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Front line defenders of the ecological niche! Screening the structural diversity of peptaibiotics from saprotrophic and fungicolous *Trichoderma/Hypocrea* species

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Abstract
Approximately 950 individual sequences of non-ribosomally biosynthesised peptides are produced by the genus *Trichoderma/Hypocrea* that belong to a perpetually growing class of mostly linear antibiotic oligopeptides, which are rich in the non-proteinogenic α-aminoisobutyric acid (Aib). Thus, they are comprehensively named peptaibiotics. Notably, peptaibiotics represent ca. 80 % of the total inventory of secondary metabolites currently known from *Trichoderma/Hypocrea*. Their unique membrane-modifying bioactivity results from amphipathicity and helicity, thus making them ideal candidates in assisting both colonisation and defence of the natural habitats by their fungal producers. Despite this, reports on the in vivo-detection of peptaibiotics have scarcely been published in the past. In order to evaluate the significance of peptaibiotic production for a broader range of potential producers, we screened nine specimens belonging to seven hitherto uninvestigated fungicolous or saprotrophic *Trichoderma/Hypocrea* species by liquid chromatography coupled to electrospray high resolution mass spectrometry. Sequences of peptaibiotics found were independently confirmed by analysing the peptaibiome of pure agar cultures.

Dedicated to Gary J. Samuels on the occasion of his 70th birthday.

Electronic supplementary material
The online version of this article (doi:10.1007/s13225-013-0276-z) contains supplementary material, which is available to authorized users.

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obtained by single-ascospore isolation from the specimens. Of the nine species examined, five were screened positive for peptaibiotics. A total of 78 peptaibiotics were sequenced, 56 (≈72%) of which are new. Notably, dihydroxyphenylalaninol and O-prenylated tyrosinol, two C-terminal residues, which have not been reported for peptaibiotics before, were found as well as new and recurrent sequences carrying the recently described tyrosinol residue at their C-terminus. The majority of peptaibiotics sequenced are 18- or 19-residue peptaibols. Structural homologies with ‘classical representatives’ of subfamily 1 (SF1)-peptaibiotics argue for the formation of transmembrane ion channels, which are prone to facilitate the producer capture and defence of its substratum.

**Keywords** HPLC/QTOF-ESI-HRMS · Metabolite profiling · Peptaibiotics · Peptaibols · Aib peptides · Trichoderma · Hypocrea

**Introduction**

Currently, the fungal genus *Trichoderma/Hypocrea* \(^1\) comprises more than 200 validly described species, which have been recognised by molecular phylogenetic analysis (Atanasova et al. 2013). This high taxonomic diversity in *Trichoderma/Hypocrea* is not only reflected in a permanently increasing number of species (Jaklitsch 2009, 2011; Jaklitsch and Voglmayr 2012; Jaklitsch et al. 2012, 2013; Chaverri et al. 2011; Samuels and Ismaiel 2011, Samuels et al. 2012a,b; Kim et al. 2012, 2013; Yamaguchi et al. 2012; Li et al. 2013; López-Quintero et al. 2013, Yabuki et al. 2014), but also in a fast-growing number of secondary metabolites of remarkable structural diversity. The latter include low-molecular-weight compounds such as pyrones (Jeleń et al. 2013), butenolides, terpenes, and steroids, but also -heterocyclic compounds and isocyanides. In addition to these relatively nonpolar and often partly volatile compounds, an impressive inventory of nonvolatile compounds, comprising some alkaloids and an imposing number of peptide antibiotics, is produced. Reino et al. (2008) reviewed 186 compounds; however, peptaibiotics (see below) were treated only marginally and incomprehensively. As of August 2013, a total of 501 entries are recorded for *Trichoderma* (461) and *Hypocrea* (40) in AntiBase, more than 300 of which are N-containing, including less than 100 in the range of 50–800 Da (Laatsch 2013). Considering recent publications in this field, which have not yet been included into AntiBase 2013 (Table 1), an estimate of 225 to 250 non-peptaibiotic secondary metabolites from *Trichoderma/Hypocrea* seems appropriate. However, the overwhelming majority of secondary metabolites obtained from this genus so far belong to a perpetually growing family of non-ribosomally biosynthesised, linear or, in a few cases, cyclic peptide antibiotics of exclusively fungal origin, comprehensively named peptaibiotics:

According to the definition, the members of this peptide family show, besides proteinogenic amino acids, \(i\) a relatively high content of the marker \(\alpha\)-aminoisobutyric acid (Aib), which is often accompanied by other \(\alpha\)-\(\alpha\)-dialkyl \(\alpha\)-amino acids such as D- and/or L-isovaline (Iva) or, occasionally, \(\alpha\)-ethynorvaline (EtNa), or 1-aminocyclop propane-1-carboxylic acid (Aec); \(ii\) have a molecular weight between 500 and 2,100 Da, thus containing 4–21 residues; \(iii\) are characterised by the presence of other non-proteinogenic amino acids and/or lipoamino acids; \(iv\) possess an acylated N-terminus, and \(v\) in the case of linear peptides, have a C-terminal residue that most frequently consists of an amide-bonded \(\beta\)-amino alcohol, thus defining the largest subfamily of peptaibiotics, named peptaibols. Alternatively, the C-terminus might also be a polyanine, amide, free amino acid, 2,5-diketopiperazine, or a sugar alcohol (Degenkolb and Brückner 2008; Stoppacher et al. 2013).

Of the approximately 1,250 to 1,300 individual sequences of peptaibiotics known as of autumn 2013 (Ayers et al. 2012; Carroux et al. 2013; Figueroa et al. 2013; Kimonyo and Brückner 2013; Röhrich et al. 2012; Röhrich et al. 2013a, b; Chen et al. 2013; Panizel et al. 2013; Ren et al. 2013; Stoppacher et al. 2013), about 950 have been obtained from *Trichoderma/Hypocrea* species, thus confirming the genus as the most prolific source of this group of non-ribosomal peptide antibiotics (Brückner et al. 1991; Degenkolb and Brückner 2008; Brückner et al. 2009).

Both the taxonomic and metabolic diversity of *Trichoderma/Hypocrea* are hypothesised to originate from mycoparasitism or hyperparasitism, which may represent the ancestral life style of this genus (Kubicek et al. 2011). The unique bioactivities of peptaibiotics, resulting from their amphipathicity and helicity, make them ideal candidates to support the parasitic life style of their fungal producers:

Under in vitro-conditions, the parallel formation of peptaibiotics such as the 19-residue trichorzianins\(^2\) and of hydrolytic enzymes, above all chitinases and \(\beta\)-1,3-glucanases (Schirmböck et al. 1994), could be demonstrated. This observation led to a widely accepted model describing the synergistic interaction of peptaibiotics and hydrolases in the course of mycoparasitism of *Trichoderma atroviride* towards *Botrytis*.

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\(^1\) Authors are aware of the drastic change of the ICBN (International Code of Botanical Nomenclature), which has been adopted at the IBC in Melbourne in July 2011 (Gams et al. 2012; Rossman et al. 2013). However, all strains used in this study were deposited at CBS in July/August 2012, and practical work for this study was finished in December 2012. For reasons of conformity with recently published contributions in the field of peptaibiotics, dual nomenclature is retained in this chemically focussed article.

\(^2\) The trichorzianin-producing strain ATCC 36042 (= CBS 391.92) has originally been identified as *T. harzianum* (el Hajji et al. 1987) but later shown to belong to *T. atroviride* (Kuhls et al. 1996).
**Table 1** Recently described, non-peptaibiotic secondary metabolites from *Trichoderma/Hypocrea* species not yet listed in AntiBase 2013

| Producing species and strains | Name of new metabolite(s) | Chemical subclass of metabolites | References |
|-------------------------------|---------------------------|---------------------------------|------------|
| *T. atroviride* G20-12        | 4'-((4,5-dimethyl-1,3-dioxolan-2-yl)methyl)phenol | D- and tetramerpenes | Lu et al. 2012 |
|                               | (3'-hydroxybutan-2'-yl)5-oxopyrrolidine-2-carboxylate Atroviridetide |                   |            |
| *T. atroviride* UB-LMA<sup>a</sup> | one bicyclic, three tetracyclic diterpenes |                  | Adelin et al. 2014 |
| *T. gamsii* SQP 79–1          | Trichalasin C, D          | Cytochalasans             | Ding et al. 2012 |
|                               |                           | Spiro-cytochalasan        | Ding et al. 2014 |
| *T. sp. FKI-6626*             | Cyto sporone S            |                  | Ishii et al. 2013 |
| *T. erinaeum* AF007           | Trichodermaerin           | Diterpenoid lactone       | Xie et al. 2013 |

<sup>a</sup>The scientific name of the producer has been misspelled as *Trichoderma atroviride* in Adelin et al. (2014)

cinerea* (Lorito et al. 1996). Despite this, reports on in vivo detection of peptaibiotics have scarcely been published in the past. Examples include the isolation of hypelcins A and B obtained from ca. 2 kg of dried, crushed stromata of the mycoparasite Hypocrea peltata (Fujita et al. 1984; Matsura et al. 1993, 1994)<sup>3</sup> as well as the detection of antiamoebins in herbivore dung, which have been produced by the coprophilous Stibella finetaria (syn. *S. erythrocephala*) (Lehr et al. 2006).

In order to close this gap, we initiated a screening project aimed at resolving the question as to whether peptaibiotic production in vivo is a common adaptation strategy of *Trichoderma/Hypocrea* species for colonising and defending ecological niches:

Several *Hypocrea* specimens were freshly collected in the natural habitat and analysed for the presence of peptaibiotics. Sequences of peptaibiotics found were independently confirmed by analysing the peptaibiome<sup>4</sup> of pure agar cultures obtained by single-ascospore isolation from the specimens. Using liquid chromatography coupled to electrospray high resolution mass spectrometry we succeeded in detecting 28 peptaibiotics from the polyporicolous *Hypocrea pulvinata* (Röhrich et al. 2012). Another 49 peptaibiotics were sequenced in *Hypocrea phellinicola*, a parasite of *Phellinus* sp., especially *Ph. ferruginosus* (Röhrich et al. 2013a).

Due to these encouraging results, our screening programme was extended to another nine specimens belonging to seven hitherto uninvestigated mycoparasitic or saprotrophic *Trichoderma/Hypocrea* species, respectively (Table 2).

