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Trichoderma brevicompactum Complex: Rich Source of Novel and Recurrent Plant-Protective Polypeptide Antibiotics (Peptaibiotics)

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Three strains of *Trichoderma brevicompactum* and another four that are closely related to that species (*Trichoderma* cf. *brevicompactum*) were analyzed for the formation of polypeptide antibiotics (peptaibiotics) by LC/ESI-MS^n. These isolates were selected because of an antagonistic potential against Eutypa dieback and Esca disease of grapevine and have not yet been investigated for the production of peptide antibiotics. Fully grown cultures on potato dextrose agar were extracted with CH_2Cl_2/MeOH, and this extract was subjected to SPE using C_{18} cartridges. The methanolic eluates were analyzed by LC/ESI-MS^n. All strains were found to produce membrane-active alamethicins F30. In addition to that, novel peptaibiotics were detected, namely, 14 12-residue trichocryptins B, 12 11-residue trichocryptins A, 19 11-residue trichobrevins A and B, 6 10-residue trichoferins, and 17 8-residue trichocompactins. These compounds may partially be responsible for the plant-protective action of the producers. Chemotaxonomic considerations also indicated the necessity to introduce another new species that is closely related to *T. brevicompactum*.

KEYWORDS: Peptaibiotic; peptaibol; alamethicin; α-aminoisobutyric acid; electrospray ionization mass spectrometry; peptide sequencing; *Trichoderma*; biocontrol

INTRODUCTION

More than 400 strains of 30 *Trichoderma* species were investigated in the course of a project aimed at preventive plant protection and biocontrol of two fungal diseases in organic viticulture: Eutypa dieback and Esca. These are latent trunk diseases that cause severe economic losses in organic grapevine production (*1*, *2*). The in vitro bioactivity of the *Trichoderma* strains against the causal agents of Eutypa dieback, *Eutypa lata*, and Esca disease, *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*, was evaluated in plate assays using crude extracts. The most active isolates were identified either as *Trichoderma brevicompactum* (*sensu stricto*, *ss*), or as *Trichoderma* cf. *brevicompactum* (*Trichoderma brevicompactum*, *sensu lato*, *sl*). Compared to the bioactivity of isolates representing well-known biocontrol *Trichoderma* species (e.g., *T. atroviride*, *T. harzianum*, *T. koningii*, and *T. viride*), crude extracts of strains belonging to the *T. brevicompactum* complex have been found to inhibit the growth of the above pathogens in vitro far more effectively (*3*). Detailed information about habitat and geographic origin of the seven isolates investigated in this study is given in **Table 1**.

T. brevicompactum, an anamorphic species with a pachybasium-like morphology, has been described from soil and tree bark in North, Central, and South America and southern Asia (*4*). The species was originally proposed to be phylogenetically closely related to *Hypocrea lutea* (*4*), but also discussed to be close to *Trichoderma minutisporum*/*Hypocrea minutispora*. As the alignment of translation−elongation factor (TEF) sequences turned out to be rather difficult, additional sequencing of the second largest RNA polymerase subunit (RPB2) has been performed. These experiments clearly indicated that the fungus known as *T. brevicompactum* comprises two phylogenetically different species that form a new lineage within the genus *Trichoderma*. These findings are further supported by the results of our work as outlined under Discussion.

Species of *Trichoderma* (teleomorphs in *Hypocrea*; *5*) are commercially used as bioprotective agents against many fungal diseases. Most commercial preparations are formulated on the basis of conidia, but application of biomass or chlamydospores

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Table 1. *Trichoderma* Strains Included in This Study

strain[a]	strains investigated[b]	habitat	geographic origin	yield[c] (mg)
1	CBS 109720 (*ex*-type)	soil in sunflower field	Geneva, NY	9.0
2	IBT 40840 (= CBS 119570)	soil	Iran	21.8
3	IBT 40839 (= CBS 119569)	soil	Qazvin, Iran	43.2
4	CBS 112445	soil	Costa Rica	4.0
5	IBT 40863 (= CBS 119577)	soil	Shar-e Kord, Chahar Mahall va Bakhtiari, Iran	14.5
6	ATCC 90237 (= CBS 119576)	micaceous clay from stream bed	Windhoek, Namibia	2.5
7	NRRL 3199[d]	unknown	unknown	17.4

[a] 1-4, *T. brevicompactum*; 5-7, *T.* cf. *brevicompactum.* [b] Abbreviations: ATCC, American Type Culture Collection, Manassas, VA; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; DSM, Deutsche Stammsammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; IBT, BioCentrum, DTU, Kgs. Lyngby, Denmark; NRRL, ARS Culture Collection, Northern Regional Research Laboratory, National Center for Agricultural Utilization Research, Peoria, IL. [c] Dry weight of the methanolic extracts obtained after cleanup over Sep-Pak C_{18} cartridges (see Experimental Procedures). [d] Alamethicin patent strain of Upjohn Co., Kalamazoo, MI.

has also been described. Choice of propagule for the preparation depends on both production system and intended application (*6*). The antifungal action of *Trichoderma* is complex. It depends on the species/strain studied and may involve competition for nutrients, plant root colonization, biofertilization, stimulation of plant resistance and defense mechanisms, rhizosphere modification, and different types of mycoparasitism. The latter may involve morphological changes such as coiling of parasite hyphae around the host and the formation of specialized appressorium-like structures (*7*−*11*).

The species of *Trichoderma* are known as saprotrophs, rare plant pathogens (*10*), or polyphagous mycoparasites, which are common in soil ecosystems. During the recent years, fungicolous fungi have attracted particular interest because of their bioactivity against economically important fungal diseases of crop plants, which cannot effectively be controlled by methods of classical plant protection (*12*).

Secondary metabolites of *Trichoderma* have extensively been reviewed (*13, 14*). Synergistic interactions between extracelluar metabolites such as wall-degrading chitinases, glucanases, and proteases, on the one hand, and antibiotics, on the other, have clearly been demonstrated in the past. The parallel formation of hydrolytic enzymes together with a group of membrane-active polypeptide antibiotics, named "peptaibiotics", and their synergistic action play an important role in mycoparasitism between *T. harzianum* and its fungal hosts such as *Botrytis cinerea* (*15, 16*). The term "peptaibiotic" was introduced by Brückner et al. (*17*) and reconsidered by Degenkolb et al. (*18*). It describes linear peptide antibiotics that (i) range from 500 to 2200 Da in molecular mass; (ii) show a high content of α-aminoisobutyric acid; (iii) are characterized by the presence of other nonproteinogenic amino acids and/or lipoamino acids; and (iv) possess an acylated N terminus, whereas the C terminus may consist of a free or methoxy-substituted 2-amino alcohol, amine, amide, free amino acid, diketopiperazine, or sugar alcohol. "Peptaibols" are regarded as a subgroup of the peptaibiotics, the N terminus of which is acetylated, whereas the C terminus is reduced to a 2-amino alcohol.

Recently, the term "peptaibiomics" was proposed by Krause et al. (*19*), describing—in analogy to proteomics—the approach to the analysis of the entirety of peptaibiotics, the so-called "peptaibiome", produced by a certain strain under defined conditions.

Peptaibiotics show interesting physicochemical and biological activities depending on particular structural properties, such as formation of pores in bilayer lipid membranes as well as antibacterial, antifungal, occasionally antiviral, insecticidal, and antiparasitic activities. Inhibition of mitochondrial ATPase, uncoupling of oxidative phosphorylation, immunosuppression, inhibition of platelet aggregation, and induction of fungal morphogenesis and neuroleptic effects have been reported (summarized in refs *18* and *19*).

Detailed information on structures of peptaibiotics and their classification into subfamilies (*20*) can be obtained from public Internet resources such as the "Peptaibol Database" (*21*). More than 250 peptaibiotics produced by members of the genus *Trichoderma* are described in the literature. Recently, a review comprising structures and properties of 186 different peptaibiotics from *Trichoderma* has been published (*14*).

Screening and sequencing of peptaibiotics with a molecular mass up to 2000 Da can be accomplished by advanced methods of tandem mass spectrometry, especially electrospray ionization (ESI-MS) techniques (for a review see ref *18*) and completed by GC/EI-MS and HPLC approaches (*22*).

As species identification of *Trichoderma* strains was demonstrated to be possible by image analysis of HPLC chromatograms (*23*), on-line coupling of HPLC and ESI-ion-trap-MS^n should therefore combine the advantages of both analytical techniques, thus providing a more reliable structural identification of compounds produced by a certain strain.

To date, 88 *Trichoderma* species have been characterized by sequencing of ribosomal DNA, and these data suggest that many species recognized on the basis of morphology have probably been misidentified in the past (*5*, *24*). Recently, the species *Trichoderma viride* was subdivided into two species—*T. viride* and *T. asperellum*—on the basis of ribosomal DNA. Antibiotic production was exclusively restricted to *T. asperellum*, whereas *T. viride* (*ss*) produced no antibiotics (*25*, *26*).

Therefore, it may be hypothesized that the production of peptaibiotics under standardized conditions might be used as a chemotaxonomic marker in support of morphological, molecular, and other (bio)chemical data for the differentiation between species of the genus *Trichoderma*.

None of the strains used in this study has been screened for peptaibol production, although six of them were previously shown to produce trichothecene-type mycotoxins, such as harzianum A and/or trichodermin (*27*). Recently, harzianum A was also detected in cultures of NRRL 3199, which is *T.* cf. *brevicompactum*.

The present study was aimed at (i) screening of selected plant-protective strains for the production of peptaibols and peptaibol-like antibiotics (peptaibiotics), (ii) sequencing of new and recurrent peptides found, and (*iii*) testing the above hypothesis concerning a possible use of the pattern of peptaibiotics for chemotaxonomy.

