REVIEW

Recent Advances and Future Prospects in Peptaibiotics, Hydrophobin, and Mycotoxin Research, and Their Importance for Chemotaxonomy of *Trichoderma* and *Hypocrea*

by **Thomas Degenkolb**[a]), **Hans von Döhren**[b]), **Kristian Fog Nielsen**[c]), **Gary J. Samuels**[d]), and **Hans Brückner***[a])

[a]) Interdisciplinary Research Centre for Biosystems, Land Use and Nutrition (IFZ), Department of Food Sciences, Institute of Nutritional Science, University of Giessen, Heinrich-Buff-Ring 26–32, D-35392 Giessen (phone: +49-641-99-39144, +49-641-99-39141; fax: +49-641-99-39149; e-mail: thomas.degenkolb@ernaehrung.uni-giessen.de, hans.brueckner@ernaehrung.uni-giessen.de)

[b]) Technische Universität Berlin, Institut für Chemie, FG Biochemie und Molekulare Biologie, Franklinstraße 29, D-10587 Berlin (phone: +49-30-314-22697; fax: +49-30-314-24783; e-mail: doehren@chem.tu-berlin.de)

[c]) Department of System Biology, BioCentrum, Technical University of Denmark, Building 221, DK-2800 Lyngby (phone: +45-45252600; fax: +45-45884922; e-mail: kfn@bio.dtu.dk)

[d]) United States Department of Agriculture (USDA), Agricultural Research Service (ARS), Systematic Mycology and Microbiology Lab, Rm 304, B-011A, BARC-W, Beltsville, MD, 20705, USA (phone: +1-301-504-8279; fax: +1-301-504-5810; e-mail: Gary.Samuels@ars.usda.gov)

Fungi of the genus *Trichoderma* with teleomorphs in *Hypocrea* are abundant producers of a group of amphiphilic, non-ribosomal **pept**ide antib**iotics**, which are rich in the non-proteinogenic amino acid **Aib** (α-aminoisobutyric acid). They are referred to as **peptaibiotics**, or **peptaibols**, if a 1,2-amino alcoh**ol** is present at the C-terminus. *Trichoderma*/*Hypocrea*, like other ascomycetous fungi, also produce hydrophobins, a class of small, cysteine-rich proteins. Advanced soft ionization mass spectrometric techniques such as LC-CID-MS, LC-ESI-MS^n, and IC-MALDI-TOF-MS enabled the high-throughput analysis, simultaneous detection and sequence determination of peptaibiotics and hydrophobins from minute quantities of fungal materials. Some *Trichoderma* species have been recognized to produce peptaibiotics as well as simple mycotoxins of the trichothecene group. The combination of sequence data of both groups of peptides with the pattern of low-molecular-weight secondary metabolites, including trichothecene-type mycotoxins, independently confirmed the results of morphological, molecular, and phylogenetic analyses. This approach established a new lineage in *Trichoderma*/*Hypocrea*, the *Brevicompactum* clade, comprising four new and one redescribed species. Notably, commercial preparations of single or mixed cultures of *Trichoderma* species, in particular *T. harzianum*, and *T. koningii*, are registered as biocontrol agents for soil and plant pathogens. In this context, it is emphasized that the four mycotoxin-producing species of the recently established *Brevicompactum* clade (*T. brevicompactum*, *T. arundinaceum*, *T. turrialbense,* and *T. protrudens*) are not closely related to any of the *Trichoderma* species currently used as biocontrol agents. Furthermore, possible health concerns about release of peptaibiotics in the biosphere are discussed with respect to their bioactivities and their use as drugs in human and veterinary medicine. Finally, future prospects regarding novel bioactivities and further research needs, including interdisciplinary taxonomic approaches, are outlined.

Peptaibiotics. – Peptaibiotics/peptaibols constitute a constantly growing family of peptide antibiotics [1–3]. Currently, more than 700 individual sequences of peptai-

