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Review:

Fungal genus *Hypocrea*/*Trichoderma*: from barcodes to biodiversity*

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Abstract: *Hypocrea*/*Trichoderma* is a genus of soil-borne or wood-decaying fungi containing members important to mankind as producers of industrial enzymes and biocontrol agents against plant pathogens, but also as opportunistic pathogens of immunocompromised humans and animals, while others can cause damage to cultivated mushroom. With the recent advent of a reliable, BarCode-aided identification system for all known taxa of *Trichoderma* and *Hypocrea*, it became now possible to study some of the biological fundamentals of the diversity in this fungal genus in more detail. In this article, we will therefore review recent progress in (1) the understanding of the geographic distribution of individual taxa; (2) mechanisms of speciation leading to development of mushroom diseases and facultative human mycoses; and (3) the possible correlation of specific traits of secondary metabolism and molecular phylogeny.

Key words: *Hypocrea*/*Trichoderma*, Biogeography, Biodiversity, Facultative human opportunists, Peptaibols, Mushroom pathogens

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INTRODUCTION

Among thousands of fungal genera, *Hypocrea* (anamorph *Trichoderma*, *Ascomycota*, *Hypocreales*) is one of those with the broadest impact on mankind. Some of its species, particularly *H. jecorina*/*T. reesei*, are known as industrial producers of enzymes and recombinant proteins, some used for the production of secondary metabolites, e.g., the coconut aroma 6-pentyl-α-pyrone by *H. atroviridis*/*T. atroviride* (Dodd *et al*., 2003), whereas others (notably *H. lixii*/*T. harzianum*, *H. virens*/*T. virens*, *H. atroviridis*/*T. atroviride* and *T. asperellum*) are used as biocontrol agents against various diseases of crops, vegetables, and fruits (Harman *et al*., 2004). More recently, this spectrum was even expanded towards the biocontrol of nematodes (Goswami *et al*., 2008; Kyalo *et al*., 2007; Dababat *et al*., 2006). On the other hand, *Trichoderma* species have been known for a long time to infect and deteriorate commercial mushroom farms (Seaby, 1998) and more recently also become opportunistic pathogens of immunocompromised humans and animals. From a basic biological perspective, members of the genus can be described as cosmopolitan and ubiquitous inhabitants of soils, decaying wood and plant debris. Many species of the genus are potent mycoparasites, and Rossmann *et al*.(1999) raised the hypothesis that during evolution, *Hypocrea*/*Trichoderma* learned to follow their hosts into decaying wood by feeding from it as a secondary colonizer. Yet their rapid growth and the ability to metabolize an amazing diversity of substrata, *Trichoderma* spp. have become predominant components of the soil mycobiota of different ecosystems, e.g., agricultural fields, pastures, forests, salt marshes, prairies, and deserts in a wide range of climatic zones (Klein and Eveleigh, 1998). The high ecological adaptability of the genus *Hypocrea*/*Trichoderma* makes a deeper understanding of its ecology and diversity worthwhile.

Understanding the ecology of *Hypocrea*/

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Trichoderma has long been hampered by the fact that the homoplasy of morphological characters made species identification difficult and prone to errors. For example, the pioneering studies on the distribution of *Trichoderma* species in soil and rhizosphere ecosystems (Danielson and Davey, 1973; Widden and Abitbol, 1980; Nelson, 1982) must be read with outmost caution, as the reported species names are in majority incorrect (C.P. Kubicek, unpublished data). This is not the authors' fault, but due to the fact that at that time only a limited number of today's known species had been recognized, and to the almost impossibility to identify *Trichoderma* spp. only by morphological examination. However, by the aid of the recently developed online identification tools *Trich*OKey and *Tricho*BLAST (ISTH, 2008), which are based on sequence analysis of the internal transcribed spacers (ITS1 and ITS2) of the rRNA gene cluster and on the long (4th) intron of translation elongation factor 1-alpha (Druzhinina *et al*., 2005; Kopchinskiy *et al*., 2005) and whose reference database is constantly updated (Druzhinina and Kopchinskiy, 2006), it is now possible to identify virtually every *Trichoderma* isolate and/or recognize it as a putative new species. This in turn now enabled studying questions on the ecology and biodiversity of individual species of this genus.