**Materials and methods**

Specimens of *Hypocrea* teleomorphs were collected from four different locations in Austria (Table 3). Pure agar cultures were obtained by single-ascospore isolations from the respective, freshly collected specimens as previously described by Jaklitsch (2009):

Parts of stromata were crushed in sterile distilled water. The resulting suspension was transferred to cornmeal agar plates (Sigma, St. Louis, Missouri) supplemented with 2 % (w/v) D(+)-glucose-monohydrate (CMD), and 1 % (v/v) of an aqueous solution of 0.2 % (w/v) streptomycin sulfate (Sigma) and 0.2 % (w/v) neomycin sulfate (Sigma). Plates were incubated overnight at 25 °C. In order to exclude possible contamination by spores of other fungal species, few germinated ascospores from within an ascus were transferred to fresh plates of CMD using a thin platinum wire. The plates were sealed with Parafilm (Pechiney, Chicago, Illinois) and incubated at 25 °C. As all species listed in Table 2 could unambiguously be identified by their morphological and growth characteristics (Jaklitsch 2009, 2011), no molecular phylogenetic analyses needed to be performed.

Detailed descriptions of chemicals, extraction and work-up procedures for specimens and agar plate cultures, cultivation methods, as well as comprehensive protocols for HPLC/QTOF-ESI-HRMS were given by Röhrich et al. (2012, 2013a). For routine screening, a high-resolution microTOF Q-II mass spectrometer with orthogonal ESI source (Bruker Daltonic, Bremen, Germany), coupled to an UltiMate 3000 HPLC ( Dionex, Idstein, Germany), was used. Samples, which have been screened negative with the above HPLC/MS system, were re-examined using a maXis 3G QTOF mass spectrometer with orthogonal ESI source (Bruker Daltonic, Bremen, Germany), coupled to an UltiMate 3000 UHPLC (Dionex, Idstein, Germany) as previously described (Röhrich et al. 2012, 2013a).

**Results and discussion**

General considerations. All strains investigated in this study represent phylogenetically well-defined species (Tables 2 and 3). This is in contrast to most of the reports published until the end of the 1990s, when peptaibiotic production by the genus *Trichoderma/Hypocrea* was — according to Rifai’s classification...
mostly attributed to one of the four common species *T. viride*, *T. koningii*, *T. harzianum*, *T. longibrachiatum*, and sometimes *T. pseudokoningii* and *T. aureoviride*. Careful inspection of the literature published prior to the turn of the millennium revealed that only three of the *Trichoderma* strains, reported as sources of ‘classical’ peptaibiotics have correctly been identified and appropriately been deposited, viz. the paracelsin-producing *T. reesei* QM 9414 (Brückner and Graf).

Table 2  Habitats and geographic distribution of *Hypocrea* species included in this study

| Species                        | Clade            | Habitat                                                                 | Geographic distribution                                      |
|--------------------------------|------------------|-------------------------------------------------------------------------|-------------------------------------------------------------|
| *Hypocrea thelephoricola*      | Chlorospora      | On and around basidiomata of *Steccherinum ochraceum*, on wood and bark | North America (USA), Europe (Austria)                       |
| *Hypocrea minutispora*         | Pachybasium      | Most common hyaline-spored species in temperate zones                   | Europe (Austria, Czech Republic, Denmark, Estonia, France, Germany, Spain, Sweden, United Kingdom) and North America (USA) |
| *Hypocrea sulphurea*           | Hypocreanum      | On basidiomes of *Exidia* spp.                                          | Europe (Eastern Austria, Ukraine), North America (USA), Japan |
| *Hypocrea citrina*             | Hypocreanum      | Spreading from stumps or tree bases on soil and debris such as small twigs, bark, leaves, dead plants; incorporating also living plants; more rarely on bark of logs on the ground. Most typically in mixed coniferous forest | widespread and locally common, mostly found from the end of August to the beginning of October. Europe (Austria, Belgium, Czech Republic, Netherlands, Sweden, United Kingdom) and North America (USA) |
| *Hypocrea voglmayrii*          | Lone lineage     | On dead, mostly corticated branches and small trunks of *Alnus alnobetula* (= *A. viridis*) and *A. incana* standing or lying on the ground | Austria (at elevations of 1,000–1,400 m in the upper montane vegetation zone of the Central Alps) |
| *Hypocrea gelatinosa*          | Lone lineage     | On medium- to well-decayed wood, also on bark and overgrowing various fungi | Europe (Austria, France, Germany, Netherlands, Slovenia, Ukraine, United Kingdom) |
| *Hypocrea parmastoi*           | Lone lineage     | On medium- to well-decayed wood and bark of deciduous trees              | Europe (Austria, Estonia, Finland, France, Germany); uncommon |

Data were compiled from Chaverri and Samuels (2003), Overton et al. (2006a, b), and Jaklitsch (2009, 2011)

(1969) – mostly attributed to one of the four common species *T. viride*, *T. koningii*, *T. harzianum*, *T. longibrachiatum*, and sometimes to *T. pseudokoningii* and *T. aureoviride*. Careful inspection of the literature published prior to the turn of the millennium revealed that only three of the *Trichoderma* strains, reported as sources of ‘classical’ peptaibiotics have correctly been identified and appropriately been deposited, viz. the paracelsin-producing *T. reesei* QM 9414 (Brückner and Graf).

Table 3  Habitat and geographic origin of *Hypocrea* isolates included in this study

| Isolate            | Substrate                      | Collecting information                                                                 | Culture          |
|--------------------|--------------------------------|---------------------------------------------------------------------------------------|------------------|
| *H. thelephoricola*| *Steccherinum ochraceum* / *Carpinus betulus* | Austria, Niederösterreich, Wien-Umgebung, Mauerbach, MTB 7763/1, 13 June 2011, W. Jaklitsch | CBS 133226       |
| *H. gelatinosa*    | *Carpinus betulus*             | Austria, Niederösterreich, Wien-Umgebung, Mauerbach, MTB 7763/1, 30 October 2011, W. Jaklitsch (Hypo 656) | not deposited*   |
| *H. minutispora*   | *Carpinus betulus*             | Austria, Vienna, Lainzer Tiergarten, near Nikolaiör, 25 September 2011, H. Voglmayr     | CBS 133242       |
| *H. sulphurea 1*   | *Exidia glandulosa* / *Carpinus betulus* | Austria, Niederösterreich, Wien-Umgebung, Mauerbach, MTB 7763/1, 13 June 2011, W. Jaklitsch | not deposited*   |
| *H. sulphurea 2*   | *Exidia glandulosa* / *Carpinus betulus* | Austria, Styria, Schladming, Untertal, at Riesachfälle, 12 June 2011, H. Voglmayr | CBS 133225       |
| *H. sulphurea 3*   | *Exidia sp.*                   | Austria, Carinthia, Obermiegler, Sabautach, MTB 9452/2, 23 September 2011, W. Jaklitsch (Hypo 654) | CBS 133244       |
| *H. parmastoi*     | *Fagus sylvatica*              |                                                                                       |                  |
| *H. voglmayrii*    | *Alnus alnobetula*             |                                                                                       |                  |
| *H. citrina*       | *Pinus sylvestris* litter, ground |                                                                                       |                  |

* a Stroma immature, isolation of single germinable ascospores impossible

b The specimens of *H. sulphurea* 1 and 2 were collected from two different trees found in the same area
1983; Brückner et al. 1984), the trichosporin/trichopolyn producer *T. polysporum* TMI 60146 (Iida et al. 1990, 1993, 1999), and the paracelsin E-producing *T. saturnisporum* CBS 330.70 (Ritienni et al. 1995). Furthermore, none of the numerous peptaibiotic-producing strains, reported to belong to those six *Trichoderma* species mentioned above, has subsequently been verified by phylogenetic analyses. Statements on the identity of the producers must therefore be regarded with great caution, unless it is being described how isolates were identified (Degenkolb et al. 2008). Unfortunately, most of the peptaibiotic-producing *Trichoderma/Hypocrea* strains investigated prior to 2000 have never been appropriately deposited either i) in a publicly accessible culture collection or ii) in an International Depositary Authority (IDA) under the

Fig. 1 Base-peak chromatograms (BPCs) analysed with the micrOTOF-Q II.

a specimen of *H. thelephorica*,
b plate culture of *H. thelephorica* on PDA. †, non-peptaibiotic metabolite(s); ‡, co-eluting peptaibiotics, not sequenced. The y-axis of all BPC chromatograms in this publication refers to relative ion intensities.
Table 4  Sequences of 11- and 18-residue peptaibiotics detected in the specimen of *Hypocrea thelephoricola*

| No. | $t_R$ [min] | $[M+H]^+$ | Residuea |
|-----|------------|-----------|----------|
|     |            |           |          | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| 1   | 37.6-37.9  | 1161.7527 | Ac Aib Gln Vxx Lxx Aib Pro Vxx Lxx Aib Pro Lxxol |
| 2   | 37.6-37.9  | 1161.7527 | Ac Aib Gln Vxx Vxx Aib Pro Lxx Lxx Aib Pro Lxxol |
| 3   | 39.3-39.5  | 1175.7712 | Ac Aib Gln Vxx Lxx Aib Pro Lxx Lxx Aib Pro Lxxol |
| 4   | 39.7-40.0  | 1175.7712 | Ac Aib Gln Lxx Lxx Aib Pro Vxx Lxx Aib Pro Lxxol |
| 5   | 41.5-41.7  | 1189.7806 | Ac Aib Gln Lxx Lxx Aib Pro Lxx Lxx Aib Pro Lxxol |
| 6   | 42.9-43.0  | 1203.7981 | Ac Vxx Gln Lxx Lxx Aib Pro Lxx Lxx Aib Pro Lxxol |
| 7   | 44.2-44.5  | 1732.0673 | Ac Aib Ala Aib Ala Vxx Gln Aib Vxx Aib Gly Lxx Aib Pro Lxx Lxx Aib Vxxol |
| 8   | 44.8-45.0  | 1746.0866 | Ac Aib Ala Aib Ala Vxx Gln Aib Lxx Aib Gly Lxx Aib Pro Lxx Lxx Aib Vxxol |
| 9   | 45.2-46.0  | 1760.1035 | Ac Aib Ala Vxx Ala Vxx Gln Aib Lxx Aib Gly Lxx Aib Pro Lxx Lxx Aib Vxxol |
| 10  | 47.5-47.8  | 1774.1161 | Ac Aib Ala Vxx Ala Vxx Gln Aib Lxx Aib Gly Lxx Aib Pro Lxx Lxx Aib Vxxol |