biotics, produced by members of 18 fungal genera, are reported in the literature. Of these *ca.* 20 genera, *Trichoderma* and *Hypocrea* are the most abundant sources of peptaibiotics with approximately half of the known peptaibiotics originating from those two genera [4]. *Trichoderma* is the asexual form of, and is phylogenetically indistinguishable from, *Hypocrea*. Thus, in this review we refer to a single genus, *Trichoderma*/*Hypocrea*. Most of the peptaibiotic-producing species formerly included in *Gliocladium* have been reclassified in *Trichoderma* [5]. A continuous problem has been the high rate of reports of biological activities of *Trichoderma*/*Hypocrea* based on misidentified species [6]. However, positive identification can be achieved by DNA sequencing which is currently accepted as the state-of-the-art approach in fungal taxonomy. Complete or partial sequences of peptaibiotics have been reported from *ca.* 25 *Trichoderma*/*Hypocrea* species [7–9], the identity of which has been verified by DNA sequencing. An additional 15 positively identified species are known to produce peptaibiotics [10] by an 'intact cell matrix assisted laser desorption/ionization-time of flight mass spectrometry' (IC-MALDI-TOF-MS) approach [11]. In a pilot study of *ca.* 60 species, we found that *ca.* 75% of these strains produced peptaibiotics on the medium tested. While *ca.* 400 species of *Hypocrea* have been described over the past 175 years, few of them have been seen since they were first described, and even fewer have been linked to *Trichoderma* stages. On the other hand, only few species of *Trichoderma* were described prior to the mid 1990s, when molecular techniques were routinely applied in taxonomy. The consequence is that *Trichoderma* stands as one of the few species-rich genera of microfungi for which all known species have been cultured, and for which sequences of two or more genomes have been deposited in GenBank. The web site of the *International Commission for Taxonomy of Fungi*, *Trichoderma* and *Hypocrea* subcommittee (http://www.isth.info/biodiversity/index.php), currently provides DNA sequences for 104 species of *Trichoderma*/*Hypocrea,* but several new species are in the process of being described. So, while only approximately half of the known *Trichoderma*/*Hypocrea* species have been investigated for the production of peptaibiotics, the full diversity of the genus has only begun to be revealed. The certain discovery and characterization of new *Trichoderma*/*Hypocrea* species in previously unexplored regions, for instance Asia [12], and in ecological niches, such as woody tissues of trees [13–15], will lead certainly to the discovery of new producers, novel constituents, and eventually uncommon building schemes of peptaibiotics.

A multiphasic, interdisciplinary approach to taxonomy has always been most convenient to discern species boundaries and the relationships among species. Taxonomy was initially strictly based on morphology of the sexual or asexual morph, which ever was in the hand of the taxonomist and depended upon the interests of the taxonomist. By the mid 19th century, it was known that ascomycetes such as *Hypocrea* had asexual morphs that were classified in different genera, but rather few taxonomists viewed the organism as a whole. Over the last 30 years of the 20th century, a greater emphasis was placed on amalgamating anamorph and teleomorph taxonomy. This amalgamation not only provided information about the respective morphs, but also, because artificial cultures were involved, taxonomic characters could be drawn from the *Petri* dish. Over time, there have been attempts to use metabolites and physiology in taxonomy of ascomycetes, such as carbon uptake [16], but interspecific variation in

these characters has limited their use in taxonomy. A major difficulty in evaluating the taxonomic utility of metabolic or other physiological characters is the high degree of misidentifications in the literature, as was mentioned above. The mid 1990s brought molecular phylogenetics together with a surge in descriptions or redescriptions of *Trichoderma* species. The DNA sequencing provided a precision in species identification and species boundaries that was lacking in purely morphological taxonomy. Thus, the newly described species were scrutinized through the lens of DNA sequencing, as have been all of the roughly 130 species known today. Prior to 2000, peptaibiotics were attributed to four 'common' species, *T. viride*, *T. koningii*, *T. harzianum*, and *T. longibrachiatum*. However, the identity of those cultures is in doubt, or has already been shown to be incorrect. Certainly, we cannot be criticized, as it has only been in the last few years that sufficient sequences of correctly identified *Trichoderma* species have been generally available through the ISTH web site and GenBank [17]. Nevertheless, statements about *Trichoderma* species must always be accepted with great caution unless the authors of those reports describe how their cultures were identified.

Peptaibiotics such as alamethicins are considered for treatment of plant diseases because of their effect on the walls of pathogens [18][19]. They may also have a role to play in taxonomy and species identification. Peptaibiomics [7–9] and IC-MALDI-TOF-MS [3][10][11] have considerably increased the screening efficiency for peptaibiotics but also contributed to progress in chemotaxonomy of *Trichoderma*/*Hypocrea*. Recently, peptaibiotics have even been isolated from natural habitats, such as dung of rabbits and from the tortoise *Testudo hermanni* [20]; and they were also considered as stable markers for the presence of fungi in the marine environment [21]. Briefly, linear peptaibiotics are polypeptide antibiotics which *i*) have a molecular weight between 500 and 2,200 Da, thus containing 5–21 residues; *ii*) show a high content of α-aminoisobutyric acid (Aib); *iii*) are characterized by the presence of other non-proteinogenic amino acids and/or lipoamino acids; *iv*) possess an acylated N-terminus, and *v*) have a C-terminal residue that, in most of them, consists of a free or acetylated amide-bonded 1,2-amino alcohol, but might also be an amine, amide, free amino acid, 2,5-dioxopiperazine, or sugar alcohol [4]. Many different peptaibiotics may be produced by a single strain of *Trichoderma*/*Hypocrea*. Peptaibiomics is the characterization of the entirety of peptaibiotics produced by a fungal strain under defined culture conditions. It also may include the time-dependent dynamics of biosynthesis and degradation of peptaibiotics [22]. An LC/MS-based peptaibiomics approach is based on the generation of so-called 'in-source fragments' by 'collision-induced decomposition' mass spectrometry (CID-MS) to the skimmer region of an ESI mass spectrometer. Varying voltages are applied to the skimmer, thus providing the possibility of clearly differentiating various types of ions. The CID-MS technique provides a possibility to partially sequence peptaibiotics. The most diagnostic difference to screen for is Δ (m/z) 85, which indicates the presence of the α-aminoisobutyric (Aib) residue being unique to all peptaibiotics. Another diagnostic difference is Δ (m/z) 99, indicative for a Vxx residue (*i.e.*, isomeric valine or isovaline). In this context, it should be pointed out again that non-proteinogenic Iva is frequently found as D- or L-isomer in peptaibiotics, whereas all other chiral constituents known so far have been reported to possess L-configuration. In some cases, both enantiomers of Iva are present in the same