In this review, we shall summarize some of the recent progress which has been made in these areas.

GEOGRAPHIC DISTRIBUTION OF *HYPOCREA*/*TRICHODERMA*

Many mitosporic fungi, such as species of *Aspergillus*, *Penicillium*, *Chaetomium*, or *Trichoderma*, are believed to be globally distributed. Finlay (2002) has postulated that organisms below a critical size (e.g., <1 mm) may have a world-wide distribution. However, data used to support this postulate have mostly been based on species identifications by morphological means which renders them subject to the criticisms raised above. The use of DNA-based species identification tools has now revised many of these claims, by showing that these species actually are composed of two or more phylogenetic species, each of which displays restricted biogeography (Koufopanou *et al*., 2001; Geiser *et al*., 1998; Burt *et al*., 1996; Kasuga *et al*., 2003). The global occurrence of *Trichoderma* was recently investigated in several studies (Kullnig *et al*., 2000; Kubicek *et al*., 2003; Wuczkowski *et al*., 2003; Gherbawy *et al*., 2004; Druzhinina *et al*., 2005; Zhang *et al*., 2005). While these studies revealed the worldwide predominance of some species such as *T. harzianum*, they also revealed a putative geographic bias for a number of other taxa. This is best exemplified in a summary of the geographic occurrence of species from section *Longibrachiatum* (Table 1), of which, only *T. longibrachiatum*, *H. orientalis*, and *T. ghanense* appear to

Table 1 Geographic distribution of taxa from section *Longibrachiatum*[*]

Taxa	Geographic distribution
T. longibrachiatum	North and South America, all Asia, Europe, Africa, Pacific
H. orientalis	North and South America, Europe, East Asia, Africa
T. citrinoviride/*H. schweinitzii*	North and South America, Asia, Siberia, Europe
T. pseudokoningii	Australia
T. ghanense	Europe, North America, Africa, Iran, South-East Asia, Australia
T. saturnisporum	USA, Europe, South Africa, Central America
T. effusum	Himalayas
T. sinensis	Thailand, Taiwan and Yunnan of China, USA
H. andinensis	Western South America, Hawai
H. novazelandia	New Zealand
T. konilangbra	Kenya, Etiopia
H. jecorina	South America, Central Africa, Indonesia, Pacific (Micronesia), Ceylon

[*]Data taken from Turner *et al*.(1997), Kullnig *et al*.(2000), Kubicek *et al*.(2003), Druzhinina *et al*.(2005), Zhang *et al*.(2005), and unpublished work in our own laboratory

fulfil the criterion of a pangaean distribution, although the latter two are more frequent in warmer and tropical areas. *H. schweinitzii*/*T. citrinoviride* is interesting as it is also widely distributed but has so far never been found in samples from Africa or the Indian Subcontinent. Also *H. jecorina* exhibits a world wide distribution with the exception that it is only found at low altitudes and within a narrow belt around the equator (±20 degrees altitude). Reports on the occurrence of its anamorph outside this range are due to confusion with a closely related sister species, with which it shares an identical ITS1 and ITS2 sequences (M. Komon-Zelazowska and C.P. Kubicek, unpublished data). All the other species of this section have so far only been found from restricted areas, such as *T. pseudokoningii* in Australia, *T. konilangbra* in Eastern Africa, *T. effusum* in the Himalayas, or *H. novazelandia* in New Zealand. Caution must, however, be applied to these examples, as most of them are based on single isolation and it may as well be that these taxa require special conditions for isolation. Examples in this direction are *T. sinensis*, which was previously found in South-East Asia only, but recently also detected on decorticated wood in South Carolina, USA (DAOM 216502; J. Bissett, personal communication). Similarly, *H. andinensis* was described on the basis of a single isolate found in the Andean mountains in South-West Venezuela, but recently also detected in the soil of Hawai (C.P. Kubicek, unpublished data).