EXPERIMENTAL PROCEDURES

Chemicals. Acetonitrile (MeCN; Chromasolve for HPLC, far UV, 99.9%) and dichloromethane (ACS reagent, 99.6%) were obtained from Sigma-Aldrich (Steinheim, Germany); methanol (MeOH; 99.8%, gradi-

Table 2. Structural Variations of Peptaibiotics from the *T. brevicompactum* Complex[a]

	residue																			
peptaibiotic[b]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
ALM F30	Ac	Aib	Pro	Aib	Ala	Aib	Ala	*Gln* *Glu*	Aib	*Aib* *Vxx* *Lxx*	Aib	Gly	*Vxx* *Lxx*	Aib	Pro	Vxx	Aib	Aib	Glu	Gln	Pheol
TCP	Ac	Aib	Gly	Ala	Lxx	*Aib* *Vxx*	*Gly* *Ala* *Ser*	*Vxx* *Lxx*	Vxx											
TBV	Ac	Aib	*Ala* *Ser*	*Aib* *Vxx*	*Aib* *Vxx* *Lxx*	Aib	Pro	Lxx	Lxx	Aib	Pro	*Alaol* *Aibol* *Vxxol* *Lxxol*								
TCT-A	Ac	*Vxx* *Lxx*	Aib	Pro	*Vxx* *Lxx*	Aib	Pro	*Aib* *Lxx*	*Aib* *Vxx* *Lxx*	Aib	Pro	Lxxol								
TCT-B	Ac	*Vxx* *Lxx*	Aib	Pro	Vxx	*Vxx* *Lxx*	Aib	Pro	*Aib* *Lxx*	*Aib* *Vxx* *Lxx*	Aib	Pro	Lxxol							
TF	MDA	Pro	*AHMOD* *desmethyl-AHMOD*	Ala	Aib	*Aib* *Vxx*	*Aib* *Vxx* *Lxx*	*Gly* *Ala* *Aib*	Aib	Aib	*AAE* *AMAE*									

[a] Exchangeable positions in a general sequence are italicized. A list of sequences of peptaibiotics detected in the individual strains is presented in the captions to **Figures 1** and **2**. [b] ALM F30, alamethicin F30; TCP, trichocompactin; TBV, trichobrevin; TCT-A, trichocryptin A; TCT-B trichocryptin B; TF, trichoferin.

ent grade, for HPLC) and trifluoroacetic acid (TFA, 98.0%) were purchased from Fluka (Steinheim, Germany). Toluene (SupraSolv, 99%, for gas chromatography) was bought from VWR International (Darmstadt, Germany). Anhydrous KH_2PO_4 and $Na_2HPO_4 \cdot H_2O$ were from Fluka, and methyl orange (helianthin) was from Riedel-de Haën (Seelze, Germany). Bidistilled water was freshly prepared from demineralized tap water prior to analysis using a quartz distil (Heraeus, Kleinostheim, Germany).

Cultivation of Strains. Cultures were grown at room temperature (23−26 °C) under ambient daylight on Difco potato dextrose agar (PDA, lot 4300389) obtained from Becton Dickinson (BD, Heidelberg, Germany). The medium was prepared according to the directions of the manufacturer and autoclaved at 121 °C for 15 min without pH adjustment. A final pH of 5.6 ± 0.2 was measured after sterilization.

Subcultures were inoculated from PDA slants used for preservation of strains, and a loop of conidia was streaked on 9.5 cm diameter plastic Petri dishes containing 20 mL of PDA. Subcultures were grown for 4 days and used for inoculation of the main culture.

Extraction of Peptaibiotics. After 6 days of cultivation, fungal cultures were extracted with a mixture of CH_2Cl_2/MeOH, 1:1. To prevent any possible contamination of the extracts by plasticizers, a ring of aluminum with a small spout on its upper margin was punched into the agar before application of the solvent mixture. A 5 mL aliquot of the solvent was applied onto the surface of each plate culture and spread with a Drigalski spatula, and the extract was poured off through the spout. This procedure was repeated twice; the combined extracts from each agar plate were transferred into Pyrex tubes and centrifuged at 2400*g* for 30 min. The supernatant was filtered and evaporated to dryness in vacuo.

Cleanup was performed using Sep-Pak C_{18} cartridges (Waters Corp., Milford, MA) as previously described (*19*). Briefly, each cartridge (dimensions: 1.5 cm × 1 cm i.d.) was conditioned by successive addition of MeOH, H_2O, and H_2O/MeOH, 2:1 (10 mL each). The sample was redissolved in H_2O/MeOH, 2:1, and centrifuged at 2400*g* for 30 min, and the supernatant was filtered; the filtrate was applied to the conditioned cartridge. The cartridge was rinsed with H_2O and H_2O/MeOH, 2:1 (10 mL each). Finally, peptaibiotics were eluted with 10 mL of MeOH. The eluate was evaporated to dryness in vacuo. The dry weight of the residue (see **Table 1**) was determined using an analytical balance. A 10 μL aliquot of a 1% methanolic solution that had been freshly prepared from the dried residue of the methanolic eluate prior to analysis was used for HPLC or ion-trap ESI-LC-MS measurements, respectively.

HPLC and Ion-Trap-ESI-LC-MS Measurements. For HPLC, a HP 1100 series instrument was used. ESI mass spectra were recorded on an LCQ instrument (Thermo Finnigan MAT, San Jose, CA). The gradient used for HPLC and ion-trap-ESI-LC-MS measurements was described previously (*19*); further details concerning the analytical equipment were given in an earlier paper (*28*). A CID energy of 45 or 65 eV was applied to generate sequence-specific *b*- and *y*-type fragments from putative $[M + H]^+$, $[M + Na]^+$, or sequence-specific fragment ions, respectively. The collision energy for MS/MS and MS^n measurements was set between 25 and 65 eV, typically at 45 eV.

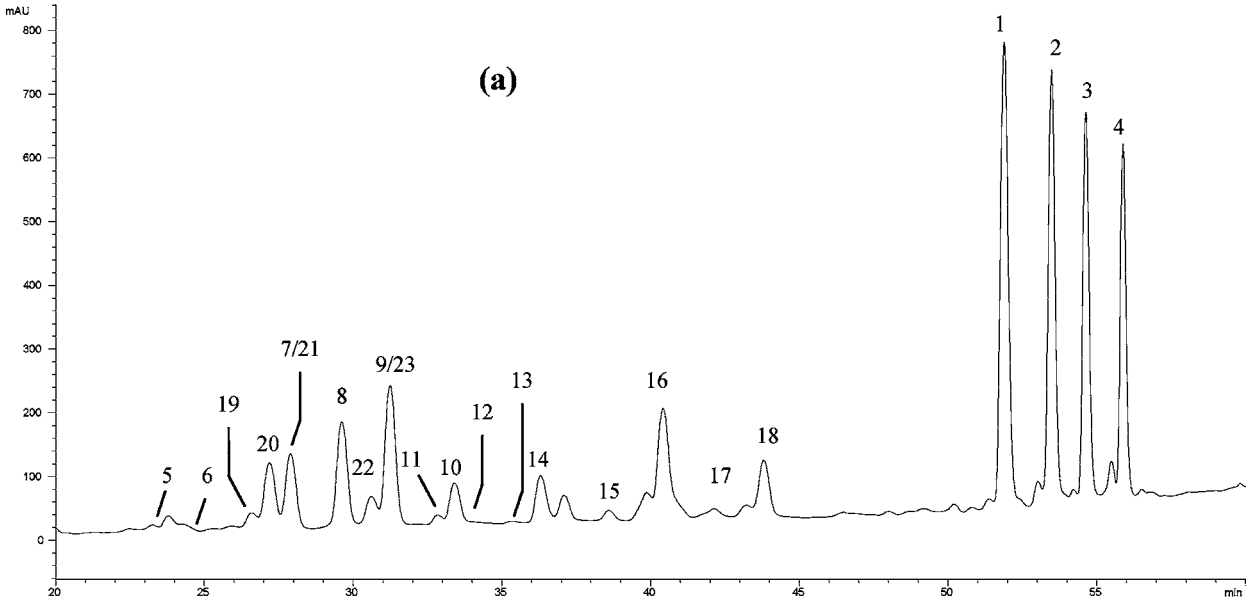
Fragment ion series were assigned in accordance with the Roepstorff/Fohlman−Biemann nomenclature used previously. In cases when the isomeric amino acids Leu/Ile or Val/Iva (Iva, isovaline) could not be distinguished, the abbreviations Lxx and Vxx were used instead (*29*, *30*).

RESULTS

Possible structural variations of all peptaibiotics investigated in this study are summarized in **Table 2**. HPLC elution profiles (detection wavelength $\lambda = 205$ nm) of the peptaibiotic-containing fraction from all strains are shown in **Figures 1** and **2**. In the following section, the HPLC/ESI-MS^n-based sequencing and structural characterization of peptaibiotics produced by *T. brevicompactum* and *T.* cf. *brevicompactum* are described.

***T. brevicompactum* CBS 109720.** The HPLC elution profile of this strain (**Figure 1a**) is dominated by four major peaks. Furthermore, MS/MS, MS^n, and CID-MS investigations and comparison of these results with the recent literature (*28*) and data obtained from experiments with authentic material from *T. viride* NRRL 3199 confirmed the structures of these compounds as the acidic alamethicins (ALM): **1**, F30/3; **2**, F30/5; **3**, F30/7; and **4**, F30/9. The strains of the *T. brevicompactum* group were not screened for the presence of neutral alamethicins F50 (ALM F50) in the course of this study. Analysis of that subgroup would have required the same conditions as described above but without TFA in the eluents. Voltage-dependent pore formation and antimicrobial activity of alamethicins have been reviewed (*31*). Alamethicins are, so far, only known from *T. viride* NRRL 3199 (*28*), which now can be classified as *T.* cf. *brevicompactum* (*3*).