peptaibiotic [4]. Complete sequencing is achieved by a combination of data from CID-MS, MS/MS, QTOF MS, and MS^n (in the case of ion-trap instruments). These data are generally needed to be combined with HPLC elution profiles, the so-called fingerprints of the samples [4][8][9].

Intact cell MALDI-TOF mass spectrometry (ICMS) permits detection of biomolecules in unfractionated biomass down to the attomolar level. Microcolonies of cyanobacteria of 50 to 100 cells have been characterized at the clonal level, differentiating strains by their peptide metabolite inventory [23–25]. We thus could expect to analyze much larger fungal cells down to the single-cell level. Analysis of bacteria at the intact-cell level by ICMS is now well-established, and generally detection of a set of ribosomal proteins as most abundant compounds permits identification at or below the species level at a comparable sensitivity as PCR techniques [26–29], while additional protein markers permit, *e.g.*, the differentiation of drug-resistant strains [30–33]. The standard ICMS sample-preparation process suspending cells in a solvent mixture disintegrates bacterial membranes releasing intracellular proteins. With fungal cells, however, solvent-soluble components of the cell wall as hydrophobins or excreted metabolites are detected [3][10][11][34][35]. Although the metabolite inventory is essential for strain characterization, this information alone is generally insufficient for the identification of microbial species and strains. Peptaibol formation has been comparatively analyzed in 32 strains of 29 species of *Trichoderma*/*Hypocrea* concluding that there is no strict correlation of phylogeny with the types and sequences of the peptaibols produced, but that the production of some groups of peptaibols appears to be found only in some clades or sections of the genus [10]. It is clear from this and another analysis including mycotoxins [36] that relevant phylogenetic information could only be drawn from the respective biosynthetic genes.

Most peptaibols range in size between 1000 and 2000 Da, and are thus easily detected without interference from low-mass metabolite signals or matrix signals. In this region, monoisotopic masses are recorded, and peptide family patterns are visible. Peptaibol production on solid media starts at the onset of sporulation, as has been shown for *H. atroviridis* [35]. In recent studies, more than 40 strains of 32 species of *Trichoderma*/*Hypocrea* have been investigated for the production of 10- to 20-residue peptaibiotics. One study defined 26 groups of characteristic mass peaks, and assigned half of these to known peptaibiotics [10]. This overview identified species with yet undescribed compounds. The purpose of the study has been to search for correlations of peptaibol type production with phylogenetically ascertained species descriptions. Taken together with all available data on peptaibiotics in *Trichoderma*/*Hypocrea*, the complexity of relations between phylogeny and peptaibol synthetases becomes obvious. Certain peptaibols are found throughout all sections and clades, while others are restricted to particular sections. As orthologous peptide synthetase domains and modules are found, and also obvious extensive regional similarities exist, possible relationships would best be analyzed at the peptide synthetase gene level.

ICMS Peptaibol patterns obviously need further analysis of molecular ions to clearly identify the sequences of the respective compounds. With the current MALDI-TOF-MS approach, the presence of these metabolites can be detected extremely rapidly from small amounts of mycelia/spores, thus also facilitating extended expression studies.

HPLC-MS combined with ESI, atmospheric-pressure chemical ionization (APCI), or other atmospheric-ionization techniques as well as UV/VIS diode array detection revolutionized the process of assaying for uncommon mycotoxins. These can be further characterized by high-resolution MS from TOF, and now also with Orbi-Trap instruments, for quickly determining the elementary composition of a given metabolite, which can be identified in databases such as Antibase, comprising 33,557 records in the 2007 version [37]. This is especially true for UV/VIS-active compounds where the UV/VIS data and original strain information can be used for validation [38]. For trace analysis, HPLC-MS/MS on triple quadrupole instruments is state-of-the art, because both the sensitivity (down to low fg levels) and specificity are excellent.

Hydrophobins. – Hydrophobins range in size from *ca.* 75 up to 400 amino acid residues containing eight positionally conserved cysteine residues forming characteristic disulfide bonds, and are divided into two classes with respect to their hydropathy profiles and cystein spacings. Hydrophobins have been considered as suitable phylogenetic markers for several reasons. Fungi contain a set of hydrophobin genes, generally with a developmentally regulated expression reflecting life conditions and interactions. Overproduction of a hydrophobin during endophytic interaction of *T. asperellum* and cucumber roots has recently been demonstrated [39]. The hydrophobins HFB1 and HFB2 have diverse functions in development of *H. jecorina* [40], including surface hydrophobicity, formation of aerial mycelia, and spore properties.