Another interesting example of biogeographic distribution was recently documented in the *T. koningii* species complex: this taxon is frequently reported as a component from soil and decaying plants, but Samuels *et al*.(2006), using a multilocus sequence analysis, were able to dissect it into 11 phylogenetic species. They exhibited a clear biogeographic bias (Table 2) that the "true" *H. koningii*/*T. koningii* is limited to eastern North America and Europe, whereas *T. koningiopsis* is a common and cosmopolitan species, although more common at tropical than at temperate latitudes (American tropics, Canada and Germany), and its teleomorph has been found in Kentucky, USA. It was also found in the rhizosphere of *Coffea arabica* from the main coffee-growing area in Ethiopia, where sampling was done from elevations of 1300~2000 m (T. Belayneh, personal communication).

Table 2 Geographic distribution of the "*koningii*" clade of section *Trichoderma*[*]

Taxa	Geographic distribution
T. austrokoningii	USA, Europe, Russia, Australia, New Zealand
T. carribeum	Puerto Rico, Guadeloupe, Ecuador
T. dingleyae	New Zealand
T. dorotheae	Australia, New Zealand
T. intricatum	Peru, Thailand
T. koningii	Europe, eastern North America
T. koningiopsis	North, Central and South America, Europe, Ghana, Etiopia
T. ovalisporum	South America
T. petersenii	North and Central America, Europe
T. rogersonii	USA, Europe
T. taiwanense	Taiwan of China

[*]Data summarized from Samuels *et al*.(2006)

T. petersenii and *T. rogersonii* are restricted to eastern North America and Central Europe; stromata of the teleomorph corresponding to *T. petersenii* have been collected also in Costa Rica. *Trichoderma intricatum* is known only from two ascospore-derived cultures that originate in Puerto Rico and Thailand, respectively. *T. austrokoningii* includes six isolates with unclear phylogenetic position because of non-concordant topologies in the trees from three loci. The sequence divergence in this clade suggests that additional sampling would resolve it into two or more species. Thus, these species represent interesting cases of both allopatric and sympatric speciation, which challenges further investigations on the biological basis for these events.

While the studies described above provide some insight into geographic preferences of individual *Trichoderma* spp. and led to the detection of a number of new species, all these studies relied only on a broad-scale sampling over a wide geographic areas. Characteristics of soil properties and vegetation of individual ecosystems have not been sufficiently considered. Towards closure of this gap of knowledge, other colleagues and we have recently performed several in-depth investigations on the occurrence of *Hypocrea*/*Trichoderma* in two selected ecosystems and attempted to correlate species occurrence with ecological parameters. The first of these ecosystems was the Tyrrhenian Island of Sardinia (Migheli *et al*., 2008). Sardinia is located in the centre of the Medi-

terranean basin, which is considered a hotspot of biodiversity and endemism (Médail and Quézal, 1999; Grill *et al*., 2006). Sardinia has become isolated since the early Miocene (7~8 Ma), and should thus have given rise to allopatric speciation events. Contrary to this expectation, we found nearly exclusively common cosmopolitan and opportunistic *Hypocrea*/*Trichoderma* species which were already known to science by isolations from many locations in Eurasia, Africa and other continents. In general, soil diversity of *Hypocrea/Trichoderma* on Sardinia was relatively low (15 species among 483 isolates, diversity index *DI*=0.03), although some sampling sites (such as the costal slope of the Limbara and of the Gennargentu mountains, respectively) exhibited higher values probably due to their exposure to winds from east and south, which may bring fungal conidia from the closely located Italian peninsula (200 km) and areas located behind it. However, it must also not be neglected that the higher diversity of *Hypocrea*/*Trichoderma* on the eastern side could be due to the fact that these locations contain the main seaports on Sardinia. This argument is supported by a relatively large number of species found in northern sampling site (close to Porto Torres, the ancient Roman colony of Turris Lybissonis) and southern pasture (located in the vicinity of Cagliari). In any case, these facts strongly argue towards an invasive origin of all species found. This also coincides with the fact that all species detected in this study had a high antagonistic potential towards other soil fungi.