| No. | Compound identical or positionally isomeric with | Ref. |
|-----|--------------------------------------------------|------|
| 1   | New                                              |      |
| 2   | Trichorovins: IIIa, IVa                         | Wada et al. 1995 |
|     | Hypomurocin A-1                                  | Becker et al. 1997 |
|     | Trichobrachins III: 5, 9b                        | Krause et al. 2007 |
|     | Tv-29-11-III g                                   | Mukherjee et al. 2011 |
|     | Hypojecorin A: 8                                | Degenkolb et al. 2012 |
| 3   | Trichobrachins III: 10a, 12a, 15b                | Krause et al. 2007 |
|     | Trichorovins: VIII, IXa                          | Wada et al. 1995 |
|     | Hypomurocin A-3                                  | Becker et al. 1997 |
|     | Tv-29-11-IV g                                    | Mukherjee et al. 2011 |
| 4   | Tv-29-11-IV e                                    | Mukherjee et al. 2011 |
| 5   | Trichobrachins III: 16a, 17, 18                 | Krause et al. 2007 |
|     | Trichorovins: XIII, XIV                         | Wada et al. 1995 |
|     | Tv-29-11-V b                                    | Mukherjee et al. 2011 |
|     | Hypomurocin: A-5, A-5a                           | Becker et al. 1997 |
|     | Trichorozin IV                                  | Iida et al. 1995 |
|     | Trichobrachins: C-I, C-II                       | Ruiz et al. 2007 |
|     | Trilongin A9                                    | Mikkola et al. 2012 |
| 6   | Trichofumin B                                   | Berg et al. 2003 |
|     | Tv-29-11-VI                                     | Mukherjee et al. 2011 |
| 7   | Thelephoricolin-1                               |      |
| 8   | Thelephoricolin-2                               |      |
| 9   | Thelephoricolin-3                               |      |
| 10  | Thelephoricolin-4                               |      |

a Variable residues are underlined in the table header. Minor sequence variants are underlined in the sequences. This applies to all sequence tables.
Table 5  Sequences of 11- and 18-residue peptaibiotics detected in the plate culture of *Hypocrea thelephoricola*

| No. | t_R [min] | [M+H]^+ | Residue^a | Ref. |
|-----|-----------|----------|-----------|-----|
| 11  | 35.6–35.8 | 1147.7443 | Ac Aib Gln Vxx Vxx Aib Pro Vxx Lxx Aib Pro Lxxol | Tv-29-11-II h Mukherjee et al. 2011 |
| 1   | 37.2–37.4 | 1161.7623 | Ac Aib Gln Vxx Lxx Aib Pro Vxx Lxx Aib Pro Lxxol | Trichobrachin III 11a Krause et al. 2007 |
| 2   | 37.7–37.9 | 1161.7652 | Ac Aib Gln Vxx Vxx Aib Pro Lxx Lxx Aib Pro Lxxol | Trichorovin Xa Wada et al. 1995 |
| 12  | 39.8–40.0 | 1175.7747 | Ac Aib Gln Lxx Vxx Aib Pro Lxx Lxx Aib Pro Lxxol | Hypomurocin A-4 Becker et al. 1997 |
| 5   | 41.5–41.7 | 1189.7893 | Ac Aib Gln Lxx Lxx Aib Pro Lxx Lxx Aib Pro Lxxol | Tv-29-11-IV f Mukherjee et al. 2011 |
| 13  | 40.6–40.8 | 1189.7996 | Ac Vxx Gln Vxx Lxx Aib Pro Lxx Lxx Aib Pro Lxxol | Trichobrachin III 11a Krause et al. 2007 |
| 6   | 42.8–43.0 | 1203.8004 | Ac Vxx Gln Lxx Lxx Aib Pro Lxx Lxx Aib Pro Lxxol | Trichorovin Xa Wada et al. 1995 |
| 8   | 44.8–44.9 | 1746.0955 | Ac Aib Ala Aib Ala Vxx Gln Aib Lxx Aib Gly Lxx Aib Pro Lxx Aib Vxx Gln Vxxol | Thelephoricolin-2 Mukherjee et al. 2011 |
| 9   | 45.5–45.7 | 1760.1104 | Ac Aib Ala Vxx Ala Vxx Gln Aib Lxx Aib Gly Lxx Aib Pro Lxx Aib Vxx Gln Vxxol | Thelephoricolin-3 Mukherjee et al. 2011 |

^a Variable residues are underlined in the table header. Minor sequence variants are underlined in the sequences. This applies to all sequence tables.
Table 6  Sequences of 11-, 18, and 19-residue peptaibiotics detected in the specimen of 
*Hypocrea gelatinosa*

| No. | t_R [min] | [M+H]+ | Residuea |
|-----|-----------|---------|----------|
|     |           |         | 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 |
| 14  | 37.1–37.3 | 1866.0929 | Ac Ala Ala Ala Ala Glx Ala Gly Lxx Ala Pro Vxx Ala Gln Glx Pheol |
| 15  | 37.7–37.8 | 1895.1067 | Ac Ala Ala Ala Ala Phe Glx Ala Ala Glx Lxx Ala Pro Vxx Ala Gln Glx Lxxol |
| 16  | 38.0–38.2 | 1908.1358 | Ac Ala Ala Ala Ala Phe Glx Ala Ala Glx Lxx Ala Pro Vxx Ala Gln Glx Lxxol |
| 17  | 38.8–38.9 | 1909.1186 | Ac Ala Ala Ala Ala Phe Glx Ala Ala Glx Lxx Ala Pro Vxx Ala Gln Glx Lxxol |
| 18  | 39.5–39.6 | 1880.1083 | Ac Ala Ala Ala Ala Glx Ala Glx Ala Lxx Ala Pro Vxx Ala Gln Glx Pheol |
| 19  | 40.2–40.4 | 1762.0856 | Ac Ala Ala Ala Ala Glx Ala Glx Ala Lxx Ala Pro Vxx Ala Gln Glx Lxxol |
| 20  | 40.9–41.1 | 1762.0840 | Ac Ala Ala Ala Ala Glx Ala Glx Ala Lxx Ala Pro Vxx Ala Gln Glx Lxxol |
| 21  | 41.2–41.4 | 1776.1023 | Ac Ala Ala Ala Ala Glx Ala Glx Ala Lxx Ala Pro Vxx Ala Gln Glx Lxxol |
| 22  | 41.9      | 1952.1674 | Ac Ala Ala Ala Ala Glx Ala Glx Ala Lxx Ala Pro Vxx Ala Gln Glx Lxxol |
| 23  | 42.1–42.3 | 1776.1023 | Ac Ala Ala Ala Ala Glx Ala Glx Ala Lxx Ala Pro Vxx Ala Gln Glx Lxxol |
| 24  | 42.9      | 1953.1515 | Ac Ala Ala Ala Ala Glx Ala Glx Ala Lxx Ala Pro Vxx Ala Gln Glx Lxxol |
| 25  | 43.0–43.1 | 1790.1199 | Ac Ala Ala Ala Ala Glx Ala Glx Ala Lxx Ala Pro Vxx Ala Gln Glx Lxxol |
| 26  | 44.6      | 1919.1568 | Ac Ala Ala Ala Ala Glx Ala Glx Ala Lxx Ala Pro Vxx Ala Gln Glx Lxxol |
| 27  | 45.8      | 1774.1299 | Ac Ala Ala Ala Ala Glx Ala Glx Ala Lxx Ala Pro Vxx Ala Gln Glx Lxxol |

No.  Compound identical or positionally isomeric with  Ref.

14  Hypopulvin-9  Röhrich et al. 2012
15  Gelatinosin-A 1 (C-terminal undecapeptide cf. hypelcins B-I and -II)  Matsuura et al. 1994
16  Gelatinosin-A 2 (C-terminal nonapeptide cf. tricholongin B-I)  Rebuffat et al. 1991
17  Gelatinosin-A 3  (cf. 16)
18  Hypopulvin-14  Röhrich et al. 2012
19  Gelatinosin-B 1  (cf. hypomurocin B-5: [Vxx]8→[Lxx]8)  Becker et al. 1997
20  Gelatinosin-B 3  (cf. hypomurocin B-3h: [Vxx]8→[Lxx]8, [Alb]11→[Vxx]11)  Becker et al. 1997
21  Gelatinosin-B 3  (cf. neoatroviridin B: [Gly]7→[Ser]7)  Oh et al. 2005
22  Gelatinosin-A 4  (cf. 16: [Gly]10→[Ser]10, [Alb]5→[Vxx]5)  Oh et al. 2005
23  Gelatinosin-B 4  (cf. hypomurocin B-4: [Alb]5→[Vxx]5)  Becker et al. 1997
24  See *H. thelephoricola*
25  Gelatinosin-A 5  (cf. 17: [Gly]10→[Ser]10, [Alb]5→[Vxx]5)  Oh et al. 2005
26  Gelatinosin-B 5  (cf. neoatroviridin D: [Gly]2→[Ser]2)  Oh et al. 2005
27  Gelatinosin-B 6  (cf. neoatroviridin D: [Gly]2→[Ala]2)  Oh et al. 2005

a Variable residues are underlined in the table header. Minor sequence variants are underlined in the sequences. This applies to all sequence tables.
| No. | Compound identical or positionally isomeric with Ref. |
|-----|-----------------------------------------------------|
| 28  | Gelatinosin-B 7 (cf. hypomurocin B-2: [Vxx]8→[Lxx]8) Becker et al. 1997 |
| 29  | Tv-29-11-IVe (positionals isomer of 4) Mukherjee et al. 2011 |
| 30  | Gelatinosin-B 8 (cf. hypomurocin B-4: [Vxx]8→[Lxx]8) Becker et al. 1997 |
| 31  | Gelatinosin-B 9 (cf. hypomurocin B-3b: [Vxx]8→[Lxx]8, [Vxxol]18→[Lxxol]18) Becker et al. 1997 |
| 32  | Gelatinosin-B 10 (cf. 25: [Gln]17→[Glu]17) Jaworski et al. 1999; Oh et al. 2005 |
| 33  | See H. thelephoricola (positionals isomer of 5) |
| 20  | Gelatinosin-B 2 (cf. hypomurocin B-4: [Aib]7→[Vxx]7, [Vxx]8→[Lxx]8) Becker et al. 1997 |
| 34  | Gelatinosin-B 11 (cf. trichovirin II 6a and neatoviridin C: [Gly]2→[Ser]2) Jaworski et al. 1999; Oh et al. 2005 |
| 6   | See H. thelephoricola |
| 25  | Gelatinosin-B 5 |
| 27  | Gelatinosin-B 6 |