A second group of six novel eight-residue peptaibiotics from ***Tricho**derma brevi**compact**um* was detected. We name these compounds **trichocompactins** (TCP) **5**, Ia; **6**, Ib; **7**, IIa; **8**, IIb;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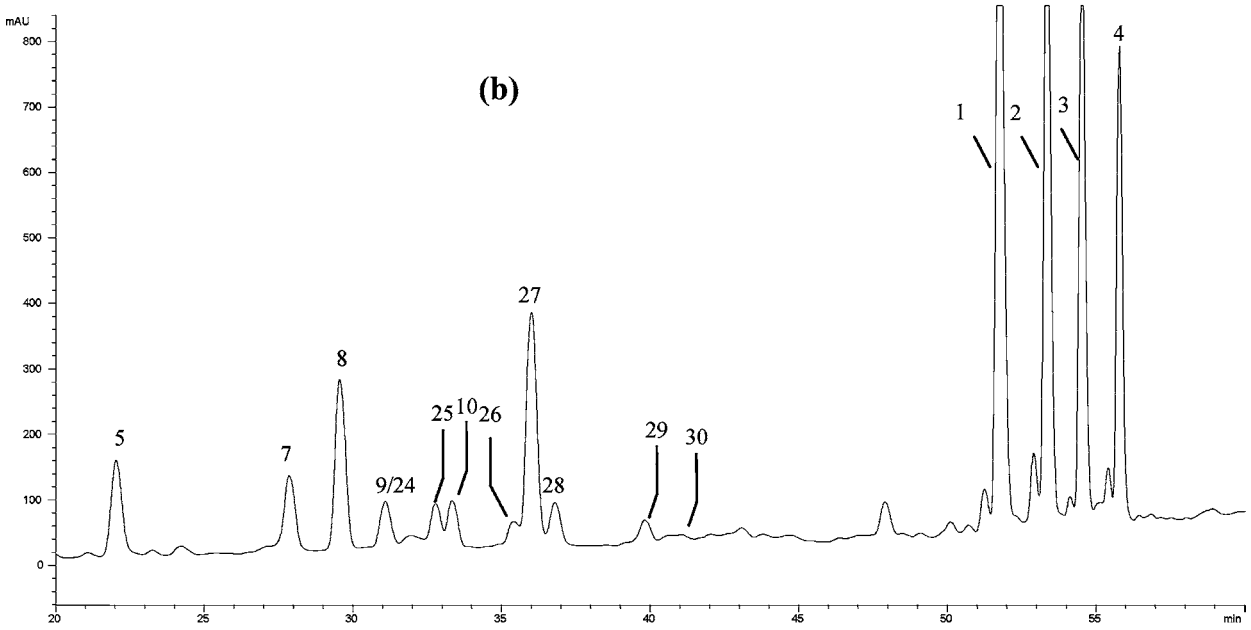
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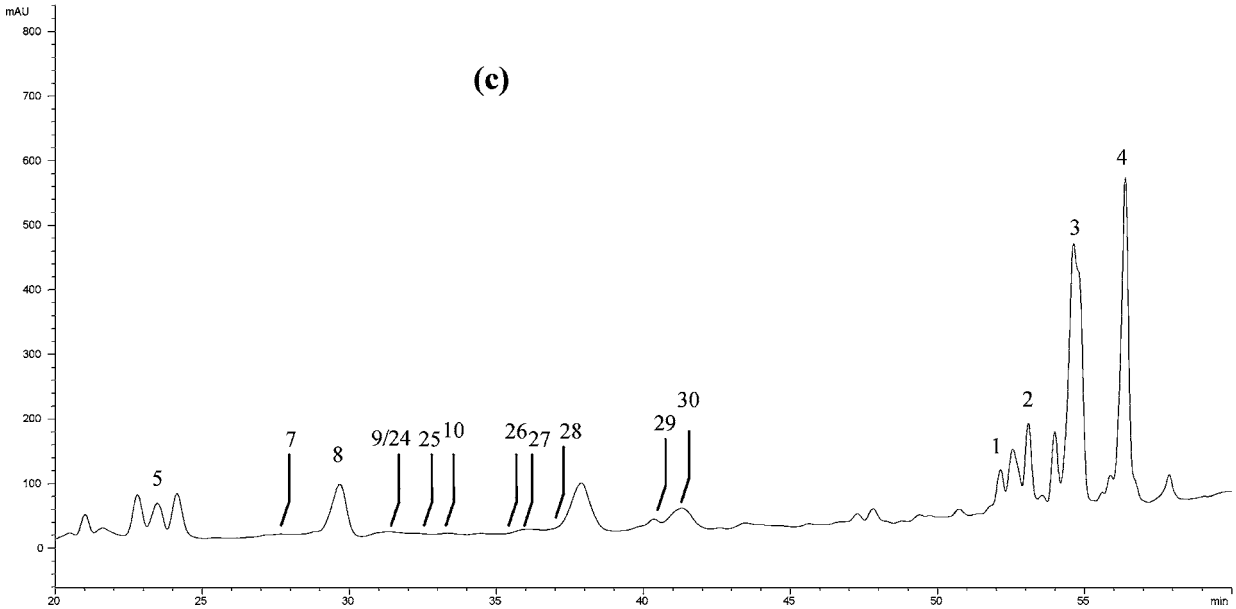
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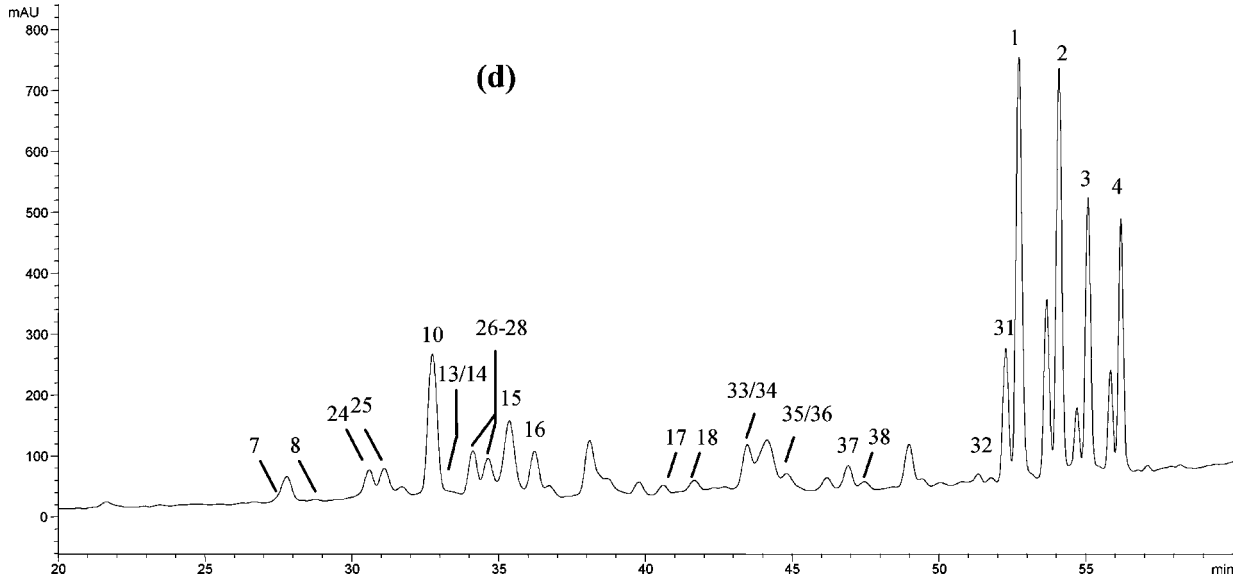

Figure 1. HPLC elution profiles of the peptaibiotic-containing fraction of *T. brevicompactum* strains (**a**) CBS 109720, (**b**) IBT 40839, (**c**) IBT 40840, and (**d**) CBS 112445. Annotations refer to consecutive numbering of peptides used in the text. Numbers separated by a slash refer to coeluting peptides. (**a**) Alamethicins (**1**–**4**), trichocompactins (**5**–**10**), trichocryptins B (**11**–**18**), trichocryptins A (**19**–**23**); (**b**) alamethicins (**1**–**4**), trichocompactins (**5**–**10**), trichocryptins A (**24**–**30**); (**c**) alamethicins (**1**–**4**), trichocompactins (**5**–**10**), trichocryptins A (**24**–**30**); (**d**) alamethicins (**1**–**4**, **31**, and **32**), trichocompactins (**5**–**10**), trichocryptins A (**23**–**28**), trichocryptins B (**13**–**18** and **33**–**38**).

9, IIIa; and **10**, IIIb. Their fragmentation patterns and sequences are listed in **Tables 3** and **4**.

The N-terminal sequence Ac-Aib-Gly-Ala-Leu-Aib was previously described for the trichovirins—peptaibols with a C-terminal Gln-Leuol motif from *T. viride* NRRL 5243 (*30*). That strain is currently deposited as *T. harzianum* sl. Furthermore, valine as a C-terminal residue is known from the trichobrachins TB IIa A and B, only, which have been isolated from *T. longibrachiatum* CBS 936.69 (*32*), a strain now reclassified as *T. paceramosum*/*T. ghanense*.

A third group of homologous peptaibols exhibited *m*/*z* 1210, 1224, 1238, and 1252, which were accompanied by *m*/*z* 1226, 1240, 1254, and 1268, respectively. It was demonstrated by CID-MS^n that the former series of ions represents the predominant $[M+ Na]^+$, whereas the latter corresponds to the $[M + K]^+$ adduct, which is present in smaller amounts. Because the $[M + H]^+$ ions of any of these compounds were never observed, the intensive sodiated adducts had to be selected as precursors for sequencing. Compounds **11**−**18** are novel 12-residue peptaibols from ***Tricho****derma*, which we name **trichocryptins B** (TCT-B) I, II, III, and IV—owing to the **crypti**c behavior of their $[M + H]^+$ ions.