In addition, all but one of the species isolated in this study exhibited ITS1 and ITS2 alleles identical to those known from pan-European and even pan-global species. Since the ITS1 and ITS2 sequences of ascomycetes are known to have a mutation rate of 1 nt per 0.6 (±0.5) per site and mio years (Kasuga *et al*., 2003), endemism in Sardinia should have led to the development of new SNPs. Only *T. hamatum* fulfilled this expectation, suggesting that this may be a truly endemic species in Sardinia.

In a second example, we investigated *Hypocrea*/*Trichoderma* populatons on the island of Tenerife in the Canarian Archipelago (Zachow *et al*., 2008). It is of recent volcanic origin (2 Ma) and represented by six different vegetation zones characterized by specific abiotic conditions and plant communities also with a high proportion of endemic plants. From 12 sampling points dispersed on the whole island, molecular investigation of fungal communities was determined by single strand conformation polymorphism (SSCP) analysis using universal and specific primers for *Hypocrea*/*Trichoderma*. While the highly diverse fungal communities were mainly characterized by ectomycorrhiza-forming *Basidiomycota* and a high proportion of yet unidentified species, *Trichoderma*-specific SSCP and ITS1 and ITS2/*tef1* sequencing of cultivated samples resulted in detection of only a very low diversity of mainly cosmopolitan species, e.g., *Hypocrea lixii*/*T. harzianum*, *T. gamsii* and *T. viridescens*. All *Trichoderma* isolates show an extraordinarily high antagonistic potential towards different groups of plant pathogens, supporting the hypothesis of extensive colonization by highly competitive *Hypocrea*/*Trichoderma* species from the nieghboring continent. Thus, Tenerife, like Sardinia, is also mainly colonized by ubiquitously and widely distributed *Hypocrea*/*Trichoderma* species and not by endemics (Table 3). Nevertheless, a species-to-species comparison of the diversity shows significant

Table 3 Comparison of distribution of *Hypocrea*/*Trichoderma* spp. in soils from Sardinia and from Tenerife*

Trichoderma section		Sardinia (%)	Tenerife (%)
Pachybasium	*T. harzianum*	**58.2**	**57.9**
	T. spirale	7.1	Yes
	T. velutinum	4.6	Yes
	T. virens	3.9	ND
	T. alnii	0.2	ND
	H. semiorbis	1	ND
	T. tomentosum	2.3	ND
	T. cf. *tomentosum*	ND	3.3
	H. cremea	ND	Yes
	T. stromaticum	ND	Yes
Trichoderma	*T. hamatum*	4.6	ND
	T. viridescens	**1**	**7.2**
	T. gamsii	**10.8**	**25**
	T. viride/*H. rufa*	ND	3.3
	T. koningii	3.9	ND
	T. koningiopsis	0.6	ND
	T. asperellum	0.6	ND
	T. rogersonii	ND	3.3
	T. atroviride	ND	Yes

*Data are taken from Migheli *et al*.(2008) and Zachow *et al*.(2008). Values printed in bold indicate species which were abundant in both studies; "Yes" indicates strains whose DNA was directly amplified from soil without subcultivation; ND: Not detected

differences. Although this may be in part due to the strongly differing number of samples in these two studies, and it cannot be ruled out that the species composition may become similar if Tenerife is also sampled as strongly as Sardinia, it could also indicate the different air flows transporting conidia to the island. The detection of *T. stromaticum*, which is known as an endophyte on *Theobroma cacao* (Samuels *et al*., 2006) but has also been found in South African soils (*Trichoderma* sp. PPRI 3559, I.S. Druzhinina and C.P. Kubicek, unpublished data), indicates some transport from either South America or Africa, and the finding of *T. cremea*, a species only known from its teleomorph in the US and New Zealand, suggests long distance dispersal as well.