Genome sequencing of *H. jecorina*/*T. reesei* revealed the presence of six hydrophobin genes, and for *H. atroviridis*/*T. atroviride* and *T. virens*, ten hydrophobin genes have been annotated so far, respectively [10][41][42]. These hydrophobic peptides can be selectively dissolved and are thus excellent biomarkers for MALDI-TOF-MS [3][10][11]. Extending genomic information, hydrophobin patterns are diverse due to posttranslational processing, as shown in the pioneering work on *H. jecorina* [43–45]. Processing involves the respective signal sequences for excreted proteins, but also defined N- and C-terminal cleavage sites [10][11]. Due to their fairly small size, sequence information of the hydrophobins can be obtained by mass spectrometry, and the respective genes are accessible by standard PCR methods. It has been noted that the variability of hydrophobin genes is fairly significant and exceeds similarities of other biomarkers proposed, *e.g.*, ubiquitins. In addition, variation of the number of hydrophobin genes provides additional information to housekeeping gene structures. Gain and loss of hydrophobin genes may also play a key role in speciation.

ICMS Patterns of the strain *H. jecorina* (syn. *T. reesei*) QM9414 have been correlated with the respective hydrophobin composition of HFB1 and HFB2 through analysis of knock out mutants [10]. Unexpectedly, two other strains, CPK 618 and CPK 665, showed differing mass peaks, which have been tentatively assigned to the expression of either HFB3 and HFB4 or differing processing of HFB1. Vegetative and sporulating mycelia differed as expected in their hydrophobin patterns. In the same study, 29 species of *Trichoderma*/*Hypocrea* have been compared, and unique patterns have been detected. In case of *H. atroviridis*/*T. atroviride* P1, mass peaks could be correlated with sporulation related hydrophobin SRH1, as also for *T. longibrachiatum* HFB3. As the results indicate proteolytic processing beyond signal peptide cleavage, further work is needed to ascertain cleavage sites and proteases involved.

We successfully linked morphological, molecular taxonomic, and phylogenetic approaches, and IC-MALDI-TOF MS, peptaibiomics, and LC/HR-ESI-MS mycotoxin screening of *Trichoderma*/*Hypocrea* to redescribe a new lineage in *Trichoderma*/*Hypocrea*, the *Brevicompactum* clade. This clade included, in addition to *T. brevicompactum*, four new species in the *T. brevicompactum* complex, and an additional new *Hypocrea* species [36]. This approach clearly supported our previous hypothesis that more than one species was involved when *T. brevicompactum* has originally been described. Combination of classical morphological mycology with DNA sequencing and profiling of mycotoxins and other small metabolites, hydrophobins, and peptaibioitics clearly delimited four new species from within the original morphological construct of *T. brevicompactum* [46]. In summary, the combination of these methods provides information about metabolites that can be used to identify species and strains.

New Bioactivities. – Sophisticated approaches are urgently required to detect novel or previously unrecognized biological activities of peptaibiotics. *Hosotani et al.* [1] reported on the inhibition of amyloid β-peptide formation in primary guinea pig cerebral cortex neuron cell cultures by a 14-residue peptaibol, SPF-5506-A. It is known that increased accumulation of amyloid β-peptides plays a decisive role in the pathogenesis of neurodegenerative dementia such as *Alzheimer*'s disease. Produced by *Trichoderma* sp. SPF-5506, peptaibol SPF-5506-A can be considered as a positional isomer of the 14-residue harzianin HC [47]. Thus, it can be hypothesized that harzianins and structurally related peptaibols might display similar bioactivity.

Biocontrol Agents. – In the past decade, peptaibiotics and the fungi producing them have gained much interest as a possible alternative to xenobiotics such as synthetic pesticides in agriculture and forestry. Recently, it has been demonstrated that five new 18-residue peptaibols, trichostromaticins A–E, produced by *Trichoderma stromaticum* [48] (teleomorph: *Hypocrea stromatica* [49]), strains CBS 101875 and CBS 101730, may contribute to the potent bioactivity of these two strains [9]. This species, which is known only as a mycoparasite of the cacao (*Theobroma cacao*) Witches' Broom disease pathogen *Moniliophthora perniciosa* [48], formerly *Crinipellis perniciosa* [50], has successfully been introduced into field control of the disease in South America. The main effect is the suppression of basidioma formation by the pathogen [51]. These two strains of *T. stromaticum* and another seven strains of *T.* cf. *strigosum*, *T. strigosum*, *T. erinaceus*, *T.* cf. *pubescens*, *T. pubescens*, and *T. spirale* were highly active against the causal agents of *Eutypa* dieback, *Eutypa lata*, and Esca disease, *Phaeomoniella chlamydospora*, and *Phaeoacremonium aleophilum*. These are latent trunk diseases that cause severe economic losses in viticulture [52]. Notably, all of the eight *Trichoderma* strains produced peptaibiotics that may contribute to their potent bioactivity against the above grapevine pathogens [9].