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Table 7  Sequences of 11- and 18-residue peptaibiotics detected in the plate culture of *Hypocrea gelatinosa*

| No. | \( t_\text{R} [\text{min}] \) | \([M+H]^+\) | Residuea |
|-----|-----------------|-------------|-----------|
| 28  | 38.0–38.1       | 1748.0789   | Ac Aib Ser Ala Lxx Aib Gln Aib Gln Aib Lxx Aib Gly Aib Aib Pro Lxx Aib Aib Gln Lxxol |
| 29  | 38.8–38.9       | 1175.7832   | Ac Aib Gln Lxx Lxx Aib Pro Vxx Lxx Aib Pro Lxxol |
| 30  | 39.2–39.3       | 1748.0789   | Ac Aib Ser Ala Lxx Aib Gln Aib Lxx Aib Gly Vxx Aib Pro Lxx Aib Aib Gln Vxxol |
| 31  | 39.4–39.7       | 1762.0802   | Ac Aib Ser Ala Lxx Aib Gln Aib Gln Vxx Lxx Aib Gly Aib Aib Pro Lxx Aib Aib Gln Lxxol |
| 32  | 40.1–40.4       | 1777.0993   | Ac Aib Ser Ala Lxx Vxx Gln Vxx Lxx Aib Gly Aib Pro Lxx Aib Aib Gln Lxxol |
| 33  | 40.8–41.0       | 1189.8026   | Ac Aib Gln Lxx Lxx Aib Pro Lxx Aib Pro Lxxol |
| 34  | 41.8–42.1       | 1776.1016   | Ac Aib Ser Ala Lxx Aib Gln Vxx Vxx Lxx Aib Gly Vxx Aib Pro Lxx Aib Aib Gln Lxxol |
| 35  | 41.4–42.9       | 1203.8234   | Ac Vxx Gln Lxx Lxx Aib Pro Lxx Aib Lxxol |
| 32  | 43.1–43.3       | 1790.1139   | Ac Aib Ser Ala Lxx Vxx Gln Vxx Lxx Aib Gly Vxx Aib Pro Lxx Aib Aib Gln Lxxol |
| 27  | 45.7–46.0       | 1774.1162   | Ac Aib Ala Ala Lxx Vxx Gln Vxx Lxx Aib Gly Vxx Aib Pro Lxx Aib Aib Gln Lxxol |

a Variable residues are underlined in the table header. Minor sequence variants are underlined in the sequences. This applies to all sequence tables.
Screening of *Hypocrea thelephoricola*. Ten peptaibols from the specimen of *H. thelephoricola* were sequenced (Fig. 1a). Six of them, compounds 1–6, are 11-residue sequences displaying the classical building scheme of subfamily 4 (SF4) peptaibols (Chugh and Wallace 2001; Degenkolb et al. 2012; Röhrich et al. 2013b). Compound 1 is new,
whereas compounds 2–6 are likely to represent 11-residue peptaibols, which have been described before (Tables 4 and 5, Table S1a and S1b). Compounds 7–10 are new 18-residue peptaibols, named thelephoricolins 1–4 sharing some structural similarity (N-terminal dipeptide, [Gln]⁶/[Aib]³, C-terminal heptapeptide) with trichotoxins A-50H and A-50-J⁵ (Brückner and Przybylski 1984). The plate culture produced predominantly 11-residue SF4-peptaibols (compounds 1, 2, 5, 6, 11–13), but only two 18-residue peptaibols, thelephoricolins 2 and 3 (Fig. 1b).

Screening of Hypocrea gelatinosa. A single strain (ICMP 5417) of this species has previously been screened positive Aib and Iva by a GC/MS-based approach (Brückner et al. 1991). From the specimen of H. gelatinosa,
Table 8  Sequences of 18- and 19-residue peptaibiotics detected in the specimen of *Hypocrea voglmayrii*

| No. | [M+H]+ (Da) | Residuea |
|-----|-------------|---------|
| 35  | 1762.0125   | Ac Aib Ala Aib Ala Aib Gln Aib Aib Ala Lxx Vxx Pro Vxx Aib Vxx Gln Gln |
| 36  | 1775.0433   | Ac Aib Ala Aib Aib Aib Gln Aib Aib Ala Lxx Vxx Pro Vxx Aib Vxx Gln Gln |
| 37  | 1924.1239   | Ac Aib Aib Aib Aib Gln Aib Aib Ala Lxx Vxx Pro Vxx Aib Vxx Gln Gln Tyrol |
| 38  | 1911.1015   | Ac Aib Ala Aib Aib Gln Aib Aib Ala Lxx Vxx Pro Vxx Aib Vxx Gln Gln Tyrol |
| 39  | 1925.1100   | Ac Aib Aib Aib Aib Aib Aib Gln Aib Aib Ala Lxx Aib Pro Vxx Aib Vxx Gln Gln |
| 40  | 1880.1041   | Ac Aib Aib Aib Gln Aib Aib Aib Ala Lxx Aib Pro Vxx Aib Vxx Gln Gln |
| 41  | 1894.1197   | Ac Aib Aib Aib Aib Aib Gln Aib Aib Aib Ala Lxx Aib Pro Vxx Aib Vxx Gln Gln |
| 42  | 1881.0933   | Ac Aib Aib Aib Aib Gln Aib Aib Aib Ala Lxx Aib Pro Vxx Aib Vxx Gln Gln |
| 43  | 1894.1218   | Ac Aib Ala Aib Aib Aib Gln Aib Aib Aib Ala Lxx Vxx Pro Vxx Aib Vxx Gln Gln |
| 44  | 1908.1391   | Ac Aib Aib Aib Aib Aib Aib Aib Gln Aib Aib Ala Lxx Vxx Pro Vxx Aib Vxx Gln Gln |
| 45  | 1909.1203   | Ac Aib Aib Aib Aib Aib Aib Aib Gln Aib Aib Ala Lxx Vxx Pro Vxx Aib Vxx Gln Gln |
| 46  | 1978.1743   | Ac Vxx Ala Aib Aib Aib Aib Gln Aib Aib Aib Ala Lxx Vxx Pro Vxx Aib Gln Gln |
| 47  | 1978.1741   | Ac Aib Aib Aib Aib Aib Aib Aib Gln Aib Aib Ala Lxx Vxx Pro Vxx Aib Vxx Gln Gln |
| 48  | 1992.1924   | Ac Aib Aib Aib Aib Aib Gln Aib Aib Aib Ala Lxx Vxx Pro Vxx Aib Vxx Gln Gln |
| 49  | 1997.1385   | Ac Aib Aib Aib Aib Aib Aib Aib Gln Aib Aib Ala Lxx Vxx Pro Vxx Aib Vxx Gln Gln |
| 50  | 1993.1762   | Ac Aib Aib Aib Aib Aib Aib Aib Gln Aib Aib Ala Lxx Vxx Pro Vxx Aib Vxx Gln Gln |
| 51  | 2007.1881   | Ac Vxx Ala Aib Aib Aib Aib Gln Aib Aib Aib Ala Lxx Vxx Pro Vxx Aib Vxx Gln Gln |

No.  Compound identical or positionally isomorphic with Ref.  
35 Voglmayrin-1 (N-terminal heptapeptide, pos. 13–15 and 18 cf. trichokonin V)  
36 Voglmayrin-2 (cf. 35: [Ala]3→[Aib]4, [Glu]17→[Gln]17: deletion sequence of 37)  
37 Voglmayrin-3 (cf. 36: + C-terminal Tyrol)  
38 Voglmayrin-4  
39 Voglmayrin-5 (cf. 37: [Gln]18→[Glu]18)  
40 Voglmayrin-6 (N-terminal nonapeptide cf. trichorzianine B-VIb, [Ser]10→[Ala]10, C-terminal nonapeptide cf. trichorzianine B-VIb, [Ile]16→[Vxx]16)  
41 Voglmayrin-7  
42 Voglmayrin-8 (homologue of 40: [Gln]18→[Glu]18)  
43 Voglmayrin-9 (homologue of 40: [Aib]12→[Vxx]12)  
44 Voglmayrin-10 (homologue of 37: [Tyrol]19→[Pheol]19)  

Huang et al. 1995  
Rebuffat et al. 1989
14 compounds 14–27, six 18-residue and eight 19-residue peptaibols, were sequenced. All of them but compounds 14 and 18 are new (Tables 6 and 7, Table S2a and S2b; Fig. 2a). The 18-residue sequences, compounds 19–21, 23, 25, and 27, named gelatinosins B 1–6, resemble hypomurocins or neoatroviridins. Two of the 19-residue sequences, compounds 14 and 18, are identical with the recently described hypopulvins from H. pulvinata (Röhrich et al. 2012). The new compounds 15–17, 22, and 24, named gelatinosins A 1–5, exhibit a partially new building scheme – the residue in position 5 of the peptide chain was assigned as Phe, based upon HR-MS/MS data. In contrast to this, the new 19-residue compound 26 displays a different building scheme, resembling trichostrigocins A/B (Degenkolb et al. 2006a). The plate culture of H. gelatinosa was shown to produce three minor 11-residue SF4-peptaibols, compounds 6, 29, and 33, and nine gelatinosins B (compounds, 19, 20, 25, 27, 28, 30–32, and 34), 18-residue peptaibols of the hypomurocin/neoatroviridin-type. However, 19-residue peptaibols have not been detected (Tables 6 and 7, Table S2a and S2b; Fig. 2b).

Compound 6 is likely to represent the second one of the partial sequences reported by Krause et al. (2006a) for H. gelatinosa CBS 724.87. In contrast, the first one, for which an unknown N-terminal residue m/z 157 was claimed (Krause et al. 2006a), could not be detected in this screening.

Screening of Hypocrea voglmayrii. The most notable species screened is by far H. voglmayrii (Fig. 3), the specimen of which produced two 18-residue deletion sequences, compounds 35 and 36, which lack the C-terminal amino alcohol, and as well as 15 19-residue peptaibols, compounds 37–51 (Tables 8 and 9, Table S3a and S3b). As all of them are new, the names voglmayrins 1–17 are introduced. They partly resemble the building schemes of trichostrigocins A/B (Huang et al. 1995) and of trichorzianins B (Rebuffat et al. 1989). Six of the major compounds (40–45) carry a C-terminal phenylalaninol (Pheol) residue, whereas three minor compounds (37–39) terminate in tyrosinol (Tyrol) – a residue that has not been described for peptaibiotics until recently (Röhrich et al. 2013a). Another six major compounds (46–51) display an additional fragment ion 68.0628 ± 2.3 mDa at their C-terminus (Fig. 4). Thus, the p-OH group of their Tyrol residue is hypothesised to be substituted by a prenyl or isoprenyl residue (C₅H₈, for details see paragraph below). In contrast to this, major 19-residue peptaibols produced by the plate culture, compounds 40, 41, 43, 44, and two additional compounds, 52 and 53, voglmayrins-18 and -19, terminate in Pheol. HR-MS data clearly confirm the presence of additional minor

### Table 8 (continued)

| No. | Compound identical or positionally isomeric with | Ref. |
|-----|-----------------------------------------------|-----|
| 45  | Voglmayrin-1 | Hypomurocin-1 (Becker et al. 1997) |
| 46  | Voglmayrin-12 | Hypomurocin-12 (Becker et al. 1997) |
| 47  | Voglmayrin-13 | Hypomurocin-13 (Becker et al. 1997) |
| 48  | Voglmayrin-15 | Hypomurocin-15 (Becker et al. 1997) |
| 49  | Voglmayrin-16 | Hypomurocin-16 (Becker et al. 1997) |
| 50  | Voglmayrin-17 | Hypomurocin-17 (Becker et al. 1997) |
| 51  | Voglmayrin-18 | Hypomurocin-18 (Becker et al. 1997) |

*Variable residues are underlined in the table header. Minor sequence variants are underlined in the sequence. This applies to all sequence tables.*

8 Hypomurocins have been isolated from strain IFO 31288 (Becker et al. 1997), originally misidentified as Hypocrea muroiana. The producer belongs, in fact, to T. atroviride (Samuels et al. 2006).