SPECIATION TOWARDS MUSHROOM PATHOGENS

Infection of commercial mushroom farms by *Trichoderma* spp. has been reported since the past several decades. Yet the first severe epidemic arose in the late 1980s in northern Ireland, UK, where farms producing *Agaricus bisporus* suffered from heavy attack by *Trichoderma* spp. (Seaby, 1998). The disease, while slowly spreading towards middle and southern Europe, was a few years later also observed in the North America. For over a decade, it was believed that this was due to *T. harzianum* (Muthumeenakshi *et al*., 1994; Castle *et al*., 1998), and some authors even suspected it to be due to the release of some *T. harzianum* biocontrol strains (Ospina-Giraldo *et al*., 1999), but Samuels *et al*.(2002) clearly showed that it was due to a new species of *Trichoderma*, i.e., *T. aggressivum*. Subtle morphophysiological differences and SNPs in ITS1 and ITS2 led them to separate the Northern American and European strains into two *formae speciales*, i.e., *T. aggressivum* f. sp. *aggressivum* and *T. aggressivum* f. sp. *europeae*. The nt differences between both of them and their next phylogenetic neighbors (*H. tawa*) in ITS1 and ITS2 are high enough (3~5 nt) to conclude that the two species are not the result of a recent clonal speciation from biocontrol strains of *T. harzianum*, but exist as separate species for at least several mio years. Interestingly, a multilocus phylogenetic analysis of *T. aggressivum* (using ITS1 and ITS2, *tef1*, and *chi18-5*) consistently placed the Northern American s. sp. basal to the European one, suggesting that it arose earlier in evolution. Thus, these two subspecies represent a case of allopatric speciation. We consider a vicariance event likely, because the 3 nt differences in ITS1 and ITS2 between the two *formae speciales* are consistent with a separation time of (1.8±1.0) Ma and thus the elimination of the land bridges between Europe and the America. This would also imply that these two species have special vectors for their distribution, and are not simply transported by air. The alternative hypothesis that they require special nutrients which are not usually met in soil (a fact which would be substantiated by the lack of detection of *T. aggressivum* in soil samples so far) appears less likely as they show very similar to the ubiquitous *T. harzianum* phenotype microarray carbon source utilization profiles (Komon-Zelazowska *et al*., 2007).

A similar epidemic occurred a few years ago, however, not on *A. bisporus* but on the oyster mushroom *Pleurotus ostreatus*. Although this epidemic was originally believed to be also caused by *T. aggressivum*, we could recently show that it is actually two different although genetically closely related species, *T. pleurotum* and *T. pleuroticola* (Park *et al*., 2006; Hatvani *et al*., 2007; Komon-Zelazowska *et al*., 2007). Interestingly, both species are closely related to the *H. lixii*/*T. harzianum* species aggregate and to *T. aggressivum*. *Pleurotus* "green mould disease" is known from South Korea and Taiwan region, as well as from Central (Poland, Hungary, Romania) and Southern Europe (Italy). In contrast to the situation with *T. aggressivum*, *T. pleurotum* and *T. pleuroticola* have been found in all of these countries and geographic regions, and they are thus the result of sympatric speciation. Some insights have been obtained into the forces driving this speciation: while *T. pleuroticola* is also found in the environment (soil, grains) and damages *Pleurotus ostreatus* mainly by competition for nutrients and overgrowing its mycelium, *T. pleurotum*, just like *T. aggressivum*, was so far never found in environmental samples. The hypothetic specialization of *T. pleurotum* for parasitism on *P. ostreatus* is also reflected by a strongly reduced carbon source assimilation profile (Komon-Zelazowska *et al*., 2007).