Strains originally identified as *T. harzianum* and *T. koningii* are currently used in commercial biological control agents (BCAs) in Europe and North America. Our own, preliminary studies on five BCAs available on the German market most likely indicate that the bioactivity of these commercial products can clearly be attributed to the presence of peptaibiotics. Taxonomy of the *Trichoderma* strains in these BCAs is currently under revision [53].

Mycotoxin Production. – Concerns have been expressed about potential toxicity of peptaibols/peptaibiotics in biological control, a fear that is reinforced by names such as 'trichotoxin'. Clearly, a biological control agent that enters the food chain should not produce toxins. Species identified as biological control agents, including *T. harzianum* and *T. viride*, are reported to produce trichothecene toxins. However, they have been reidentified as *T. brevicompactum* and *T. arundinaceum*, closely related species, both of which belong to the *T. brevicompactum* complex that was mentioned above. This lineage is distant from any biological control species. Only *T. brevicompactum* and three closely related species within the *T. brevicompactum* complex [8][36] have been proven to produce trichothecene-type mycotoxins (trichodermin, harzianum A), and neither in [54], nor in [9] were trichothecenes reported in any species outside of the *T. brevicompactum* complex.

Note, that the 11- and 14-residue peptaibols harzianins HB [55] and HC [47] must not be confused with the non-peptidic, trichothecene-type mycotoxins harzianum A [54][56][57] and harzianum B [58]. Studies reporting other types of trichothecenes than the one with only a OH group in the 4- and perhaps 15-position are probably wrong, as these other types, *e.g.*, from *Fusarium,* are biosynthesised *via* trichotriol, rather than trichodermol [59], as is probably the case in *Stachybotrys*, *Myrothecium*, *Memnoniella*, and *Podostroma* (= *Hypocrea*) [54][59][60], all of which are members of the Hypocreales along with *Trichoderma*/*Hypocrea*. Gliotoxin is produced by *Trichoderma virens*, which is commonly reported in the literature as *Gliocladium virens* [61].

Toxicity of Peptaibiotics. – Recently, it has been reported [62][63] that the 20-residue peptaibols alamethicin and paracelsin, the 16-residue antiamoebin, and other 11-residue trichobrachins [2][64] were highly toxic in three *in vitro* invertebrate models, *viz*. *Crassostrea gigas*, *Artemia salina*, and *Daphnia magna*. An alternative explanation of the toxicity reported in [62] and [63] might be that the batch of the alamethicin standard used (*Sigma-Aldrich*, product number A-4665) may have been contaminated with the trichothecene mycotoxin harzianum A that is produced by the strain of *Trichoderma* cf. *brevicompactum* used for alamethicin fermentations [8]. In our own work, we have found that this particular *Sigma* alamethicin contains harzianum A; and this group of trichothecene toxins is highly toxic to *Artemia salina* [36].

In contrast, oral administration of antiamoebin [65][66], trichotoxin A [67], aibellin [68][69], ampullosporin [70], and trichofumin [71] to rodents and ruminants revealed a very low toxicity. This was explained by the almost complete resistance of peptaibiotics towards any kind of proteolytic cleavage with the result that their high molecular weight prevented them from passing through the intestinal cell. The microheterogeneous mixtures of antiamoebin or aibellin were administered in huge amounts to cows or goats, respectively. The idea behind these experiments was to inhibit protozoa and other microbial competitors in order to increase cellulose digestibility by rumen bacteria. Antiamoebin was also tested for antiprotozoal and anthelminthic activities in man and against *Trypanosoma evansii* in laboratory animals. While its LD_{50} value was very high: 155–165 mg/kg body weight/animal, only 10 mg/kg killed the pathogen [65], a concentration far below the level that would affect the test animal.

Trichotoxin and alamethicin have been reported to cause haemolysis of erythrocytes [72]. This action, however, is common for many amphiphilic detergent-like molecules, including soaps and saponins, and requires direct contact with cell membranes. Analogously, the reported uncoupling of oxidative phosphorylation in mitochondria caused, for instance, by efrapeptins [73] requires direct interaction under experimental conditions.

From an application point of view, the potential 'toxic' effect of peptaibiotics appears to be more theoretical than real, perhaps no more than that of common amphiphilic detergents, and well below the threshold of human consequence. On the other hand, the reported synergistic effects of peptaibiotics and cellulases by strains of *Trichoderma* and *Hypocrea,* and the antibiotic activities of peptaibiotics which are of interest for plant protection [18][19] enhance their potential for biological control application.