Fragmentation of $[M + Na]^+$, as illustrated in **Table 5**, exclusively generated a sodiated *y*-type series of daughter ions (y_2−y_8), which was dominated by the corresponding series of sodiated *x*-type ions, thus leading to complete suppression of N-terminal fragments. Loss of water from the $[M + Na]^+$ ions indicated the presence of a C-terminal amino alcohol. The first sequence-specific pair of fragment ions is y_2/x_2. The diagnostic difference of either *m*/*z* 201 or 215 supports the presence of a C-terminal Pro-Vxxol or Pro-Lxxol, which is followed by an Aib residue. The extremely labile tertiary Aib−Pro bond is preferably cleaved (*33*), thus explaining the absence of y_1/x_1 fragments. Cleavage of the Aib−Pro bond between positions 6 and 7 is the reason for the generation of an additional intensive sodiated *y*-type fragment comprising amino acids 7−12 (cf. **Tables 2** and **6**). Further sequence information was obtained from CID-MS experiments: Application of a CID energy of 45 and 65 eV generated the diagnostic fragments b_2−b_6 and their corresponding *y*-type ions. The structure of these *b*- and *y*-type ions was confirmed by CID-MS^n experiments. However, attempts to detect the b_1 fragment by CID-MS^n of the ions b_6 and b_5 were unsuccessful. Moreover, the intensity of b_2−b_4 was insufficient to perform further CID-MS^n investigations. Despite this, literature data revealed a single sequence, corresponding only to the pair of b_2/b_3 ions *m*/*z* 241/338 present in compounds **12** and **14**−**18**: the N-terminal fragment Ac-Leu-Aib-Pro has previously been described for the cervinins I and II—12-residue peptaibol antibiotics from *Mycogone cervina* A09-02, parasitizing *Helvella* (*Paxina*) *acetabulum* (*34*). Assuming structural homology, the b_2/b_3 ion pair *m*/*z* 227/324 could represent Ac-Val-Aib-Pro as an N-terminal sequence of compounds **11** and **13**. The sequence Ac-Val-Aib is known from the protonophoric bergofungin A from *Emericellopsis donezkii* HKI 0059 (*35*) as well as from the antiprotozoic/antihelminthic antiamoebins XIII and XIV from *Stilbella fimetaria* (syn. *Stilbella erythrocephala*) ATCC 28144 (*22*). The corresponding isoforms Ac-Ile-Pro and Ac-Iva-Pro have not been described as N termini of peptaibiotics, yet.

The partial sequence Pro-Aib-Leu-Aib-Pro-Leuol is known as the C terminus of harzianins HC I, HC VI, HC XI, and HC XIV from *T. harzianum* M-903614 and M-903603 (*33*), whereas the other C-terminal sequences listed in **Table 6** represent new structural variations.

The strain produces a fourth group of homologous peptaibols displaying *m*/*z* 1125 (compounds **19**−**21**) and 1139 (compounds **22**/**23**, all $[M + Na]^+$). Basically, the mass spectrometric fragmentation of these substances follows the same general scheme described above for compounds **11**−**18**. The CID-MS experiments generated a series of the diagnostic fragments b_2−b_5. Briefly, the—presumably invariable—Vxx residue at position 4 of the peptide chain is lost, thus leading to the appearance of novel 11-residue peptaibols, which we name **trichocryptins A** (TCT-A) I and II. Fragmentation patterns and sequences of these compounds are listed in **Tables 5** and **6**, respectively. Additional homologues and positional isomers of compounds **11**−**23** are

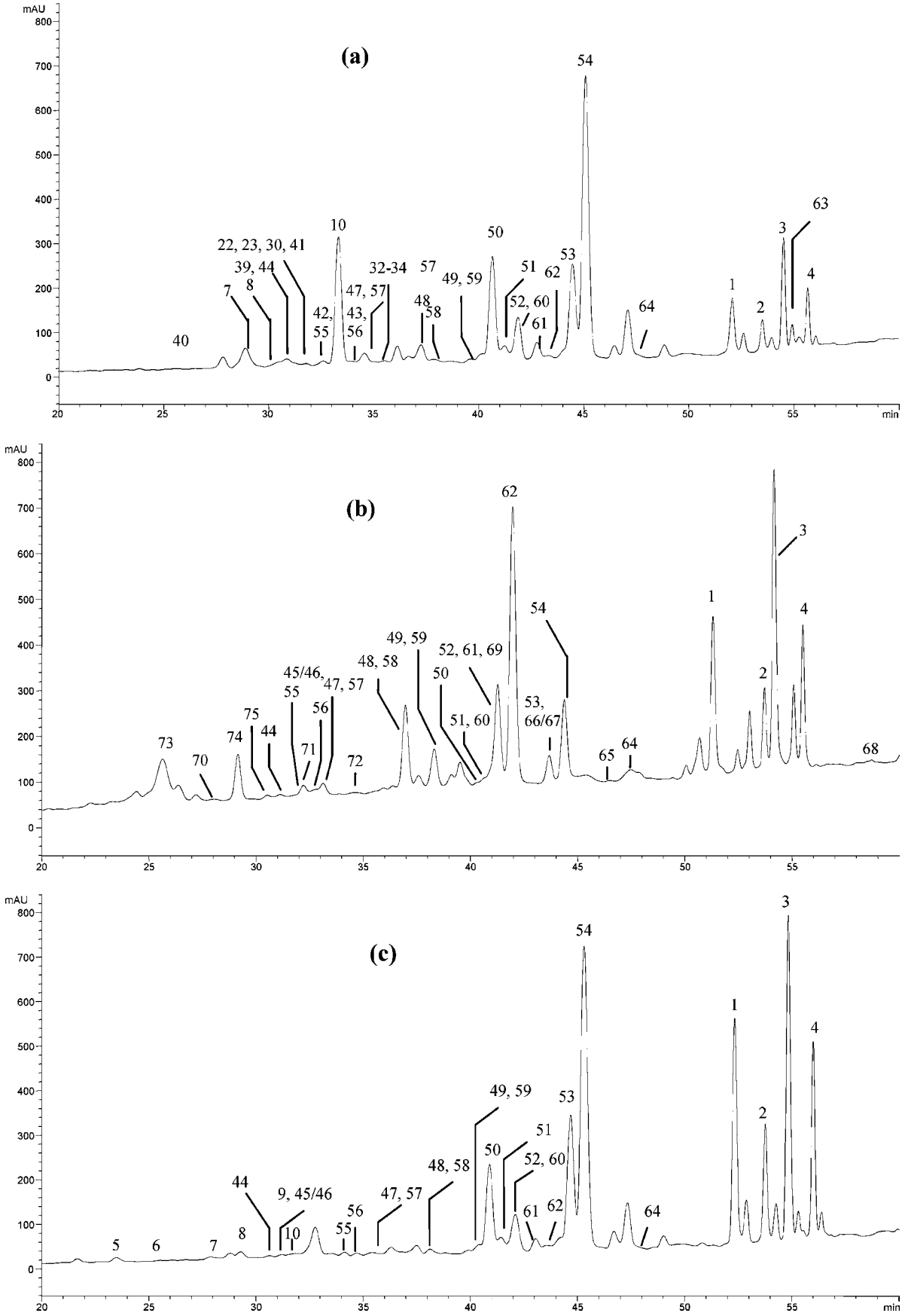

Figure 2. HPLC elution profiles of the peptaibiotic-containing fraction of *T.* cf. *brevicompactum* strains (**a**) ATCC 90237, (**b**) NRRL 3199, and (**c**) IBT 40863. Annotations refer to consecutive numbering of peptides used in the text. Numbers separated by a slash refer to coeluting peptides. (**a**) Alamethicins (**1**–**4** and **63**), trichocompactins (**5**–**8**, **10**, and **39**–**43**), trichocryptins B (**15**–**18** and **33**–**36**), trichobrevins A and B (**44**–**62**), trichoferin A (**64**); (**b**) alamethicins (**1**–**4**), trichocompactins (**70**–**75**), trichobrevins (**44**–**62**); (**c**) alamethicins (**1**-**4**), trichocompactins (**5**–**10**), trichocryptins B (**13**–**18**), trichcryptins A (**22**–**28**), trichobrevins A and B (**44**–**62**), trichoferin A (**64**).

Table 3. Diagnostic Fragment Ions (*m*/*z*) of Trichocompactins[a] Produced by Members of the *T. brevicompactum* Complex

	5	**6**	**7**	**8**	**9**	**10**	**40**	**39**	**41**	**42**	**43**
ion	Ia	Ib	IIa	IIb	IIIa	IIIb	IV	Va	Vb	VIa	VIb
$[M + H]^+$	726	726	740	740	754	754	756	770	770	784	784
$[M - H_2O]^+$	708	708	722	722	nd	736	738	752	752	766	766
$[M + Na]^+$	748	748	762	762	776	776	778	792	792	806	806
b_1	nd[b]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
b_2	184	nd	184	184	184	nd	184	184	184	nd	184
b_3	255	269	255	255	255	nd	255	255	255	nd	255
b_4	368	354	368	368	368	368	368	368	368	381	368
b_5	453	439	453	453	467	467	453	453	453	467	467
$b_6 - H_2O$	nd	nd	nd	nd	nd	nd	522	522	522	nd	nd
b_6	510	496	510	510	524	524	540	540	540	nd	554
$b_7 - H_2O$	nd	nd	nd	nd	nd	nd	621	635	635	649	649
b_7	609	609	623	623	637	637	639	653	653	667	667
a_4	340	nd	340	nd	340	340	340	340	340	nd	nd
$y_3 - H_2O$	nd	nd	nd	nd	nd	nd	nd	300	nd	nd	nd
y_3	nd	nd	287	nd	nd	287	nd	318	nd	nd	nd
$y_4 - H_2O$	nd	nd	nd	nd	nd	nd	nd	nd	385	nd	nd
y_4	nd	nd	372	nd	nd	nd	nd	nd	nd	nd	nd
y_5	nd	nd	nd	485	nd	nd	nd	516	nd	nd	nd

	70	**71**	**72**	**73**	**74**	**75**
ion	VII	VIIIa	VIIIb	IX	Xa	Xb
$[M + H]^+$	740	754	754	756	770	770
$[M - H_2O]^+$	722	736	736	nd	752	752
$[M + Na]^+$	762	776	776	778	792	792
b_1	nd	nd	nd	nd	nd	nd
b_2	184	184	184	184	184	184
b_3	255	255	255	255	255	255
b_4	368	368	368	368	368	368
$b_5 - H_2O$	nd	nd	nd	nd	nd	nd
b_5	453	453	453	453	453	453
$b_6 - H_2O$	nd	nd	nd	522	522	522
b_6	524	524	524	nd	540	540
$b_7 - H_2O$	nd	nd	nd	621	635	635
b_7	623	637	637	nd	653	653
a_4	340	nd	nd	340	340	340
$y_3 - H_2O$	nd	nd	nd	nd	nd	300
y_3	nd	302	nd	nd	nd	nd
y_5	485	nd	nd	nd	516	nd
y_6	nd	nd	nd	nd	nd	601

[a] Arabic numbers in bold refer to the consecutive numbering of peptides used throughout the text and in **Figures 1** and **2**. Roman numerals followed by lower case Arabic letters in **Tables 3**–**8** refer to the abbreviations used for the individual trichocompactins, trichocryptins, and trichobrevins. Capital letters in **Tables 9** and **10** refer to the abbreviations used for the individual trichoferins. Capital letters for characterization of trichoferins are introduced for conformity reasons in the nomenclature of lipoaminopeptides. [b] Not detected.