These two examples show that species of the

genus *Hypocrea/Trichoderma* can use different strategies for speciation towards aggressive mycoparasitism. We should like to note that in both cases, tests for recombination such as the phi-test or the Index of Association did not support the assumption of recombination (C.P. Kubicek, unpublished data), and *T. aggressivum*, *T. pleurotum* and *T. pleuroticola* appear to propagate therefore predominantly in an asexual way.

IDENTITY AND ORIGIN OF THE FACULTATIVE HUMAN PATHOGENIC *TRICHODERMA* SPECIES

The occurrence of *Trichoderma* spp. as facultative pathogens of humans is a different story. Of the more than one million fungal species that are estimated to exist in nature, only a few hundred have been associated with human and animal diseases (Walsh and Groll, 1999). However, case reports on infections by common mold fungi have increased in the last decade, due to HIV/AIDS and the use of immunosuppressive therapies for organ transplantation and cancer treatments. Species from the fungal genus *Hypocrea*/*Trichoderma* have also become members of this emerging list of opportunistic pathogens. A detailed list of case reports about *Trichoderma* infections have been summarized by Kredics *et al*.(2003). Typically, these include several isolates from the peritoneal effluent of dialysis patients, infections of immunocompromised transplant recipients, and patients suffering from leukemia, brain abscesses and HIV (Furukawa *et al*., 1998; Hennequin *et al*., 2000; Munoz *et al*., 1997; Myoken *et al*., 2002). The causal agents of these infections have previously been attributed to several different species of *Hypocrea*/*Trichoderma*, such as *H. rufa*/*T. viride*, *H. lixii*/*T. harzianum*, *H. koningii*/*T. koningii*, *H. schweinitzii*/*T. citrinoviride* and *T. longibrachiatum*. Sequence analysis of ITS1 and ITS2, however, identified all strains isolated from clinical patients, with a few exceptions, as *T. longibrachiatum* (Kuhls *et al*., 1999; Kredics *et al*., 2003).

The increase in *Trichoderma* infection of humans could be due to various reasons, including events in the population history of *T. longibrachiatum*. We have therefore investigated the phylogenetic relatedness and population genetics origin of *T. longibrachiatum*, using both "clinical" and "non clinical" strains. We also included its putative teleomorph *H. orientalis* and investigated the mode of reproduction of this fungal sample (Druzhinina *et al*., 2008). The results from this work was surprising because it showed clearly that not only *T. longibrachiatum* but also *H. orientalis* infect immunocompromised patients, and that *H. orientalis* is not the teleomorph of *T. longibrachiatum* but constitutes a different species. While *T. longibrachiatum* propagated essentially clonal, *H. orientalis* exhibited a history of sexual recombination. The clinical isolates from both species shared identical multilocus haplotypes with isolates of the same species from soil and plant materials, and *Trichoderma* invasive mycoses may therefore be potentially nosocomial. Thus, not a single population of *T. longibrachiatum* is responsible for the opportunistic attack on humans but that presumably every isolate of *T. longibrachiatum* or *H. orientalis* is potentially able to do so. Interestingly, clinical isolates in the *H. orientalis* clade were recovered during the last 5 years, whereas isolates recovered earlier were only *T. longibrachiatum*.

The results from this study present evidence that sexual reproduction indicated by recombination occurs in one of the two opportunistic pathogenic species of *Hypocrea*/*Trichoderma*, i.e., *H. orientalis*. This is a significant finding, because it allows the fungus to respond faster to environmental challenges, thereby combating disease treatment by exchange of antibiotic resistance genes and virulence factors (Milgroom, 1996; Nielsen and Heitman, 2007; Normark *et al*., 2003; Paoletti *et al*., 2005). The phylogenetic analysis presented in this paper suggests that *T. longibrachiatum* and *H. orientalis* evolved in parallel from the common ancestor, forming two sympatric species. Thus, their pathogenic ability would be rather a result of a heritage from a recent ancestor than from a convergent evolution.