Conclusions. – The introduction of novel methodical approaches during the past two years such as peptaibiomics and IC-MALDI-TOF-MS have remarkably enhanced screening efficiency for peptaibiotics and has extended their application to chemotaxonomic questions. More than half of the currently known sequences of peptaibiotics were determined, between 2002 and 2007, mostly by HPLC-MS approaches. Likewise, HPLC-MS has revolutionized mycotoxin analysis in the past decade, not only lowering detection limits but also with the introduction of high-resolution MS screening, and fast identification/dereplication of mycotoxins without reference standards are now possible. Novel test assays have been used to detect new or previously unrecognized bioactivities of peptaibiotics such as neuroleptic [70], anti-HIV integrase I [74], and anti-*Alzheimer* [1] effects. Combined use of molecular biology, direct-infusion MS, HPLC-MS, and MALDI-TOF-MS has also been proven to be beneficial for characterization of new fungal species [36][38][75].

Certainly, trichothecene-producing strains of *Trichoderma* and *Hypocrea* must not be introduced into biological control of fungal pathogens in the field or greenhouse. Thanks to a growing phylogenetic understanding of *Trichoderma*/*Hypocrea*, we are able to predict biological properties of species, and avoid those that are related to known toxigenic species. Mycotoxin-free *Trichoderma* species such *T. stromaticum* that can be regarded as safe are already in use for crop protection [51].

Financial support by the *Studienstiftung Mykologie* (D-Cologne) and the *Erwin-Stein-Stiftung* (D-Gießen) is gratefully acknowledged.

Note Added in Proof. Recently, a new subfamily of natural peptaibiotics, named cyclopeptaibiotics, was introduced, currently comprising seven 4-membered and two 7-membered cyclic peptides, which contain at least one Aib or Iva residue [76].

REFERENCES

[1] N. Hosotani, K. Kumagai, S. Honda, A. Ito, T. Shimatani, I. Saji, *J. Antibiot.* **2007**, *60*, 184.
[2] N. Ruiz, G. Wielgosz-Collin, L. Poirier, O. Grovel, K. E. Petit, M. Mohamed-Benkada, T. Robiou du Pont, J. Bissett, P. Vérité, G. Barnathan, Y. F. Pouchus, *Peptides* **2007**, *28*, 1351.
[3] T. Neuhof, A. Berg, H. Besl, T. Schwecke, R. Dieckmann, H. von Döhren, *Chem. Biodivers.* **2007**, *4*, 1103.