Table 4. Sequences of Trichocompactins I–X Produced by Members of the *T. brevicompactum* Complex[a]

			residue								
			1	2	3	4	5	6	7	8	$[M + H]^+$
5	Ia	Ac	Aib	Gly	Ala	Lxx	Aib	Gly	Vxx	Vxx	726
6	Ib				[269]	Aib	Aib	Gly	Lxx	Vxx	726
7	IIa	Ac	Aib	Gly	Ala	Lxx	Aib	Gly	Lxx	Vxx	740
8	IIb	Ac	Aib	Gly	Ala	Lxx	Aib	Gly	Lxx	Vxx	740
9	IIIa	Ac	Aib	Gly	Ala	Lxx	Vxx	Gly	Lxx	Vxx	754
10	IIIb	Ac	Aib	Gly	Ala	Lxx	Vxx	Gly	Lxx	Vxx	754
40	IV	Ac	Aib	Gly	Ala	Lxx	Aib	Ser	Vxx	Vxx	756
39	Va	Ac	Aib	Gly	Ala	Lxx	Aib	Ser	Lxx	Vxx	770
41	Vb	Ac	Aib	Gly	Ala	Lxx	Aib	Ser	Lxx	Vxx	770
42	VIa	Ac	Aib	Gly	Ala	Lxx	Aib	Ser	Lxx	Vxx	784
43	VIb	Ac	Aib	Gly	Ala	Lxx	Vxx	Ser	Lxx	Vxx	784
70	VII	Ac	Aib	Gly	Ala	Lxx	Aib	Ala	Vxx	Vxx	740
71	VIIIa	Ac	Aib	Gly	Ala	Lxx	Aib	Ala	Lxx	Vxx	754
72	VIIIb	Ac	Aib	Gly	Ala	Lxx	Aib	Ala	Lxx	Vxx	754
73	IX	Ac	Aib	Gly	Ala	Lxx	Aib	Ser	Lxx	Vxx	756
74	Xa	Ac	Aib	Gly	Ala	Lxx	Aib	Ser	Lxx	Vxx	770
75	Xb	Ac	Aib	Gly	Ala	Lxx	Aib	Ser	Lxx	Vxx	770

[a] Bold numbers in the first column refer to consecutive numbering of peptides used throughout the text. Abbreviations of compound names used in the second column refer to the individual compounds introduced in the text.

expected from the corresponding TIC traces. However, their structures could not be determined due to their low abundance in the mixture.

***T. brevicompactum* IBT 40839.** Analysis of the four major peaks displayed in the HPLC elution profile (**Figure 1b**) and comparison of these data with those obtained for strain CBS 109720 confirmed the presence of compounds **1**−**5** and **7**−**10**.

In contrast to what was found in strain CBS 109720, strain IBT 40839 did not produce any of the compounds **11**−**23**, but a mixture of peptaibols with molecular masses *m*/*z* 1153 (**24**/**25**), 1167 (**26**−**28**), and 1181 (**29**/**30**, all $[M + Na]^+$), representing higher homologues of trichocryptins A I and A II. Fragmentation and sequences of these trichocryptins A III, IV, and V are listed in **Tables 5** and **6**. The C-terminal motif of trichocryptins A IV b, IV c, V a, and V b, that is, Pro-Leu-Leu-Aib-Pro-Leuol, has previously been described for harzianin HK VI from *T. pseudokoningii* MVHC 662 (*36*) and the hypomurocins A I, A II, A IV, and A V—hemolytic peptaibols from strain IFO 31288 (*37*). That strain was originally described as *Hypocrea muroiana*, but recently demonstrated to be *Trichoderma atroviride*/*Hypocrea atroviridis* by internal transcript spacer (ITS) and elongation factor (EF) sequencing.

***T. brevicompactum* IBT 40840.** Analysis of the four major peaks displayed in the HPLC elution profile (**Figure 1c**) and comparison of these data with those obtained for strain CBS 109720 confirmed the presence of compounds **1**−**9**. The strain also produces peptaibols with molecular masses *m*/*z* 1153, 1167, and 1181 (all $[M + Na]^+$), having the same retention time(s) and thus supposed to be identical or positionally isomeric with compounds **24**−**30** described for strain IBT 40839.

***T.* cf. *brevicompactum* CBS 112445.** Analysis of the four major peaks displayed in the HPLC elution profile (**Figure 1d**) and comparison of these data with those obtained for strain CBS 109720 confirmed the presence of compounds **1**−**4**. Additional ALMs are present, the sequence of which could only partially be assigned by MS/MS, which is due to their low abundance in the mixture. For example, fragmentation of *m*/*z* 1950 $[M + H]^+$ at $t_R = 50.4$ and comparison with literature data reported for ALMs F30 (*28*) indicated that alamethicin F30/2 (compound **31**) could be present as a minor compound. Another novel minor compound, **32**, was detected during fragmentation of *m*/*z* 1964 ($[M + H]^+$) at $t_R = 50.9$. Again, assuming structure homology with literature data deduced for the ALM F30 peptides (*28*), including invariability of amino acid residues 1 and 2, the following possible sequences are proposed for this new ALM F30/11 as the intensity of the fragment ions obtained during MS^3 was insufficient to perform further MS^n experiments. According to structure homologies with compounds **1**−**4**, the variable positions 3, 5, and 8 in compound **32** consist of either Ala or Aib, respectively, whereas positions 9−20 are invariable. Theoretically, three positional isomers are possible. Compounds **5**−**10** are also present—the latter displaying a particularly intense peak in that part of the HPLC elution profile. Sodiated molecular ions *m*/*z* 1139, 1153, and 1167 were detected, which may represent compounds **22**−**28** or homologues thereof.

A fourth group of peptaibols with molecular masses *m*/*z* 1224, 1238, and 1252 may consist of homologues and positional isomers of compounds **13**−**18**. In contrast to what has been

Table 5. Diagnostic Fragment Ions (*m*/*z*) of Trichocryptins A and B Produced by Members of the *T. brevicompactum* Complex[a]

ion	**11** B-Ia	**12** B-Ib	**13** B-IIa	**14** B-IIb	**15** B-IIIa	**16** B-IIIb	**17** B-IVa	**18** B-IVb	**33** B-Va	**34** B-Vb	**35** B-Vc	**36** B-Vd	**37** B-VIa	**38** B-VIb
$[M + Na]^+$	1210	1210	1224	1224	1238	1238	1252	1252	1266	1266	1266	1266	1280	1280
$[M + K]^+$	1226	1226	1240	1240	1254	1254	1268	1268	1282	1282	1282	1282	1296	1296
$[M + Na - H_2O]^+$	1192	1192	nd	nd	nd	1220	1234	1234	nd	nd	nd	nd	1262	1262
b_1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
b_2	227	nd	227	241	nd	241	nd	241	241	241	241	241	241	241
b_3	324	nd	324	338	338	338	nd	338	338	338	338	338	338	338
b_4	423	nd	423	437	437	437	437	437	437	437	437	437	437	437
b_5	522	536	522	536	536	536	550	550	550	550	536	550	550	550
b_6	607	621	607	621	621	621	635	635	635	635	621	635	635	635
$[Pro\text{-}Vxx + H]^+$	197	nd	197	197	197	197	197	197	197	197	197	197	197	197
$[Pro\text{-}Vxx\text{-}Vxx + H]^+$	296	nd	296	296	296	296	nd	nd	nd	nd	296	296	296	296
$[Pro\text{-}Vxx\text{-}Lxx + H]^+$	nd	nd	nd	nd	nd	nd	310	310	310	310	nd	nd	310	310
$[Pro\text{-}Vxx\text{-}Vxx\text{-}Aib + H]^+$	381	nd	381	381	381	381	nd	nd	nd	nd	381	nd	nd	nd
$[Pro\text{-}Vxx\text{-}Lxx\text{-}Aib + H]^+$	nd	nd	nd	nd	nd	nd	395	395	395	395	nd	nd	395	nd
$[Pro\text{-}Vxx\text{-}CO + H]^+$	169	nd	169	169	169	169	169	169	169	169	169	169	nd	nd
$[Pro\text{-}Vxx\text{-}Vxx\text{-}CO + H]^+$	268	nd	268	268	268	268	nd	nd	nd	nd	nd	nd	nd	nd
$[Pro\text{-}Vxx\text{-}Lxx\text{-}CO + H]^+$	nd	nd	nd	nd	nd	nd	282	282	282	282	nd	nd	282	nd
$[Pro\text{-}Vxx\text{-}Vxx\text{-}Aib\text{-}CO + H]^+$	nd	nd	353	nd	nd	353	nd	nd	nd	nd	nd	nd	nd	nd
$[Pro\text{-}Vxx\text{-}Lxx\text{-}Aib\text{-}CO + H]^+$	nd	nd	nd	nd	nd	nd	nd	367	nd	nd	nd	nd	367	nd
$[Pro\text{-}Aib\text{-}Aib\text{-}Aib\text{-}Pro\text{-}Lxxol + Na]^+$	nd	589	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
$[Pro\text{-}Aib\text{-}Vxx\text{-}Aib\text{-}Pro\text{-}Lxxol + Na]^+$	603	nd	nd	603	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
$[Pro\text{-}Aib\text{-}Lxx\text{-}Aib\text{-}Pro\text{-}Lxxol + Na]^+$	nd	nd	617	nd	617	617	617	617	nd	nd	nd	nd	nd	nd
$[Pro\text{-}Lxx\text{-}Lxx\text{-}Aib\text{-}Pro\text{-}Vxxol + Na]^+$ or $[Pro\text{-}Vxx\text{-}Lxx\text{-}Aib\text{-}Pro\text{-}Lxxol + Na]^+$	nd	nd	nd	nd	nd	nd	nd	nd	631	631	nd	631	nd	nd
$[Pro\text{-}Lxx\text{-}Lxx\text{-}Aib\text{-}Pro\text{-}Lxxol + Na]^+$	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	645	nd	645	645
$[Pro\text{-}Aib + H]^+$	nd	nd	183	183	nd	183	183	183	nd	nd	nd	nd	nd	nd
$[Pro\text{-}Aib\text{-}Lxx + H]^+$	nd	nd	296	nd	296	296	296	296	nd	nd	nd	nd	nd	nd
$[Pro\text{-}Aib\text{-}Lxx\text{-}Aib + H]^+$	nd	nd	381	nd	381	381	381	381	nd	nd	nd	nd	nd	nd
$(y_8 + Na)^+$	nd	nd	nd	nd	nd	445	459	459	459	459	nd	nd	459	459
$(y_7 + Na)^+$	nd	nd	544	558	558	558	572	572	572	572	558	nd	572	nd
$(y_6 + Na)^+$	629	nd	629	643	643	643	657	657	657	657	643	nd	657	nd
$(y_5 + Na)^+$	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	754	nd
$(y_4 + Na)^+$	811	825	811	825	825	825	839	839	853	867	853	867	867	867
$(y_3 + Na)^+$	910	910	924	924	938	938	952	952	966	980	966	980	980	980
$(y_2 + Na)^+$	995	995	1009	1009	1023	1023	1037	1037	1051	1065	1051	1065	1065	1065
$(y_1 + Na)^+$	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
$(x_8 + Na)^+$	417	nd	nd	nd	nd	417	431	431	nd	nd	431	431	431	nd
$(x_7 + Na)^+$	516	530	516	530	530	530	544	544	544	544	530	544	544	544
$(x_6 + Na)^+$	601	615	601	615	615	615	629	629	629	629	615	629	629	629
$(x_5 + Na)^+$	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
$(x_4 + Na)^+$	783	797	783	797	797	797	811	811	825	839	825	839	839	839
$(x_3 + Na)^+$	882	882	896	896	910	910	924	924	938	952	938	952	952	952
$(x_2 + Na)^+$	967	967	981	981	995	995	1009	1009	1023	1037	1023	1037	1037	1037
$(x_1 + Na)^+$	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