7 The neoatroviridin producer T. atroviride F80317 (Oh et al. 2005) has neither been deposited with an IDA, nor has its identity been verified phylogenetically.
Table 9  Sequences of 11- and 19-residue peptaibiotics detected in the plate culture of *Hypocrea voglmayrii*

| No. | t_R [min] | [M+H]+ | Residuea |
|-----|-----------|---------|----------|
|     |           |         |          | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
| 52  | 35.2–35.6 | 1852.0739 | Ac Aib Ala Ala Aib Aib Gln Ala Aib Aib Ala Lxx Aib Pro Vxx Aib Vxx Gln Gln Pheol |
| 53  | 35.6–35.8 | 1866.0884 | Ac Aib Ala Ala Aib Aib Gln Ala Aib Aib Ala Lxx Aib Pro Vxx Aib Vxx Gln Gln Pheol |
| 40  | 37.3–37.6 | 1880.1099 | Ac Aib Ala Ala Aib Aib Gln Aib Aib Aib Ala Lxx Aib Pro Vxx Aib Vxx Gln Gln Pheol |
| 41  | 37.7–37.8 | 1894.1237 | Ac Aib Ala Aib Aib Aib Gln Aib Aib Aib Ala Lxx Aib Pro Vxx Aib Vxx Gln Gln Pheol |
| 43  | 39.6–39.7 | 1894.1238 | Ac Aib Ala Aib Aib Aib Gln Aib Aib Aib Ala Lxx Aib Pro Vxx Aib Vxx Gln Gln Pheol |
| 44  | 40.0      | 1908.1395 | Ac Aib Ala Aib Aib Aib Gln Aib Aib Aib Ala Lxx Aib Pro Vxx Aib Vxx Gln Gln Pheol |
| 54  | 40.7–41.0 | 1052.7130 | Oc Aib Gly Lxx Aib Gly Gly Vxx Aib Gly Lxx Lxxol |
| 55  | 42.8–43.1 | 1066.7288 | Oc Aib Gly Lxx Aib Gly Gly Lxx Aib Gly Lxx Lxxol |

| No. | Comment (compound identical or positionally isomeric with) | Ref. |
|-----|----------------------------------------------------------|-----|
| 52  | Voglmayrin-18 (homologue of 53; [Vxx]^16→[Aib]^16; N-terminal hexapeptide cf. trichorzianine B-VIIb; C-terminal nonapeptide cf. trichosporins B) | Rebuffat et al. 1989; Iida et al. 1990 |
| 53  | Voglmayrin-19 (homologue of 40; [Aib]^17→[Ala]^17; C-terminal nonapeptide cf. polysporin D) | New et al. 1996 |
| 40  | Voglmayrin-20 | |
| 41  | Voglmayrin-21 | |
| 43  | Voglmayrin-22 | |
| 44  | Voglmayrin-23 | |
| 54  | cf. lipostrigocins B-04 and B-05 | Degenkolb et al. 2006a |
| 55  | cf. trichogin A-IV | Auvin-Guette et al. 1992; Degenkolb et al. 2006a |

*a Variable residues are underlined in the table header. Minor sequence variants are underlined in the sequences. This applies to all sequence tables*
Fig. 4 HR-MS/MS sequencing of diagnostic, C-terminal γ-ions, displaying novel and recurrent residues of β-amino alcohols. a phenylalaninol (Pheol); b tyrosinol (Tyrol); c O-prenylated tyrosinol (Tyr(C5H8)ol); d dihydroxyphenylalaninol (DOPAol)
Table 10  Sequences of 19-residue peptaibiotics detected in the specimen of *Hypocrea minutispora*

| No. | $t_{R}$ [min] | $[M+H]^+$ | Residue$^a$ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
|-----|----------------|------------|-------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 56  | 34.5–34.7      | 1847.1051  | Ac Aib Ala  | Aib Gly| Aib | Gln | Aib | Lxx | Aib | Gly | Lxx | Aib | Pro | Vxx | Aib | Vxx | Glu | Gln | Lxxol |
| 57  | 37.5–38.1      | 1846.1192  | Ac Aib Ala  | Aib | Aib| Aib | Aib | Gln | Aib | Lxx | Aib | Gly | Lxx | Aib | Pro | Vxx | Aib | Aib | Gln | Gln | Lxxol |
| 58  | 38.5–38.6      | 1846.1099  | Ac Aib Ala  | Aib | Ala| Aib | Gln | Aib | Lxx | Aib | Gly | Lxx | Aib | Pro | Vxx | Aib | Vxx | Glu | Gln | Lxxol |
| 59  | 39.1–39.4      | 1860.1278  | Ac Aib Ala  | Aib | Aib| Aib | Aib | Gln | Aib | Lxx | Aib | Gly | Lxx | Aib | Pro | Vxx | Aib | Vxx | Glu | Gln | Lxxol |
| 60  | 39.8–40.1      | 1861.1130  | Ac Aib Ala  | Aib | Gln | Aib | Aib | Lxx | Aib | Gly | Lxx | Aib | Pro | Vxx | Aib | Vxx | Glu | Gln | Lxxol |
| 61  | 40.9–41.0      | 1874.1420  | Ac Aib Ala  | Aib | Ala| Aib | Aib | Vxx | Glu | Aib | Lxx | Aib | Lxx | Aib | Pro | Vxx | Aib | Vxx | Glu | Gln | Lxxol |
| 62  | 41.5–41.6      | 1875.1390  | Ac Aib Ala  | Aib | Aib| Aib | Aib | Gln | Aib | Lxx | Aib | Gly | Lxx | Aib | Pro | Vxx | Aib | Vxx | Glu | Gln | Lxxol |
| 63  | 41.9–42.0      | 1875.1284  | Ac Aib Ala  | Aib | Ala| Aib | Aib | Lxx | Aib | Lxx | Aib | Vxx | Glu | Aib | Vxx | Glu | Gln | Lxxol |

* Variable residues are underlined in the table header. Minor sequence variants are underlined in the sequences. This applies to all sequence tables.
Table 11  Sequences of 19-residue peptaibiotics detected in the plate culture of Hypocrea minutispora

| No. | $t_R$ [min] | $[M+H]^+$ | Residue$^a$ |
|-----|-------------|------------|-------------|
| 64  | 36.1–36.3   | 1832.1060  | Ac Aib Ala Aib Ala Aib Gln Aib Lxx Aib Gly Lxx Aib Pro Vxx Aib Aib Gln Gln Vxxol |
| 65  | 37.3–37.5   | 1832.1025  | Ac Aib Ala Aib Gly Aib Gln Aib Lxx Aib Gly Lxx Aib Pro Vxx Aib Vxx Gln Gln Vxxol |
| 66  | 37.5–37.9   | 1846.1196  | Ac Aib Ala Aib Ala Aib Gln Aib Lxx Aib Gly Vxx Aib Pro Vxx Aib Vxx Gln Gln Lxxol |
| 57  | 37.8–38.0   | 1846.1199  | Ac Aib Ala Aib Ala Aib Gln Aib Lxx Aib Gly Lxx Aib Pro Vxx Aib Aib Gln Gln Vxxol |
| 67  | 38.6–38.7   | 1847.1135  | Ac Aib Ala Aib Ala Aib Gln Aib Lxx Aib Gly Lxx Aib Pro Vxx Aib Aib Gly Aib Vxx Gln Gln Lxxol |
| 59  | 39.0–39.2   | 1860.1318  | Ac Aib Ala Aib Ala Aib Gln Aib Lxx Aib Gly Lxx Aib Pro Vxx Aib Aib Gln Gln Vxxol |
| 60  | 39.8–40.0   | 1861.1271  | Ac Aib Ala Aib Ala Aib Gln Aib Lxx Aib Gly Lxx Aib Pro Vxx Aib Aib Gly Aib Vxx Gln Gln Lxxol |
| 68  | 40.4–40.6   | 1874.1492  | Ac Aib Ala Aib Ala Aib Gln Aib Lxx Aib Gly Lxx Aib Pro Lxx Aib Vxx Gln Gln Lxxol |
| 61  | 40.6–40.9   | 1874.1554  | Ac Aib Ala Aib Ala Vxx Gln Aib Lxx Aib Gly Lxx Aib Pro Vxx Aib Aib Vxx Gln Gln Lxxol |

| No. | Compound identical or positionally isomeric with | Ref. |
|-----|-----------------------------------------------|-----|
| 64  | Minutisporin-9 (pos. 1, 6–10, 12–19; [Pro]$^7$→[Ala]$^9$, [Aib]$^{11}$→[Lxx]$^{11}$ and deletion of [Aib]$^5$: cf. stilbollavin B-5) | Jaworski and Brückner 2001b |
| 65  | Minutisporin-10 (positionally isomer of 64: [Ala]$^9$→[Gly]$^9$, [Aib]$^{11}$→[Vxx]$^{11}$) | |
| 66  | Minutisporin-11 (positionally isomer of 57: [Lxx]$^1$→[Vxx]$^{11}$, [Aib]$^{10}$→[Vxx]$^{10}$) | |
| 57  | Minutisporin-2 | |
| 67  | Minutisporin-12 (positionally isomer of 57: [Gln]$^7$→[Glu]$^{17}$ and of 56: [Aib]$^7$→[Gly]$^7$, [Aib]$^{10}$→[Vxx]$^{10}$) | |
| 59  | Minutisporin-4 | |
| 60  | Minutisporin-5 | |
| 68  | Minutisporin-13 (positionally isomer of 61: [Aib]$^7$→[Vxx]$^7$) | |
| 61  | Minutisporin-6 | |

$^a$ Variable residues are underlined in the table header. Minor sequence variants are underlined in the sequences. This applies to all sequence tables.
components carrying a C-terminal Tyrol or prenylated Tyrol residue, respectively. Unfortunately, the intensities were too low for MS/MS sequencing of the respective \( y_6 \) ions. Two 11-residue lipopeptaibols, compound 54 and 55, resembling lipostrigocin B-04/B-05 (Degenkolb et al. 2006a) and trichogin A IV (Auvin-Guette et al. 1992), have also been sequenced.