[4] T. Degenkolb, J. Kirschbaum, H. Brückner, *Chem. Biodivers.* **2007**, *4*, 1052.
[5] K. H. Domsch, W. Gams, T.-H. Anderson, 'Compendium of soil fungi', 2nd taxonomically revised edn., Ed. W. Gams, IHW-Verlag, Eching, 2007.
[6] C. M. Kullnig, T. Krupica, S. L. Woo, R. L. Mach, M. Rey, M. Lorito, C. P. Kubicek, *Mycol. Res.* **2001**, *105*, 770.
[7] C. Krause, J. Kirschbaum, H. Brückner, *Amino Acids* **2006**, *30*, 435.
[8] T. Degenkolb, T. Gräfenhan, H. I. Nirenberg, W. Gams, H. Brückner, *J. Agric. Food Chem.* **2006**, *54*, 7047.
[9] T. Degenkolb, T. Gräfenhan, A. Berg, H. I. Nirenberg, W. Gams, H. Brückner, *Chem. Biodivers.* **2006**, *3*, 593.
[10] T. Neuhof, R. Dieckmann, I. S. Druzhinina, C. P. Kubicek, H. von Döhren *Microbiology* **2007**, *153*, 3417.
[11] T. Neuhof, R. Dieckmann, I. S. Druzhinina, C. P. Kubicek, T. Nakari-Setälä, M. Penttilä, H. von Döhren, *FEBS J.* **2007**, *274*, 841.
[12] J. Bissett, G. Szakacs, C. A. Nolan, I. Druzhinina, C. Gradinger, C. P. Kubicek, *Can. J. Bot.* **2003**, *81*, 570.
[13] G. J. Samuels, S. Dodd, B. Lu, O. Petrini, H.-J. Schroers, I. S. Druzhinina, *Stud. Mycol.* **2006**, *56*, 67.
[14] G. J. Samuels, C. Suarez, K. Solis, K. A. Holmes, S. E. Thomas, A. A. Ismaiel, H. C. Evans, *Mycol. Res.* **2006**, *110*, 381.
[15] W. M. Jaklitsch, G. J. Samuels, S. L. Dodd, B.-S. Lu, I. Druzhinina, *Stud. Mycol.* **2006**, *56*, 137.
[16] C. P. Kubicek, J. Bissett, I. Druzhinina, C. M. Kullnig-Gradinger, G. Szakacs, *Fungal Genet. Biol.* **2003**, *38*, 310.
[17] G. J. Samuels, *Phytopathology* **2006**, *96*, 195.
[18] M. Schirmböck, M. Lorito, Y. L. Wang, C. K. Hayes, C. Arisan-Atac, F. Scala, G. E. Harman, C. P. Kubicek, *Appl. Environ. Microbiol.* **1994**, *60*, 4364.
[19] M. Lorito, V. Varkas, S. Rebuffat, B. Bodo, C. P. Kubicek, *J. Bacteriol.* **1996**, *178*, 6382.
[20] N.-A. Lehr, A. Meffert, L. Antelo, O. Sterner, H. Anke, R. W. S. Weber, *FEMS Microbiol. Ecol.* **2006**, *55*, 106.
[21] L. Poirier, M. Montagu, A. Landreau, M. Mohamed-Benkada, O. Grovel, C. Sallenave-Namont, J.-F. Biard, C. Amiard-Triquet, J.-C. Amiard, Y. F. Pouchus, *Chem. Biodivers.* **2007**, *4*, 1116.
[22] C. Krause, J. Kirschbaum, H. Brückner, *Chem. Biodivers.* **2007**, *4*, 1083
[23] J. Fastner,M. Erhard, H. von Döhren, *Appl. Environ. Microbiol.* **2001**, *67*, 5069.
[24] M. Welker, M. Bruhnke,K. Preussel, I. Lippert, H. von Döhren, *Microbiology* **2004**, *150*, 1785.
[25] M. Welker, M. Erhard, *J. Mass Spectrom.* **2007**, *42*, 1062.
[26] Z. Du, R. Yang, Z. Guo, Y. Song, J. Wang, *Anal. Chem.* **2002**, *74*, 5487.
[27] M. P. Kumar, M. Vairamani, R. P. Raju, C. Lobo, N. Anbumani, C. P. Kumar, T. Menon, S. Shanmugasundaram, *Indian J. Med. Res.* **2004**, *119*, 283.
[28] 'Identification of Microorganisms by Mass Spectrometry', Eds. C. L. Wilkins, J. O. Lay Jr., John Wiley & Sons, 2006.
[29] R. Dieckmann, I. Kaesler, I. Graeber, U. Szewzyk, H. von Döhren, *Appl. Microbiol. Biotechnol.* **2005**, *67*, 539.
[30] K. Bernardo, N. Pakulat, M. Macht, O. Krut, H. Seifert, S. Fleer, F. Hünger, M. Krönke, *Proteomics* **2002**, *2*, 747.
[31] P. A. Majcherczyk, T. McKenna, P. Moreillon, P. Vaudaux, *FEMS Microbiol. Lett.* **2006**, *255*, 233.
[32] E. R. Castanja, A. Fox, K. F. Fox, *J. Microbiol. Meth.* **2006**, *67*, 230.
[33] C. Friedrichs, A. C. Rodloff, G. S. Chhatval, W. Schellenberger, K. Eschrich, *J. Clin. Microbiol.* **2007**, *45*, 2392.
[34] T. Neuhof, Ph.D. Thesis, Technical University of Berlin, Berlin, 2002.
[35] M. Komon-Zelazowska, T. Neuhof, R. Dieckmann, H. von Döhren, R. Herrera-Estrella, C. P. Kubicek, I. S. Druzhinina, *Eukaryotic Cell* **2007**, *6*, 2332.
[36] T. Degenkolb, R. Dieckmann, K. F. Nielsen, T. Gräfenhan, C. Theis, D. Zafari, P. Chaverri, A. Ismaiel, H. Brückner, H. von Döhren, U. Thrane, G. J. Samuels, *Mycol. Prog.* **2008**, in press.
[37] H. Laatsch, 'Antibase 2007 SciDex – The Natural Products identifier', Wiley-VCH, Weinheim, 2007.
[38] T. O. Larsen, J. Smedsgaard, K. F. Nielsen, M. E. Hansen, J. C. Frisvad, *Nat. Prod. Rep.* **2005**, *22*, 672.