ion	**19** A-Ia	**20** A-Ib	**21** A-Ic	**22** A-IIa	**23** A-IIb	**24** A-IIIa	**25** A-IIIb	**26** A-IVa	**27** A-IVb	**28** A-IVc	**29** A-Va	**30** A-Vb
$[M + Na]^+$	1125	1125	1125	1139	1139	1153	1153	1167	1167	1167	1181	1181
$[M + K]^+$	1141	1141	1141	1155	1155	1169	1169	1185	1185	1185	1197	1197
$[M + Na - H_2O]^+$	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
b_1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
b_2	227	227	241	241	241	241	241	241	241	241	241	241
b_3	324	324	338	338	338	338	338	338	338	338	338	338
b_4	423	423	437	437	437	437	437	451	437	437	451	451
b_5	508	508	522	522	522	522	522	536	522	522	536	536
$[Pro\text{-}Vxx + H]^+$	197	197	197	197	197	197	197	197	197	197	nd	nd
$[Pro\text{-}Vxx\text{-}Aib + H]^+$	282	282	282	282	282	282	282	nd	282	282	nd	nd
$[Pro\text{-}Vxx\text{-}Vxx + H]^+$	nd	nd	nd	nd	nd	nd	nd	296	nd	nd	nd	nd
$[Pro\text{-}Vxx\text{-}Lxx + H]^+$ or $[Pro\text{-}Lxx\text{-}Vxx + H]^+$	nd	nd	nd	nd	nd	nd	310	nd	nd	nd	nd	nd
$[Pro\text{-}Lxx\text{-}Lxx + H]^+$	nd	nd	nd	nd	nd	324	nd	nd	324	324	nd	nd
$[Pro\text{-}Lxx\text{-}Lxx\text{-}Aib + H]^+$	nd	nd	nd	nd	nd	nd	nd	nd	409	409	409	nd
$[Pro\text{-}Vxx\text{-}Vxx\text{-}Aib + H]^+$	nd	nd	nd	nd	nd	nd	nd	381	nd	nd	nd	nd
$[Pro\text{-}Vxx\text{-}Lxx\text{-}Aib + H]^+$	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
$[Pro\text{-}Vxx\text{-}CO + H]^+$	169	169	169	169	169	169	169	169	169	169	nd	nd
$[Pro\text{-}Vxx\text{-}Aib\text{-}CO + H]^+$	254	nd	nd	nd	254	254	254	nd	nd	nd	nd	nd
$[Pro\text{-}Vxx\text{-}Vxx\text{-}CO + H]^+$	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
$[Pro\text{-}Vxx\text{-}Lxx\text{-}CO + H]^+$	nd	nd	nd	nd	nd	nd	282	nd	282	282	nd	nd
$[Pro\text{-}Lxx + H]^+$	nd	nd	nd	nd	nd	211	211	nd	211	211	211	211
$[Pro\text{-}Lxx\text{-}Aib + H]^+$	nd	nd	nd	nd	nd	nd	296	nd	nd	nd	296	296
$[Pro\text{-}Lxx\text{-}CO + H]^+$	nd	nd	nd	nd	nd	183	183	183	183	183	183	nd
$[Pro\text{-}Lxx\text{-}Aib\text{-}CO + H]^+$	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
$[Pro\text{-}Vxx\text{-}Vxx\text{-}Aib\text{-}CO + H]^+$	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Table 5 (Continued)

ion	**19** A-Ia	**20** A-Ib	**21** A-Ic	**22** A-IIa	**23** A-IIb	**24** A-IIIa	**25** A-IIIb	**26** A-IVa	**27** A-IVb	**28** A-IVc	**29** A-Va	**30** A-Vb
[Pro-Vxx-Lxx-Aib-CO + H]$^+$	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
[Pro-Aib-Aib-Aib-Pro-Lxxol + Na]$^+$	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
[Pro-Aib-Vxx-Aib-Pro-Lxxol + Na]$^+$	nd	nd	631	nd	nd	nd	nd	nd	nd	nd	nd	nd
[Pro-Aib-Lxx-Aib-Pro-Lxxol + Na]$^+$	617	617	nd	617	617	nd	nd	nd	nd	nd	nd	nd
[Pro-Lxx-Vxx-Aib-Pro-Vxxol + Na]$^+$	nd	nd	nd	nd	nd	nd	nd	617	nd	nd	nd	nd
[Pro-Lxx-Lxx-Aib-Pro-Vxxol + Na]$^+$ or [Pro-Vxx-Lxx-Aib-Pro-Lxxol + Na]$^+$	nd	nd	nd	nd	nd	631	631	nd	nd	nd	nd	nd
[Pro-Lxx-Lxx-Aib-Pro-Lxxol + Na]$^+$	nd	nd	nd	nd	nd	nd	nd	nd	645	645	645	645
[Pro-Aib + H]$^+$	nd	nd	183	183	nd	183	183	183	nd	nd	nd	nd
[Pro-Aib-Lxx + H]$^+$	nd	nd	296	nd	296	296	296	296	nd	nd	296	296
[Pro-Aib-Lxx-Aib + H]$^+$	nd	nd	381	nd	381	381	381	381	nd	nd	nd	nd
(y_8 + Na)$^+$	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
(y_7 + Na)$^+$	nd	nd	nd	nd	431	431	431	nd	nd	nd	nd	nd
(y_6 + Na)$^+$	530	530	516	530	516	544	544	nd	nd	nd	nd	nd
(y_5 + Na)$^+$	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
(y_4 + Na)$^+$	712	712	726	726	726	754	754	754	nd	754	768	nd
(y_3 + Na)$^+$	825	825	825	839	839	867	853	867	881	867	881	881
(y_2 + Na)$^+$	910	910	910	924	924	952	938	952	966	952	966	966
(y_1 + Na)$^+$	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
(x_8 + Na)$^+$	nd	nd	nd	nd	nd	403	nd	nd	nd	431	445	nd
(x_7 + Na)$^+$	502	502	nd	502	nd	516	516	516	nd	516	530	530
(x_6 + Na)$^+$	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
(x_5 + Na)$^+$	684	684	698	698	698	726	726	726	740	726	740	740
(x_4 + Na)$^+$	797	797	797	811	811	839	825	839	853	839	853	853
(x_3 + Na)$^+$	882	882	882	896	896	924	910	924	938	924	938	938
(x_2 + Na)$^+$	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
(x_1 + Na)$^+$	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

[a] See **Table 3** footnote.