Screening of *Hypocrea minutispora*. The specimen of *H. minutispora* has been shown to produce a mixture of eight new 19-residue peptaibols, compounds 56–63, named...
Table 12  Sequences of 19-residue peptaibiotics detected in the specimen of Hypocrea citrina

| No. | \( t_R \) [min] | \([M+H]^+\) | Residue<sup>a</sup> |
|-----|-----------------|-------------|-------------------|
|     |                 |             | 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 |
| 69  | 31.6–31.7       | 1926.1036   | Ac Aib Ala Aib Ala Gln Aib Lxx Aib Gly Lxx Aib Pro Vxx Aib Vxx Gln Gln di-OH-Pheol |
| 70  | 32.0–32.1       | 1896.0937   | Ac Aib Ala Aib Ala Aib Gln Aib Lxx Aib Gly Lxx Aib Pro Vxx Aib Gln Gln Tyrol |
| 71  | 32.9–33.1       | 1910.1084   | Ac Aib Ala Aib Ala Aib Gly Lxx Aib Pro Vxx Aib Vxx Gln Gln Tyrol |
| 72  | 33.6–33.9       | 1880.0971   | Ac Aib Ala Aib Gly Aib Gln Aib Lxx Aib Gly Lxx Aib Pro Vxx Aib Vxx Gln Gln Tyrol |
| 73  | 34.6–34.7       | 1880.0975   | Ac Aib Ala Aib Ala Aib Gln Aib Lxx Aib Gly Lxx Aib Pro Vxx Aib Vxx Gln Gln Tyrol |
| 74  | 36.4–36.6       | 1880.0999   | Ac Aib Ala Aib Ala Aib Gln Aib Lxx Aib Gly Lxx Aib Pro Vxx Aib Vxx Gln Gln Tyrol |
| 75  | 37.7–37.9       | 1880.1050   | Ac Aib Ala Aib Ala Aib Gln Aib Lxx Aib Gly Lxx Aib Pro Vxx Aib Vxx Gln Gln Tyrol |
| 76  | 38.2–38.4       | 1880.1018   | Ac Aib Ala Aib Ala Aib Gln Aib Lxx Aib Gly Lxx Aib Pro Vxx Aib Vxx Gln Gln Tyrol |
| 77  | 38.8–39.1       | 1894.1241   | Ac Aib Ala Aib Ala Aib Gln Aib Lxx Aib Gly Lxx Aib Pro Vxx Aib Vxx Gln Gln Tyrol |
| 78  | 39.7–39.9       | 1895.1083   | Ac Aib Ala Aib Ala Aib Gln Aib Lxx Aib Gly Lxx Aib Pro Vxx Aib Vxx Glu Glu Tyrol |

| No. | Compound identical or positionally isomeric with | Ref. |
|-----|-----------------------------------------------|------|
| 69  | Hypocitrin-1 (homologue of hypophellin-15: [Tyrol]<sup>19</sup>→[di-OH-Pheol]<sup>19</sup>) | Röhrich et al. 2013a |
| 70  | Hypocitrin-2 (homologue of hypophellin-15: [Vxx]<sup>17</sup>→[Aib]<sup>17</sup>) | Röhrich et al. 2013a |
| 71  | Hypophellin-15 | Röhrich et al. 2013a |
| 72  | Hypocitrin-3 (positional isomer of 73, 74, and 76; [Ala]<sup>3</sup>→[Aib]<sup>3</sup>, [Ala]<sup>4</sup>→[Gly]<sup>4</sup>) | Röhrich et al. 2013a |
| 73  | Hypocitrin-4 (positional isomer of 75 and 77, homologue of hypophellin-17: [Vxx]<sup>17</sup>→[Aib]<sup>17</sup>) | Röhrich et al. 2013a |
| 74  | Hypocitrin-5 (positional isomer of 73 and 77, homologue of hypophellin-17: [Vxx]<sup>17</sup>→[Aib]<sup>17</sup>) | Röhrich et al. 2013a |
| 75  | Hypophellin-18 | Röhrich et al. 2013a |
| 76  | Hypocitrin-6 (positional isomer of 73 and 75, homologue of hypophellin-17: [Vxx]<sup>17</sup>→[Aib]<sup>17</sup>) | Röhrich et al. 2013a |
| 77  | Hypophellin-20 | Röhrich et al. 2013a |
| 78  | Hypocitrin-7 (homologue of 77: [Gln]<sup>17</sup>→[Glu]<sup>17</sup>) | Röhrich et al. 2013a |

<sup>a</sup>Variable residues are underlined in the table header. Minor sequence variants are underlined in the sequences. This applies to all sequence tables.
minutisporins 1–8 (Tables 10 and 11, Table S4a and S4b; Fig. 5a), resembling the recently described hypophellins (Röhrich et al. 2013a). Analysis of the plate culture (Fig. 5b) revealed that compounds 59–61 were recurrently isolated along with another five new 19-residue sequences, minutisporins 9–13 (compounds 64–68).

Screening of Hypocrea citrina. The specimen of H. citrina was shown to be a prolific producer of 19-residue peptaibols, compounds 69–78, of which seven are new, viz. compounds 69, 70, 72–74, 76, and 78. The names hypocitrins 1–7 were selected in order to avoid possible confusion with the mycotoxin citrinin and its derivatives. The remaining three were identified as hypophellin-15, 18, and 20, respectively (Röhrich et al. 2013a). Notably, compound 69, hypocitrin-1, exhibits a C-terminal substituent, which is novel to peptaibiotics, dihydroxyphenylalaninol (Table 12 and Table S5; Fig. 6). Compound 70, hypocitrin-2, a homologue of hypophellin-15 (compound 73), also terminates in Tyrol (Fig. 4). Due to exceptionally high background noise of unknown origin, the methanolic extract of the well-grown H. citrina plate culture could not be interpreted appropriately.

Screening of Hypocrea sulphurea. All three specimens of H. sulphurea were negatively screened for peptaibiotics. From two of them, plate cultures could be obtained; however, those were also screened negatively (data not shown).

Screening of Hypocrea parmastoi. Neither specimen, nor plate culture of H. parmastoi displayed the presence of peptaibiotics (data not shown).

Screening of specimens collected in the natural habitat(s) corroborated the distinguished importance of the genus Trichoderma/Hypocrea as the currently richest source of peptaibiotics. Five of the nine specimens were screened positively, and the results of this screening confirmed by the sequences obtained from screening of the plate cultures. Notably, 56 of the 78 peptaibiotics (72 %) detected represent new sequences.

Screening of H. voglmayrii and H. citrina revealed five peptaibols (compounds 37–39, 70, and 73) carrying a C-terminal Tyrol, a residue quite recently described for H. phellinicola (Röhrich et al. 2013a), which is considered comparatively rare. The additional substituent of the C-terminal Tyrol of voglmayrins 12–17 (compounds 46–51), which has tentatively been assigned as a prenyl or isoprenyl (C5H8) residue, is hypothesised to be located at the p-hydroxy group. A regiospecific O-prenylation at the 4-position of the aromatic ring has recently been demonstrated for SirD (Zou et al. 2011), a tyrosine O-prenyltranferase (Kremer and Li 2010) catalysing the first pathway-specific step in the biosynthesis of the phytotoxin sirodesmin PL. The latter is produced by Leptosphaeria maculans (anamorph: Phoma lingam), the causal agent of blackleg of canola (Brassica napus). Recently, O-prenyltyrosine diketopiperazines have been described from Fusarium sp. and Penicillium crustosum (Guimarães et al. 2010).

Another notable structural element, dihydroxy-Phelol was found at the C-terminus of hypocitrin-1 (compound 69). While the presence of either Phelol or Tyrol may be assumed to originate from the relaxed substrate specificity in the terminal adenylate domain of the respective peptaibol synthetase, the direct incorporation of dihydroxy-Phel, presumably 3,4-dihydroxy-L-Phel (DOPA), is one possible biosynthetic route. Fungal tyrosinases are known to oxidise not only Tyr and various other monophenols, e.g. in the route to melanins, but also act on tyrosyl residues within peptides and proteins, leading to the formation of inter- and intra-molecular crosslinks (Selinheimo et al. 2007). Thus, Tyrol-containing peptaibols could be further oxidised by tyrosinases, and even

Fig. 6 Base-peak chromatograms (BPCs) of the specimen of H. citrina analysed with the micrOTOF-Q II. †, co-eluting peptaibiotics, not sequenced.
become attached to components of the fungal cell wall (Mattinen et al. 2008).

Considering the sequences of all species screened, including those of *H. pulvinata* and *H. phellinicola*, a general building scheme for those SF1-peptaibiotics can be given (Table 13):

As can be seen from above, all structural features (Röhrich et al. 2012) required for ion channel formation (Grigoriev et al. 2003), are present in the 17-, 18-, 19-, and 20-residue peptaibiotics sequenced. Multiple bioactivities of pore-forming 20-residue SF1-peptaibiotics (Röhrich et al. 2013a) and of 11-residue SF4-peptaibiotics (Bobone et al. 2013; Röhrich et al. 2013b) have recently been compiled.

The results of our screening programme further extend the list of peptaibiotic-producing species of *Trichoderma/Hypocrea* compiled in Table 14. Most notably, the sequences of peptaibiotics produced by the freshly collected specimens are either identical to those found in the plate cultures, or represent—at least—closely related homologues and positional isomers of the latter. Thus, our LC-MS/MS screening approach confirmed that all peptaibiotic-producing specimens and plate cultures obtained thereof represent one and the same species. Consequently, the same type (= subfamily) of peptaibiotics is produced both in the natural habitat and under artificial (= laboratory) conditions—a fact, which is important for the application of *Trichoderma* formulations in biocontrol and integrated pest management schemes. A *Trichoderma/Hypocrea* species capable of producing peptaibiotics under the conditions of its natural habitat may defend its ecological niche more effectively compared to a non-producing species, as will be outlined below. At present, ca. 15 % of the phylogenetically verified *Trichoderma/Hypocrea* species have been positively screened for peptaibiotics; however, it appears that the inventory of peptaibiotics of the remaining 85 % is still waiting to be scrutinised by state-of-the-art bioanalytical—particularly mass spectrometric—methods. Of approximately 130 *Trichoderma/Hypocrea* species pre-screened by LC/HRMS (Nielsen et al. 2011), ca. 60 were found to produce peptaibiotics. Thus, the production of peptaibiotics in the natural habitat seems to be independent of the habitat preference, i.e. mycoparasitism vs. saprotrophy (Chaverri and Samuels 2013), but neither predictable per se nor universal.