[39] A. Viterbo, I. Chet, *Mol. Plant Pathol.* **2007**, in press.
[40] S. Askolin, M. Penttilä, H. A. Wösten, T. Nakari-Setälä, *FEMS Microbiol. Lett.* **2005**, *253*, 281.
[41] C. P. Kubicek, S. E. Baker, C. Gamauf, I. S. Druzhinina, submitted to GenBank, 2007/8.
[42] M. B. Linder, G. R. Szilvay, T. Nakari-Setälä, M. Penttilä, *FEMS Microbiol. Rev.* **2005**, *29*, 877.
[43] T. Nakari-Setälä, N. Aro, M. Ilmen, G. Munoz, N. Kalkkinen, E. Atalo, M. Penttilä, *Eur. J. Biochem.* **1997**, *248*, 415.
[44] E. Rintala, M. Linder, T. Nakari-Setälä, 8th European Conference on Fungal Genetics, Vienna, April 8–11, 2006, Abstract VII p-9.
[45] S. Askolin, Ph.D. Thesis, University of Helsinki, Helsinki, 2006.
[46] G. F. Kraus, I. Druzhinina, W. Gams, J. Bissett, D. Zafari, G. Szakacs, A. Koptchinski, H. Prillinger, R. Zare, C. P. Kubicek, *Mycologia* **2004**, *96*, 1059.
[47] S. Rebuffat, C. Goulard, B. Bodo, *J. Chem. Soc., Perkin Trans. 1* **1995**, 1849.
[48] G. J. Samuels, R. Pardo-Schultheiss, K. P. Hebbar, R. D. Lumsden, C. N. Bastos, J. C. B. Costa, J. L. Bezerra, *Mycol. Res.* **2000**, *104*, 760.
[49] J. L. Bezerra, J. C. B. Costa, C. N. Bastos, F. G. Faleiro, *Fitopatol. Bras.* **2003**, *28*, 408.
[50] M. C. Aime, W. Phillips-Mora, *Mycologia* **2005**, *97*, 1012.
[51] J. T. de Souza, A. W. V. Pomella, J. H. Bowers, C. P. Pirovani, L. L. Loguercio, K. P. Hebbar, *Phytopathology* **2006**, *96*, 61.
[52] T. Gräfenhan, Ph.D. Thesis, Humboldt-University, Berlin, 2006.
[53] T. Degenkolb, G. J. Samuels, H. Brückner, in preparation.
[54] K. F. Nielsen, T. Gräfenhan, D. Zafari, U. Thrane, *J. Agric. Food Chem.* **2005**, *53*, 8190.
[55] I. Augeven-Bour, S. Rebuffat, C. Auvin, C. Goulard, Y. Prigent, B. Bodo, *J. Chem. Soc., Perkin Trans. 1* **1997**, 1587.
[56] D. G. Corley, M. Miller-Wideman, R. C. Durley, *J. Nat. Prod.* **1994**, *57*, 422.
[57] H.-B. Lee, Y. Kim, H.-Z. Jin, J.-J. Lee, C.-J. Kim, J.-Y. Park, H.-S. Jung, *Lett. Appl. Microbiol.* **2005**, *40*, 497.
[58] H.-Z. Jin, J.-H., W.-D. Zhang, H.-B. Lee, Y.-S. Hong, Y.-H. Kim, J.-J. Lee, *J. Asian Nat. Prod. Res.* **2007**, *9*, 203.
[59] K. F. Nielsen, U. Thrane, *J. Chromatogr, A* **2001**, *929*, 75.
[60] Y. Saikawa, H. Okamoto, T. Inui, M. Makabe, T. Okuno, T. Suda, K. Hashimoto, M. Nakata, *Tetrahedron* **2001**, *57*, 8277.
[61] S. A. Rehner, G. J. Samuels, *Mycol. Res.* **1994**, *98*, 625.
[62] L. Poirier, F. Quiniou, N. Ruiz, M. Montagu, J.-C. Amiard, Y. F. Poirier, *Aquat. Toxicol.* **2007**, *83*, 254.
[63] M. Favilla, L. Macchia, A. Gallo, C. Altomare, *Food Chem. Toxicol.* **2006**, *44*, 1922.
[64] M. Mohamed-Benkada, M. Montagu, J. F. Biard, F. Mondeguer, P. Vérité, M. Dalgalarrondo, J. Bissett, Y. F. Pouchus, *Rapid Commun. Mass Spectrom.* **2006**, *20*, 1176.
[65] M. J. Thirumalachar, *Hindustan Antibiot. Bull.* **1968**, *10*, 287.
[66] A. Jaworski, H. Brückner, *J. Pept. Sci.* **2000**, *6*, 149.
[67] C. T. Hou, A. Ciegler, C. W. Hesseltine, *Appl. Microbiol.* **1972**, *23*, 183.
[68] T. Hino, H. Saitoh, T. Miwa, M. Kanda, S. Kumazawa, *J. Diary Sci.* **1994**, *77*, 3426.
[69] S. Kumazawa, M. Kanda, H. Aoyama, M. Utagawa, J. Kondo, S. Sakamoto, H. Ohtani, T. Mikawa, I. Chiga, T. Haysase, *J. Antibiot.* **1994**, *47*, 1136.
[70] M. Ritzau, S. Heinze, K. Dornberger, A. Berg, W. F. Fleck, B. Schlegel, A. Härtl, U. Gräfe, *J. Antibiot.* **1997**, *50*, 722.
[71] A. Berg, P. A. Grigoriev, T. Degenkolb, T. Neuhof, B. Schlegel, U. Gräfe, *J. Pept. Sci.* **2003**, *9*, 810.
[72] G. Irmscher, G. Jung, *Eur. J. Biochem.* **1977**, *80*, 165.
[73] R. L. Cross, W. E. Kohlbrenner, *J. Biol. Chem.* **1978**, *253*, 4865.
[74] S. B. Singh, K. Herath, Z. Guan, D. L. Zink, A. W. Dombrowski, J. D. Polishook, K. C. Silverman, R. B. Lingham, P. J. Felock, D. J. Hazuda, *Org. Lett.* **2002**, *4*, 1431.
[75] J. Smedsgaard, H. C. Frisvad, *J. Microbiol. Meth.* **1996** *25*, 5.
[76] T. Degenkolb, W. Gams, H. Brückner, *Chem. Biodivers.* **2008**, *5*, 693.

Received December 10, 2007