MOLECULAR PHYLOGENY AND BIODIVERSITY

The availability of a safe molecular identification tool for *Hypocrea*/*Trichoderma* can also be used to answer the question whether phenotypic traits of industrial relevance could be predicted from a known

species identity. In other words, would the ability to produce a certain enzyme or metabolite be the property of only a single or a defined group of species? Previous work already suggested that the answer may be "yes", for Kubicek *et al*.(1996) selected strains from *Trichoderma* section *Longibrachiatum* species for their ability to produce cellulases, and found a clearly enhanced production by isolates of *H. jecorina* and *T. longibrachiatum*. Arisan-Atac *et al*.(1995) showed that the ability to biocontrol *Cryphonectria parasitica* causing cancer of chestnut trees is restricted to only a few molecular species of *Hypocrea*/*Trichoderma*. We have therefore recently tested this possibility with two examples, secondary metabolite production (Neuhof *et al*., 2007) and chitinase formation (Nagy *et al*., 2007).

Secondary metabolites have been used as taxonomic tools in the pre-molecular area. Today, there is still a debate whether phylogeny is paralleled by uniqueness in secondary metabolite profiles or not, and conflicting results have been published. Little is known about this in the genus *Hypocrea/Trichoderma*. The peptaibols or peptaibiotics (Szekeres *et al*., 2005; Whitmore and Wallace, 2004) can be used as model secondary metabolites for this purpose. They are linear, non-ribosomally formed peptides with 5~20 residues, characteristically highly abundant in alpha-aminoisobutyric acid (Aib), and containing an N-acyl (usually acetyl)-terminal and a C-terminal alcohol such as phenylalaninol or leucinol (Benedetti *et al*., 1982; Brückner and Graf, 1983; Brückner *et al*., 1984). They are unique for *Hypocrea/Trichoderma* and closely related genera. Whether the biodiversity of peptaibols is indeed species-correlated is not only an academic question, but also of practical importance because the peptaibols are often named after a putative producer species ("harzianins", "longibrachins", "koningiins", etc.). However, the species identity of most of the strains producing the presently known peptaibols is highly uncertain, for they were only "identified" by morphological analysis. Only recently, strains with defined species identity have been investigated for the peptaibols produced by them, which led to the identification of several new compounds (Wiest *et al*., 2002; Degenkolb *et al*., 2006a; 2006b; Vizcaíno *et al*., 2006). In the latter study, peptaibols produced by five defined species of *Trichoderma* (*T. pubescens*, *T. strigosum*, *T. spirale*, *T. erinaceum* and *T. stromaticum*) were compared, and the authors concluded that the type of peptaibols produced correlates only poorly with the taxonomy of the species. This claim must be accepted with caution, however, as Degenkolb *et al*.(2006b) only investigated 5 out of more than 100 presently accepted species. Moreover, they included 3 species (*T. pubescens*, *T. erinaceum* and *T. strigosum*) that are phylogenetically closely related. Consequently, we have investigated peptaibol production in a collection of 28 *Hypocrea*/*Trichoderma* species thereby covering a wide range of the diversity within these genera (Neuhof *et al*., 2007). We could show that there is some correlation between the types of peptaibols formed and clade phylogeny, but the phylogeny based on the amino acid sequence of parts of the peptaibol synthase proteins (i.e., the Aib, Gln and Pro adenylation domains) is discordant with established phylogenies at the species level.