Given that peptaibiotics are readily biosynthesised in the natural habitat of the producers, they could significantly contribute to the complex interactions of phytoprotective *Trichoderma* species, which are used in commercial or semi-commercial biocontrol agents (BCAs) against plant pathogenic fungi (Harman et al. 2004; Viterbo et al. 2007; Vinale et al. 2008a, b). Examples of successful biocontrol approaches using *Trichoderma* strains include ‘Tricovab’, a Brazilian formulation recently approved (Anonymous 2012) for integrated management of *Crinipellis*

| Residue | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|
| Ae | Aib | Ala | Aib | Ala | Aib | Ala | Gln | Aib | Lxx | Aib | Gly | Lxx | Aib | Pro | Vxx | Aib | Vxx | Gln | Gln | Pheol |
| Aib | Ala | Aib | Vxx | Aib | Vxx | Gln | Gln | Pheol |

Minor sequence variants are parenthesised.
8 Nielsen KF, Samuels GJ (2013) unpublished results.

Table 13 General building scheme of the sequences of *Hypocrea* *Trichoderma* SF1-peptaibiotics screened (Röhrich et al. 2012, 2013a, this study)
Table 14 Phylogenetically verified peptaibiotic-producing strains and species of *Trichoderma*/*Hypocrea*. NB: Species and strains for which only MALDI-TOF-MS screening data have been published are not considered for inclusion

| Species                  | Positively screened strains | Peptaibiotics found                  | References      |
|--------------------------|-----------------------------|--------------------------------------|-----------------|
| *T. arundinaceum*         | CBS 119575 (ex-type)        | alamethicins F30                     | Degenkolb et al. 2008 |
|                          |                             | alamethicins F50                     |                 |
|                          |                             | trichobrevis A                       |                 |
|                          |                             | trichobrevis B                       |                 |
|                          |                             | trichocompactins                     |                 |
|                          |                             | trichoferins A                       |                 |
|                          | CBS 119576 (= ATCC 90237)   | trichobrevis A                       | Degenkolb et al. 2006b |
|                          |                             | trichobrevis B                       |                 |
|                          |                             | alamethicins F30                     |                 |
|                          |                             | trichocompactins                     |                 |
|                          |                             | trichoferins A                       |                 |
|                          |                             | trichoferins B                       |                 |
|                          |                             | trichoferins B                       |                 |
|                          | CBS 119577                  | trichobrevis A                       | Degenkolb et al. 2008 |
|                          |                             | alamethicins F30                     |                 |
|                          |                             | trichobrevis B                       |                 |
|                          |                             | trichocompactins                     |                 |
|                          |                             | trichoferins A                       |                 |
|                          | CBS 121153                  | alamethicins F30                     |                 |
|                          |                             | alamethicins F50                     |                 |
|                          |                             | trichobrevis A                       |                 |
|                          |                             | trichobrevis B                       |                 |
|                          |                             | trichocompactins                     |                 |
|                          |                             | trichoferins A                       |                 |
|                          | CBS 123793 (= NRRL 3199)    | alamethicins F30                     | Kirschbaum et al. 2003; |
|                          |                             | alamethicins F50                     | Psurek et al. 2006; |
|                          |                             | trichobrevis A                       | Degenkolb et al. 2006b, |
|                          |                             | trichobrevis B                       | Degenkolb et al. 2008 |
|                          |                             | trichocompactins                     |                 |
|                          |                             | trichoferins A                       |                 |
| *T. brevicompactum*       | CBS 109720 (= DAOM 231232,  | alamethicins F30                     | Degenkolb et al. 2006b |
|                          | ex-type)                    | trichoferins A                       |                 |
|                          |                             | trichoferins B                       |                 |
|                          |                             | trichocompactins                     |                 |
|                          | CBS 112444                  | alamethicins F30                     |                 |
|                          |                             | trichocompactins                     |                 |
|                          |                             | trichoferins A                       |                 |
|                          |                             | trichoferins                         |                 |
|                          | CBS 112446                  | alamethicins F30                     | Degenkolb et al. 2008 |
|                          |                             | alamethicins F50                     |                 |
|                          |                             | trichobrevis A                       |                 |
|                          |                             | trichobrevis B                       |                 |
|                          |                             | trichocompactins                     |                 |
|                          | CBS 112447                  | alamethicins F30                     |                 |
|                          |                             | alamethicins F50                     |                 |
|                          |                             | trichobrevis A                       |                 |
|                          |                             | trichobrevis B                       |                 |
|                          |                             | trichocompactins                     |                 |
|                          | CBS 119569                  | alamethicins F30                     | Degenkolb et al. 2006b |
|                          |                             | trichobrevis A                       |                 |
|                          |                             | trichobrevis B                       |                 |
|                          | CBS 119570                  | trichobrevis A                       |                 |
|                          |                             | trichobrevins A                      |                 |
|                          |                             | trichobrevins B                      |                 |
|                          |                             | trichocompactins                     |                 |
| (syn. *Moniliophthora* perniciosa), the causal agent of Witches’ broom of cacao (Pomella et al. 2007; Loguercio et al. 2009; Medeiros et al. 2010). Notably, ‘Tricovab’ contains a peptaibiotic-producing strain (Degenkolb et al. 2006a) of the hyperparasitic endophyte *Trichoderma stromaticum*. Moreover, the in vivo-detection of peptaibiotics corroborates the recently demonstrated pro-apoptotic in vitro-activities of the 19-residue peptaibols trichokonin VI from *Trichoderma pseudokoningii* SMF2

Trichokonin VI is identical to gliodeliquestin A that has been isolated from *Gliocladium deliquescens* NRRL 1086 (Brückner et al. 1988) and not from NRRL 3091 (Brückner and Przybylski 1984). According to phylogenetic data, *G. deliquescens* NRRL 1086 (= CBS 228.48=ATCC 10097) was re-identified as *G. viride*, see (www.straininfo.net/strains/260309).
Table 14  (continued)

| Species             | Positively screened strains | Peptaibiotics found                      | References                      |
|---------------------|-----------------------------|------------------------------------------|---------------------------------|
| *T. turrialbense*   | CBS 112445 (ex-type)        | alamethicins F30                         | Degenkolb et al. 2006b;         |
|                     |                             | trichocryptins A                         | Degenkolb et al. 2008           |
|                     |                             | trichocryptins B                         |                                 |
|                     |                             | trichocompactins                         |                                 |
|                     | CBS 122554                  | alamethicins F30                         | Degenkolb et al. 2008           |
|                     |                             | alamethicins F50                         |                                 |
|                     |                             | trichocryptins C                         |                                 |
|                     |                             | trichocryptins D                         |                                 |
|                     |                             | trichocompactins                         |                                 |
|                     |                             | trichoferin A (trichobrevins A)          |                                 |
|                     |                             | (trichobrevins B)                        |                                 |
| *T. protrudens*     | CBS 121320 (ex-type)        | trichobrevins A                          | Degenkolb et al. 2008           |
|                     |                             | trichobrevins B                          |                                 |
|                     |                             | alamethicins F30                         |                                 |
|                     |                             | alamethicins F50                         |                                 |
|                     |                             | trichocryptins                          |                                 |
|                     |                             | trichoferins                             |                                 |
| *T. strigosum*      | CBS 348.93 (ex-type)        | tricholongins                            |                                 |
|                     |                             | trichobrevins                            |                                 |
|                     |                             | trichostrigocins                         |                                 |
|                     |                             | trikoningins                             |                                 |
|                     |                             | trichogin A IV                           |                                 |
| *T. cf. strigosum*  | CBS 119777                  | tricholongins                            |                                 |
|                     |                             | lipostrigocins A                         |                                 |
|                     |                             | lipostrigocins B                         |                                 |
|                     |                             | trichostrigocins                         |                                 |
|                     |                             | trikoningin KB II                        | Degenkolb et al. 2006a          |
| *T. erinaceus*      | CBS 117088 (= DAOM 230019, | tricholongins                            |                                 |
|                     | ex-type)                    | lipostrigocins B                         |                                 |
|                     |                             | trichostrigocins                         |                                 |
| *T. pubescens*      | CBS 345.93 (= DAOM 166162, | tricholongins                            |                                 |
|                     | ex-type)                    | lipostrigocins                           |                                 |
|                     |                             | lipopubescin                             |                                 |
| *T. cf. pubescens*  | CBS 119776                  | trichostromaticins                       |                                 |
| *T. stromaticum*    | CBS 101875 (holotype)       | trichocompactins                         |                                 |
|                     | CBS 101730                  |                                          |                                 |
| *T. spirale*        | CBS 346.93 (ex-type)        | trichobrevins B                          |                                 |
| *H. rodmanii*       | CBS 109719                  | hypocompactins                           | Degenkolb et al. 2008           |
|                     | CBS 120897                  | hyporodicins                             |                                 |
|                     |                             | trichokonins                             |                                 |
| *T. asperellum*     | CBS 361.97^b (= ATCC 38501, | trichotoxins A-50                        | Przybylski et al. 1984          |
|                     | NRRL 5242)                  | trichotoxins A-40                        | Jaworski and Brückner 1999      |
|                     | CBS 433.97 (ex-type)        | trichotoxins A-50                        | Krause et al. 2006              |
|                     | T32                         | trichotoxins                             | Chutrakul et al. 2008           |
|                     | Y19-07                      | asperelines                              | Ren et al. 2009; 2013;          |
|                     |                             |                                          | Chen et al. 2013                |
| *T. harzianum*      | CBS 354.33 (= CECT 2413,    | 11-, 14-, and 18- residue peptaibols      | Vizcaíno et al. 2006            |
|                     | = ATCC 48131)               | (not sequenced)                          |                                 |
| Species                  | Positively screened strains | Peptaibiotics found                     | References                                      |
|--------------------------|-----------------------------|-----------------------------------------|------------------------------------------------|
| *T. cf. harzianum*       | CBS 130670<sup>c</sup>      | trichovirins II                        | Jaworski et al. 1999                          |
|                         | (ATCC 90200, NRRL 5243)     |                                         |                                                 |
| *T. virens*              | Tv29-8                      | trichorzins (18-residue peptaibols),    | Wiest et al. 2002                              |
|                         |                             | 11- and 14-residue peptaibols          |                                                |
| *T. polysporum*          | TMI 60146                   | trichopolys                             | Fuji et al. 1978; Fujita et al. 1981; Iida et al. 1991; Iida et al. 1993 |
|                         |                             | trichosporins-B                        |                                                |
| *T. reesei* (H. jecorina) | FKI-4452                   | trichosporins-B                        | Iwatsuki et al. 2010                          |
|                         | CBS 392.92                  | paracelins                              | Brückner and Graf 1983; Brückner et al. 1984   |
|                         | (ATCC 2692, QM 9414)       |                                         |                                                 |
| *T. parareesei*          | C.P.K. 618                  | hypojecorins-A                          | Degenkolb et al. 2012                          |
|                         | C.P.K. 665                  | hypojecorins-B                          |                                                 |
|                         |                             | paracelins                               |                                                 |
| *T. saturnisporum*       | CBS 330.70                  | paracelins E                            | Ritieni et al. 1995                           |
| (ex-type)                |                             |                                         |                                                 |
| *T. atroviride*          | IFO 31288<sup>d</sup>      | hypomurocins A                          | Becker et al. 1997                            |
|                         |                             | hypomurocins B                          |                                                 |
|                         |                             | trichorzianins                          | El Hajji et al. 1987                           |
|                         | CBS 391.92<sup>e</sup>     | trichorzianins,                         |                                                 |
|                         | (= ATCC 36042)              | trichortrokontins                       |                                                 |
|                         | ATCC 74058<sup>f</sup>     |                                         |                                                 |
|                         | (= P1) and mutants          |                                          |                                                 |
|                         | thereof                     |                                          |                                                 |
|                         | MMS 639                     | unprecedented                           |                                                 |
|                         | MMS 925                     | 17-residue peptaibiotics and             |                                                 |
|                         | MMS 927                     | 19-residue peptaibols                   |                                                 |
|                         | MMS 1295                    |                                          |                                                 |
|                         | MMS 1513                    |                                          |                                                 |
| *T. atroviride*          | NF16                        | new and recurrent trichorzianins        | Panizel et al. 2013                            |
| *T. citrinoviride*       | IMI 91968<sup>g</sup>      | trichoaureocins                         | Jaworski and Brückner 2001a                    |
|                         | S25                         | 20-residue peptaibols                   | Maddau et al. 2009                            |
| *T. longibrachiatum*     | DAOM 234100                 | 11-residue peptaibols                   | Mohamed-Benkada et al. 2006; Ruiz et al. 2007 |
|                         | (= MMS 151)                 | 11- and 20-residue trilongins           | Mikkola et al. 2012                            |
|                         | Thb                         |                                          |                                                 |
|                         | Tbd                         |                                          |                                                 |
|                         | CNM-CM 2171                 |                                          |                                                 |
|                         | (= C.P.K. 1696)             |                                          |                                                 |
|                         | CNM-CM 2277                 |                                          |                                                 |
|                         | (= C.P.K. 2277)             |                                          |                                                 |
|                         | IMI 291014                  |                                          |                                                 |
|                         | (= C.P.K. 1303)             |                                          |                                                 |
|                         | CECT 2412                   |                                          |                                                 |
|                         | (= C.P.K. 2062)             |                                          |                                                 |
|                         | CECT 20105                  |                                          |                                                 |
|                         | (= C.P.K. 1698 = IMI 297702)|                                          |                                                 |
towards plant fungal pathogens such as *Fusarium oxysporum* (Shi et al. 2012). The value of peptaibiotics for chemotaxonomy of *Trichoderma/Hypocrea* has scarcely been scrutinised in the past (Neuhof et al. 2007; Degenkolb et al. 2008). To exhaustively answer this question, a larger number of strains, belonging to recently described species, are required to be included in an LC-MS/MS-based study.

