depositor of the UNITE database.

et al., 2009; Samuels et al., 2006; Zachow et al., 2009). Meiser et al. (2014) compared results from studies dealing with the entire fungal community from soil, phyllosphere or indoor habitats and did not find globally distributed OTUs, even when comparing fungal communities from similar habitats. This suggested that high local fungal diversity scales up to high global diversity. However, fungal communities are affected by dominant vegetation, e.g. the entire fungal community of forest trees shared 15–45% of the fungal OTUs (Urbanová et al., 2015); whereas the *Trichoderma*-specific communities showed no correlation to plant communities (Zachow et al., 2009). The host generality is discussed for endemic plants and maize.

Our next hypothesis – that endemic plants harbour a high proportion of specific *Trichoderma* strains and antagonists to protect themselves against pathogens as a result of their long-time plant-microbe relationship – was also confirmed. The number of antagonists of plant pathogens was significantly higher in endemic plants compared to maize. These findings support the theory of naturally occurring plants with a balanced microbiome as resource for antagonists (Opelt et al., 2007). The biocontrol potential of *Trichoderma* spp. and their ability to induce plant drought tolerance has been recognized in several publications (Calvo et al., 2014 Atanasova et al., 2013a). In comparison with other studies, especially those involving bacterial antagonists (Berg et al., 2002, 2005), the antagonistic potential of the rhizosphere fungal communities was very high and included approximately one third of the fungal and more than half of the *Trichoderma* populations. Whole genome sequences are becoming increasingly important with the use of biological control (*T. atroviride, T. harzianum, T. virens, T. longibrachiatum, T. citrinoviride, T. asperellum*) and industrial strains (*T. reesei*) for industrial applications. Genome comparisons with the mycoparasitic *T. atroviride* and *T. virens* species vs. the saprophytic *T. reesei* identified components predicted to contribute to a parasitic lifestyle. Recently Atanasova et al. (2013a) revisited the mycoparasitism of *Trichoderma* at the molecular physiology level. They display different strategies to antagonize their host or prey and the key to successful antagonism it is more likely the regulation of gene expression than the availability of specific genes. The one is for antagonistic activity and the other one for nutrient acquisition, and consequently it would be of interest to evaluate the molecular base and strategy of *Trichoderma* from endemic and cosmopolitan plants on a global scale.

5. Conclusion

In this study, we explored areas of high diversity at different global locations in an effort to contribute to the knowledge of *Trichoderma* from cosmopolitan and endemic plants at a global scale. A global core *Trichoderma*-biome associated with all analysed plants exists; whereas the endemic plants harboured highly specific *Trichoderma* communities and maize acquired specific *Trichoderma*-OTUs shared with all plants independent from site. Endemic plants, as an indicator for natural ecosystems, harbour an extraordinary high proportion of antagonistic *Trichoderma* species with hot spots in Madagascar and New Zealand. Another reason to protect and maintain natural ecosystems is the existence of hidden and often completely unknown diversity in plant biomes.

Author contributions

CZ, CB, HM, JM and GB designed the study. CZ and HM performed the molecular, microbial and bioanalytical work. CB, JM and GB were responsible for sampling worldwide.

CZ, HM and GB drafting the article, CB and GB revising it critically for important intellectual content and all authors approved the final version to be published.

Acknowledgement

The authors would like to thank Barbara Fetz, Monika Schneider-Trampitsch, Stefanie Maier and Melanie Marek (Graz) for valuable technical assistance. We are grateful to Jacky Andriatiana from Parc Botanique et Zoologique de Tsimbazaza, Antananarivo, Madagascar, for valuable field support and to Meg Starcher (Washington DC.) for language support. This work has been supported by a grant to G.B. from the Federal Ministry of Economy, Family and Youth (BMWFJ), the Federal Ministry of Traffic, Innovation and Technology (bmvit), the Styrian Business Promotion Agency SFG, the Standortagentur Tirol and ZIT—Technology Agency of the City of Vienna through the COMET-Funding Program managed by the Austrian Research Promotion Agency FFG.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jbiotec.2016.03.049.

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