Ten peptaibol groups could be strictly correlated with single species (*H. rufa*/*T. viride*, *H. semiorbis*, *T. flavofuscum*, *H. lixii*/*T. harzianum*, *H. minutispora*/*T. minutisporum*, *H. atroviridis*/*T. atroviride*, *T. strigosum*, *H. koningii*/*T. koningii*), but other groups were abundant and distributed throughout all phylogenetic clades. On the clade level, some groups were consistently found in some phylogenetic clades only. When the chain length of the peptaibols in the respective subfamilies was taken into consideration, it became apparent that members of 'Pachybasium B' clade within *Hypocrea*/*Trichoderma* produced only 18-residue peptaibols, whereas 19-residue peptaibols were only formed by members of section *Trichoderma*, and 20-residue compounds formed by the *Polysporum* clade, section *Trichoderma* and section *Longibrachiatum*. When the sequence of peptaibols within the same subtypes was compared, however, these clades exhibited no relationship to gene phylogeny. This was tested with groups 25 and 26 of the 20-residue peptaibols (section *Longibrachiatum*: longibrachins, trichobrachins, trichoaureocins, paracelsins, and saturnisporins; section *Trichoderma* atroviridines; section *Pachybasium B* polysporins and alamethicins). Thus the biodiversity of peptaibols of *Trichoderma* is not concordant with the evolution of the genus, but still contains a background of ancient species-specific patterns.

This assumption was recently confirmed by

Degenkolb *et al*.(2008); they investigated one of the species clades in *Hypocrea*/*Trichoderma*, the *Lutea* clade, with a combination of sequence data of peptaibols and of hydrophobins, also including trichothecene-type mycotoxins. Researches could show that the occurrence of secondary metabolites strictly correlated with results of morphological, molecular, and phylogenetic analyses. This combined approach led them to dissect *T. brevocompactum* (Kraus *et al*., 2004) into four species, i.e., *T. brevicompactum*, *T. arundinaceum*, *T. turrialbense*, and *T. protrudens*. Therefore, at this level, secondary metabolite biodiversity perfectly matches evolutionary history.

One of the difficulties to correlate secondary metabolites with phylogeny may be the fact that their synthesis is usually encoded by several genes which may undergo different rates of evolution. Although the peptaibols are encoded by single gene, their individual domains are under different selection pressures (C.P. Kubicek, unpublished data) and, moreover, display a degenerate substrate specificity, two facts probably responsible for the only partial correlation of peptaibol diversity with phylogeny. In order to test the above hypothesis therefore with other molecules, we used *H. lixii*/*T. harzianum* and chitinase production as a model. We tested whether fungal strains with superior enzyme production may be diagnosed by DNA Bar-Codes (Nagy *et al*., 2007). To this end, we sequenced two phylogenetic marker loci, i.e., ITS1 and ITS2 and *tef1*, from 48 isolates of *H. lixii*/*T. harzianum*, which were tested for their ability to produce chitinases in solid state fermentation (SSF) using wheat bran and crude chitin as a substrate. A statistically supported superior chitinase production was obtained for strains carrying one of the observed ITS1 and ITS2 and *tef1* alleles corresponding to *T. harzianum* type strain CBS 226.95. Biolog Phenotype MicroArray analysis identified the lack of *N*-acetyl-β-*D*-mannosamine utilization as a specific trait of strains with the chitinase overproducing haplotype, and was used to develop a plate screening assay for rapid microbiological identification of these strains. The data illustrate that desired industrial properties like enzyme production can be an attribute of certain populations within a species, and screening procedures should thus ensure to include a balanced mixture of all genotypes of a given species.

CONCLUSION AND PERSPECTIVE

The findings reviewed in this article illustrate how the availability of a safe and universally applicable identification system for a fungal genus can be used to obtain information about the biogeography, ecology, mechanisms of speciation and diversity in these fungi. Clearly, the examples described represent only a first step towards a comprehensive understanding of this important genus, and rather serve as an illustration what can already be done. Consequently, the next steps will be to concentrate in more detail on the specific ecological niches and physiochemical requirements of different species, using cultivation independent molecular techniques (such as metagenomic or oligonucleotide array approaches), and using population genetic strategies to identify the history of migration of species. In view of the fact that *Hypocrea/Trichoderma* is a genus that is exceptionally well covered by diagnostic sequence information, it could well become a model case for such studies.

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