Table 6. Sequences of Trichocryptins A and B Produced by Members of the *T. brevicompactum* Complex[a]

		residue												
	A		1	2	3	4	5	6	7	8	9	10	11	[M + Na]$^+$
19	Ia	Ac	Vxx	Aib	Pro	Vxx	Aib	Pro	Aib	Lxx	Aib	Pro	Lxxol	1125
20	Ib	Ac	Vxx	Aib	Pro	Vxx	Aib	Pro	Aib	Lxx	Aib	Pro	Lxxol	1125
21	Ic	Ac	Lxx	Aib	Pro	Vxx	Aib	Pro	Aib	Vxx	Aib	Pro	Lxxol	1125
22	IIa	Ac	Lxx	Aib	Pro	Vxx	Aib	Pro	Aib	Lxx	Aib	Pro	Lxxol	1139
23	IIb	Ac	Lxx	Aib	Pro	Vxx	Aib	Pro	Aib	Lxx	Aib	Pro	Lxxol	1139
24	IIIa	Ac	Lxx	Aib	Pro	Vxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Vxxol	1153
25	IIIb	Ac	Lxx	Aib	Pro	Vxx	Aib	Pro	Lxx	Vxx	Aib	Pro	Lxxol	1153
26	IVa	Ac	Lxx	Aib	Pro	Vxx	Vxx	Pro	Lxx	Vxx	Aib	Pro	Vxxol	1167
27	IVb	Ac	Lxx	Aib	Pro	Vxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Lxxol	1167
28	IVc	Ac	Lxx	Aib	Pro	Vxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Lxxol	1167
29	Va	Ac	Lxx	Aib	Pro	Lxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Lxxol	1181
30	Vb	Ac	Lxx	Aib	Pro	Lxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Lxxol	1181

		residue													
	B		1	2	3	4	5	6	7	8	9	10	11	12	[M + Na]$^+$
11	Ia	Ac	Vxx	Aib	Pro	Vxx	Vxx	Aib	Pro	Aib	Vxx	Aib	Pro	Lxxol	1210
12	Ib	Ac	Lxx	Aib	Pro	Vxx	Vxx	Aib	Pro	Aib	Aib	Aib	Pro	Lxxol	1210
13	IIa	Ac	Vxx	Aib	Pro	Vxx	Vxx	Aib	Pro	Aib	Lxx	Aib	Pro	Lxxol	1224
14	IIb	Ac	Lxx	Aib	Pro	Vxx	Vxx	Aib	Pro	Aib	Vxx	Aib	Pro	Lxxol	1224
15	IIIa	Ac	Lxx	Aib	Pro	Vxx	Vxx	Aib	Pro	Aib	Lxx	Aib	Pro	Lxxol	1238
16	IIIb	Ac	Lxx	Aib	Pro	Vxx	Vxx	Aib	Pro	Aib	Lxx	Aib	Pro	Lxxol	1238
17	IVa	Ac	Lxx	Aib	Pro	Vxx	Lxx	Aib	Pro	Aib	Lxx	Aib	Pro	Lxxol	1252
18	IVb	Ac	Lxx	Aib	Pro	Vxx	Lxx	Aib	Pro	Aib	Lxx	Aib	Pro	Lxxol	1252
33	Va	Ac	Lxx	Aib	Pro	Vxx	Lxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Vxxol	1266
34	Vb	Ac	Lxx	Aib	Pro	Vxx	Lxx	Aib	Pro	Vxx	Lxx	Aib	Pro	Lxxol	1266
35	Vc	Ac	Lxx	Aib	Pro	Vxx	Vxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Lxxol	1266
36	Vd	Ac	Lxx	Aib	Pro	Vxx	Lxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Vxxol	1266
37	VIa	Ac	Lxx	Aib	Pro	Vxx	Lxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Lxxol	1280
38	VIb	Ac	Lxx	Aib	Pro	Vxx	Lxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Lxxol	1280

[a] See **Table 4** footnote.

described for strain CBS 109720, strain CBS 112445 did not produce peptaibols with molecular masses *m*/*z* 1210. However, additional higher homologues displaying *m*/*z* 1266 and 1280 (all [M + Na]$^+$) are present. Fragmentation patterns and sequences of these trichocryptins (**33**, B-Va; **34**, B-Vb; **35**, B-Vc; and **36**, B-Vd; as well as **37**, B-VIa, and **38**, B-VIb) are listed in **Tables 5** and **6**. The C-terminal motif, Pro-Iva-Leu-Aib-Pro-Leuol, is known from harzianin HB I (*38*) and from

some of the previously described harzianins HC from *T. harzianum* M-903603 and M-903614 (*33*). The C-terminal motif, Pro-Ile-Leu-Aib-Pro-Valol, was reported for the trichorozins I and II isolated from a strain of *T. harzianum* (*39*).

***T.* cf. *brevicompactum* ATCC 90237.** This strain shows a quite different and much more diverse pattern (**Figure 2a**) compared to the other isolates investigated in this study: The CID-MS and MS/MS of *m/z* 756, 770, and 784 (all $M + H^+$) revealed the presence of homologous trichocompactins, which have been sequenced as follows below. The peak of compound **39**, named TCT Va, is comparatively intense in the total ion current (TIC), although its UV absorbance is rather low. Compared to compounds **5**−**10**, these pseudomolecular ions display a mass difference of +30 Da. This fact leads to the hypothesis that a Gly residue in the molecule might be substituted by a Ser residue.

Serine-containing peptaibiotics often tend to display additional b'_n fragments resulting from the loss of water from the corresponding b_n fragments—a feature that is important for the detection of that particular amino acid in CID-MS, MS/MS, and MS^n experiments. The proposed fragmentation pattern has, in fact, been observed, leading to the assignment of the structures indicated in **Tables 3** and **4**. Assuming structural homology of *m/z* 184, the N-terminal fragment of these compounds could consist of Ac-Aib-Gly. Thus, compound **40**, named TCT IV, is a homologue of compound **5**. Compounds **39** and **41** (TCT Vb) might be interpreted as homologues of compounds **7** and **8**, whereas compounds **42** and **43** (TCT VIa and VIb) may represent homologues of compounds **9** and **10** with Gly in position 6 substituted by Ser. However, compounds **5**−**8** and **10** are also present, with **10** displaying the most abundant peak of this part of the HPLC elution profile. Fragmentation patterns and sequences of all trichocompactins are described in **Tables 3** and **4**.

Small amounts of substances with molecular masses of *m/z* 1238, 1252, and 1266 have also been detected, which may represent compounds **15**−**18** and **33**−**36**, whereas *m/z* 1210, 1224, and 1280 (all $[M + Na]^+$) have not been observed. The pattern of peptaibiotics with molecular masses between 1100 and 1200 Da is completely different from those of *T. brevicompactum* CBS 109720, IBT 40840, IBT 40839, and CBS 112445: *T.* cf. *brevicompactum* ATCC 90237 produced two additional series of homologous peptaibols—the former comprising *m/z* 1099, 1113, 1127, and 1141 and the latter, *m/z* 1129, 1143, and 1157. The fragmentation behavior of these ions was very similar to that observed for trichocryptins A and B. The molecular ions mentioned above again represented $[M + Na]^+$ adducts. They generated a series of sodiated *y*-type ions dominated by the corresponding sodiated *x*-type fragments, as proven by MS^n investigations. As previously observed for trichocryptins A and B, any diagnostic N-terminal fragments were completely suppressed in the collision chamber. However, CID-MS revealed the ions b_2−b_5, but the b_1 fragment could not be detected. Despite this, a difference of *m/z* 199 most probably corresponds to the N-terminal sequence Ac-Aib-Ala, which is very common among peptaibols produced by *Trichoderma* spp. (*20*). For instance, an alanyl residue in position 2 has previously been described for the trichocellins from *T. viride* ATCC 20672 (*40*). Compounds from ***Tricho*derma** cf. ***brevicompactum*** displaying *m/z* 1099 (**44**−**47**), 1113 (**48**/**49**), 1127 (**50**−**52**), and 1141 (**53**/**54**, all $[M + Na]^+$) contain Ala in position 2 and were named **trichobrevins A**. The latter two compounds, **53**, trichobrevins A-IVa, and **54**, A-IVb, as well as compound **50**, trichobrevin A-IIIa, are the most abundant peaks in the HPLC elution profile.

In the case of *m/z* 1129, 1143, and 1157, the CID fragments b_2−b_4 are accompanied by the corresponding $b_n - H_2O$ ions. This diagnostic phenomenon has previously been observed for the Ser-containing trichocompactins described above. Thus, the Ala residue in position 2 is exchanged by a seryl residue. These compounds exhibiting $[M + Na]^+$ ions *m/z* 1129 (**55**−**57**), 1143 (**58**/**59**), and 1157 (**60**−**62**) were named **trichobrevins B**. The fragmentation scheme and sequences of trichobrevins A and B are shown in **Tables 7** and **8**.

The C termini of trichobrevin compounds **45**, A-Ib, and **47**, A-Id, consist of a Vxxol residue. Interestingly, MS/MS data indicate that compound **46**, trichobrevin A-Ic, carries a C-terminal Aibol residue, whereas compound **44**, trichobrevin A-Ia, terminates in Alaol. However, the occurrence of Aibol and Alaol remains tentative. Detailed investigations, preferably on the isolated compounds, are required to unequivocally prove the presence of these distinctive structural elements as such C termini have not been previously reported in the literature.

Compounds **1**−**4** have also been detected. Partial sequences of a minor compound *m/z* 1992 were determined. Diagnostic fragments observed in the MS/MS and MS^n spectra give reason for the assumption that ALM F30/8 (compound **63**, cf. ref *28*) could be present.

***T.* cf. *brevicompactum* IBT 40863.** *T.* cf. *brevicompactum* IBT 40863 (**Figure 2c**) produces compounds **1**−**4** as main components and a number of minor ALMs, the structures of which have not been investigated in detail. Pseudomolecular ions *m/z* 1139, 1153, and 1167 could represent compounds **22**−**28** or their positional isomers. Homologues displaying *m/z* 1125 and 1181 $[M + Na]^+$ were not detected. Minor amounts of *m/z* 1224, 1238, 1252, 1266, and 1280 (all $[M + Na]^+$) are present, which could represent compounds **13**−**18** and **33**−**38** or positional isomers thereof. The pattern of compounds **44**−**62** is supposed to be identical or very closely related to that of *T.* cf. *brevicompactum* ATCC 90237—as deduced from the elution order of the respective pseudomolecular ions.

***T.* cf. *brevicompactum* NRRL 3199.** As previously mentioned, this strain is known as the "classical" source of alamethicins (*28*), mostly producing compounds **1**−**4** (**Figure 1b**). Further alamethicin-like compounds are present in minor amounts, the sequence of which could not be determined due to their comparatively low abundance in the mixture. To date, several hundred studies dealing with research on this particular peptaibol have been published. Thus, alamethicin is regarded as the most thoroughly investigated peptaibiotic. Compound **64** displays a rather low UV absorption at 205 nm, but a remarkably good ionization in positive ESI-MS. MS/MS studies on the pseudomolecular ion *m/z* 1207 revealed obvious structural homology to helioferins (*41*) and roseoferins (*42*), nine-residue lipoaminopeptides from the fungicolous *Mycogone rosea* strains DSM 8822 and DSM 12973.