| Species                     | Positively screened strains | Peptaibiotics found     | References                        |
|-----------------------------|----------------------------|-------------------------|-----------------------------------|
| *T. ghanense* (syn. *T. parceramosum*) | CBS 936.69<sup>1</sup> | trichobrachins          | Brückner et al. 1993; Krause et al. 2007 |
| *H. pulvinata*              | CBS 133228                |                         | Röhrich et al. 2012              |
|                            | CBS 133229                | hypopulvins             |                                    |
|                            | CBS 133230                |                         |                                    |
| *H. phellinicolana* (ex-type) | CBS 119283                | hypophellins            | Röhrich et al. 2013              |
| *H. peltata*                | Not deposited             | hypelcins               | Fujita et al. 1984; Matsuura et al. 1993, 1994 |
| *T. deliqueszens* (= *G. deliqueszens* (= ATCC 10097)                     | CBS 228.48                | gliodeliquescin A           | Brückner and Przybylski 1984    |
| (*= G. viride)<sup>1</sup> |                           |                         |                                    |
| *T. flavofuscum* (ex-type: syn. *T. viridus*: Chaverri = DSM 3500 and Samuels [2003]) | CBS 248.59 | trichofumins | Berg et al. 2003 |
| *T. asperellum*             | CBS 433.97                |                         |                                    |
| *T. aggressivum var. europaeum* | CBS 100526               | only partial             |                                    |
| *T. inhamatum*              | CBS 345.96                | given, for              |                                    |
| *H. dichromospora*          | CBS 337.69                | comments on             |                                    |
| *H. vinosae*                | CBS 247.63                | sequencing/putative     |                                    |
| *H. semiorbis*              | CBS 244.63                | identification of       |                                    |
| *H. citrina* (syn. *H. lactea*) | CBS 853.70               | peptaibiotics, see      | Krause et al. (2006)              |
| *H. nigricans*              | MUCL 28439                |                         |                                    |
| *H. lactea*                 | IFO 8434                  | screened positive for peptidic Aib and Iva | Brückner et al. 1991 |
| *H. swceinitzii*            | ICMP 5421                 |                         |                                    |

<sup>a</sup> Accession numbers under which the peptaibiotic-producing strain was first published are highlighted in bold.

<sup>b</sup> Originally misidentified as *T. viride* (Hou et al. 1972).

<sup>c</sup> Originally misidentified as *T. viridus* (Hou et al. 1972).

<sup>d</sup> Originally misidentified as *H. muroiana*, for taxonomic revision see Samuels et al. (2006).

<sup>e</sup> Originally misidentified as *T. harzianum* (el Hajji et al. 1987), for reidentification see Kühls et al. (1996).

<sup>f</sup> Originally misidentified as *T. harzianum*.

<sup>g</sup> Originally misidentified as *T. aureoviride*, data taken from http://www.lahan.info/Herbinia/specimen.HTM?IMI=91968

<sup>h</sup> Not identical to those trichobrachins reported by Brückner et al. (1993) and Krause et al. (2007) from *T. ghanense* CBS 936.69.

<sup>i</sup> Originally misidentified as *T. longibrachiatum*.

<sup>j</sup> For taxonomic recombination of *G. deliqueszens*, the anamorph of *H. lutea*, see Jaklitsch (2011).
aimed at analysing the peptaibiotome of strains and species within different clades of Trichoderma/Hypocrea. However, statements on peptaibiotic production by a particular Trichoderma/Hypocrea species must always be treated with great caution as they are highly habitat-, isolate-, and/or cultivation-dependent. Furthermore, ‘peptaibol subfamilies’ were introduced at a time when the total number of peptaibiotics described did not exceed 200 (Chugh and Wallace 2001) – less than a sixth of the currently known sequences. Notably, the additional 1,000–1,100 individual peptaibiotics published since then exhibit both new building schemes and constituents. This issue becomes even more complex as ‘peptaibol subfamilies’ were published when phylogenetic methods have not yet been recognised as an indispensable tool in fungal taxonomy. Thus, a considerable number of peptaibiotics, the sequences of which have been elucidated correctly, cannot be linked to an unambiguously identified producer that is deposited in a publicly accessible culture collection. These facts illustrate the urgent need to reconsider the classification into the nine subfamilies – a task that has to be completed before the aforementioned study can be performed.

Currently, any approach for a peptaibiotics-based chemotaxonomy of Trichoderma/Hypocrea must be regarded as extremely complicated – even within a defined clade –, because i) peptaibiotics only represent one single class of secondary metabolites produced by Trichoderma/Hypocrea, ii) most of the producers reported in literature have never been deposited appropriately, and iii) the persistently high degree of misidentification makes any comparison between members of different clades problematic and challenging. This is illustrated by the following examples (references are compiled in Table 14):

i) The 20-residue alamethicins (ALMs) have hitherto been found in four species belonging to the Brevicompactum clade of Trichoderma; however, it is not yet possible to estimate if the Pro\(^2\) residue of the ALMs could be regarded as a structurally highly conserved position, comparable to the Pro\(^{14}\) residue. Chemotaxonomy of the Brevicompactum clade encompassed the comparison of hydrophobins, peptaibiotics, and low-molecular weight secondary metabolites, including simple trichothecone-type mycotoxins.

ii) The 18-residue trichotoxins (TXT) A-50 and A-40, for example, have been obtained from Trichoderma asperellum NRRL 5242, whereas Trichoderma asperellum Y 19-07 did not produce TXTs but 9- and 10-residue peptaibols instead (and vice versa).

iii) Trichoderma citrinoviride strains S 25 and IMI 91968 are rich sources of 20-residue peptaibols of the paracelsin/saturnisporin/trichocellin/suzukacillin/tricho Aureocin-type. These are the only two strains of T. citrinoviride that have been investigated for peptaibiotics. Hypocrea schweinitzii ICMP 5421, which has also been verified phylogenetically (Réblová and Seifert 2004), had only been screened positive for Aib by GC/MS; but – to the best of the authors’ knowledge – specimens of that species have never been investigated for its inventory of peptaibiotics. Parcelsins, which have been isolated from T. reesei QM 9414, are also produced by a member of the Longibrachiatum clade. However, the producer of saturnisporin (T. saturnisporum MNHN 903578: Rebuffat et al. 1993) has never been made publicly available, nor has its identity been verified phylogenetically. The producers of both trichocellins and suzukacillins A (Krause et al. 2006b) have not been deposited in a publicly available culture collection; thus, their identification as T. ‘viride’ is highly questionable.

iv) T. flavofuscum CBS 248.59 is the only species of Trichoderma/Hypocrea, which produces 13-residue sequences – notably trichofumins C and D are the only two peptaibols of that chain length reported to date. They display the rare Gln-Gln motif in positions 5 and 6. Looking at the sequences, their biosynthesis seems to be distantly related to that one of trichofumins A and B (and positional isomers thereof). The latter are 11-residue SF4-peptaibols and widespread amongst Trichoderma/Hypocrea species.

v) T. virens strain Tv29-8 produces common 11- and 14-residue peptaibols, and it is the only phylogenetically verified source of 18-residue peptaibols of the trichorzintype.

However, the results of our LC-MS/MS screening are also of interest for analysis of environmental samples as well as extraterrestrial materials such as carbonaceous meteorites as their contamination by propagules of soil- or airborne peptaibiotic-producing fungi has to be taken into account (Brückner et al. 2009; Elsila et al. 2011).

To sum up, production of peptaibiotics may generally be regarded as a sophisticated ecological adaptation for the producing fungus providing it with an obvious advantage over non-producing fungal and other competitors. This group of ‘chemical weapons’ in their ‘armoury’ may effectively assist a remarkable number of strains currently identified as belonging to ca. 30 Trichoderma/Hypocrea species in colonising and defending their ecological niches.

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