An additional diagnostic fragment, *m/z* 266, is found in CID-MS spectra recorded at a CID energy of 45 and 65 eV, respectively, as well as in MS^3 spectra of the MS^2 fragment ion *m/z* 550. Assuming structural homology with helio- and roseoferins, the difference of *m/z* 213 could correspond to an AHMOD residue. At present, this lipoamino acid is known as a unique constituent of most of the leucinostatins, of trichopolyns, helioferins, roseoferins, and acremostatins (reviewed in ref *18*). Thus, the fragment *m/z* 1135 should indicate the loss of the *n*-butyl side chain ($[M + H - 72]^+$) from the AHMOD residue by α-cleavage—a diagnostic feature observed in positive

Table 7. Diagnostic Fragment Ions (*m*/*z*) of Trichobrevins A and B Produced by Members of the *T. brevicompactum* Complex[a]

	44	**45**	**46**	**47**	**48**	**49**	**50**	**51**	**52**	**53**	**54**
ion	A-Ia	A-Ib	A-Ic	A-Id	A-IIa	A-IIb	A-IIIa	A-IIIb	A-IIIc	A-IVa	A-IVb
$[M + Na]^+$	1099	1099	1099	1099	1113	1113	1127	1127	1127	1141	1141
$[M + K]^+$	1115	1115	1115	1115	1129	1129	1143	1143	1143	1157	1157
$[M + H]^+$	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
$[M + Na - H_2O]^+$	1081	1081	1081	1081	1095	1095	1109	1109	1109	1123	1123
b_1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
b_2	199	nd	nd	nd	199	199	199	199	199	199	199
b_3	298	nd	nd	284	298	284	298	298	298	298	298
b_4	411	nd	nd	383	397	383	411	397	397	411	411
b_5	496	468	nd	468	482	468	496	482	482	496	496
[Pro-Lxx-Lxx-Aib-Pro-Alaol + Na]$^+$	603	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
[Pro-Lxx-Lxx-Aib-Pro-Aibol + Na]$^+$	nd	nd	617	nd	nd	nd	nd	nd	nd	nd	nd
[Pro-Lxx-Lxx-Aib-Pro-Vxxol + Na]$^+$ or [Pro-Vxx-Lxx-Aib-Pro-Lxxol + Na]$^+$	nd	631	nd	631	631	nd	631	nd	nd	nd	nd
[Pro-Lxx-Lxx-Aib-Pro-Lxxol + Na]$^+$	nd	nd	nd	nd	nd	645	nd	645	645	645	645
$(y_7 + Na)^+$	nd	nd	nd	405	419	nd	433	418	418	433	433
$(y_6 + Na)^+$	nd	nd	nd	490	504	nd	518	504	504	518	nd
$(y_5 + Na)^+$	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
$(y_4 + Na)^+$	728	nd	nd	700	714	700	728	714	714	728	728
$(y_3 + Na)^+$	841	nd	827	813	827	813	841	827	827	841	841
$(y_2 + Na)^+$	926	nd	nd	898	912	898	926	912	912	926	926
$(y_1 + Na)^+$	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
$(x_7 + Na)^+$	nd	nd	nd	377	nd	377	405	nd	nd	405	405
$(x_6 + Na)^+$	490	462	476	462	476	462	490	476	476	490	490
$(x_5 + Na)^+$	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
$(x_4 + Na)^+$	700	672	686	672	686	672	700	686	686	700	700
$(x_3 + Na)^+$	813	785	799	785	799	785	813	799	799	813	813
$(x_2 + Na)^+$	898	870	884	870	884	870	898	884	884	898	898
$(x_1 + Na)^+$	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

	55	**56**	**57**	**58**	**59**	**60**	**61**	**62**
ion	B-Ia	B-Ib	B-Ic	B-IIa	B-IIb	B-IIIa	B-IIIb	B-IIIc
$[M + Na]^+$	1129	1129	1129	1143	1143	1157	1157	1157
$[M + K]^+$	1145	1145	1145	1159	1159	1173	1173	1173
$[M + H]^+$	nd	nd	nd	nd	nd	nd	nd	nd
$[M + Na - H_2O]^+$	1111	1111	1111	1125	1125	1139	1139	1139
b_1	nd	nd	nd	nd	nd	nd	nd	nd
$b_2 - H_2O$	197	nd	197	197	197	197	197	197
b_2	nd	nd	215	215	215	215	215	215
$b_3 - H_2O$	296	nd	296	296	296	296	296	296
b_3	314	nd	314	314	314	314	314	314
$b_4 - H_2O$	409	nd	nd	409	395	409	409	409
b_4	427	399	413	427	413	427	427	427
$b_5 - H_2O$	nd	nd	nd	494	nd	nd	nd	494
b_5	512	484	498	512	498	512	512	512
[Pro-Lxx-Lxx-Aib-Pro-Aibol + Na]$^+$	617	nd	nd	nd	nd	nd	nd	nd
[Pro-Lxx-Lxx-Aib-Pro-Vxxol + Na]$^+$ or [Pro-Vxx-Lxx-Aib-Pro-Lxxol + Na]$^+$	nd	nd	631	631	nd	nd	nd	nd
[Pro-Lxx-Lxx-Aib-Pro-Lxxol + Na]$^+$	nd	645	nd	nd	645	645	645	645
$(y_7 + Na)^+$	nd	nd	nd	nd	nd	nd	449	nd
$(y_6 + Na)^+$	nd	506	520	nd	nd	534	534	nd
$(y_5 + Na)^+$	nd	nd	nd	nd	nd	nd	nd	nd
$(y_4 + Na)^+$	744	716	730	744	730	744	744	744
$(y_3 + Na)^+$	857	829	843	857	843	857	857	857
$(y_2 + Na)^+$	942	914	928	942	928	942	942	942
$(y_1 + Na)^+$	nd	nd	nd	nd	nd	nd	nd	nd
$(x_7 + Na)^+$	nd	nd	nd	431	nd	421	421	421
$(x_6 + Na)^+$	506	nd	492	516	492	nd	506	nd
$(x_5 + Na)^+$	nd	nd	nd	nd	nd	nd	nd	nd
$(x_4 + Na)^+$	716	688	702	716	702	716	716	716
$(x_3 + Na)^+$	829	801	815	829	815	829	829	829
$(x_2 + Na)^+$	914	886	900	914	900	914	914	914
$(x_1 + Na)^+$	nd	nd	nd	nd	nd	nd	nd	nd

[a] See **Table 3** footnote.

ES-MS of the lipoaminopeptide antibiotics mentioned above. The C terminus of helio- and roseoferins consists of either a 2-[(2′-**a**minopropyl)-**m**ethyl**a**mino]-**e**thanol (**AMAE**, *m*/*z* 132) or a 2-(2′-**a**minopropyl)**a**mino-**e**thanol (**AAE**, *m*/*z* 118) residue. Furthermore, the presence of a C-terminal AMAE is indicated by the loss of C_3H_9NO from the pseudomolecular ion. Consequently, $[M + H - 75]^+$ should be formed—a fragment that is present at *m*/*z* 1132. Moreover, *m*/*z* 1189 indicates the loss of water from $[M + H]^+$—a typical feature of C-terminal (amino) alcohols. CID-MS/MS investigations on *m*/*z* 266 revealed its corresponding *a*-type fragment *m*/*z* 238. Further diagnostic fragments were not observed, due to the comparatively low

Table 8. Sequences of Trichobrevins A and B Produced by Members of the *T. brevicompactum* Complex[a]

		residue											
			1	2	3	4	5	6	7	8	9	10	11	$[M + Na]^+$
44	A-Ia	Ac	Aib	Ala	Vxx	Lxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Alaol	1099
45	A-Ib	Ac	Aib	Ala	Aib	Vxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Vxxol	1099
46	A-Ic	Ac	Aib	Ala	Vxx	Vxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Aibol	1099
47	A-Id	Ac	Aib	Ala	Aib	Vxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Vxxol	1099
48	A-IIa	Ac	Aib	Ala	Vxx	Vxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Vxxol	1113
49	A-IIb	Ac	Aib	Ala	Aib	Vxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Lxxol	1113
50	A-IIIa	Ac	Aib	Ala	Vxx	Lxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Vxxol	1127
51	A-IIIb	Ac	Aib	Ala	Vxx	Vxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Lxxol	1127
52	A-IIIc	Ac	Aib	Ala	Vxx	Vxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Lxxol	1127
53	A-IVa	Ac	Aib	Ala	Vxx	Lxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Lxxol	1141
54	A-IVb	Ac	Aib	Ala	Vxx	Lxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Lxxol	1141
55	B-Ia	Ac	Aib	Ser	Vxx	Lxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Aibol	1129
56	B-Ib	Ac	Aib	Ser	Vxx	Aib	Aib	Pro	Lxx	Lxx	Aib	Pro	Lxxol	1129
57	B-Ic	Ac	Aib	Ser	Vxx	Vxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Vxxol	1129
58	B-IIa	Ac	Aib	Ser	Vxx	Vxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Lxxol	1143
59	B-IIb	Ac	Aib	Ser	Vxx	Lxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Vxxol	1143
60	B-IIIa	Ac	Aib	Ser	Vxx	Lxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Lxxol	1157
61	B-IIIb	Ac	Aib	Ser	Vxx	Lxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Lxxol	1157
62	B-IIIc	Ac	Aib	Ser	Vxx	Lxx	Aib	Pro	Lxx	Lxx	Aib	Pro	Lxxol	1157

[a] See **Table 4** footnote.

abundance of *m*/*z* 266, which was not sufficient to perform further CID-MS^n experiments. Considering the obvious structural homologies with helio- and roseoferins, the ion *m*/*z* 266 could consist of a Pro linked to an α-methyldecanoic acid residue. Positional isomers or higher homologues of compound **64** have not been detected. However, homologues displaying a mass difference of −14 Da (*m*/*z* 1193, $[M + H]^+$) are present. The C-terminal AMAE residue of compound **64** is replaced by AAE in compound **65**—as previously described as a structural variation of helio- and roseoferins (*40*, *41*). The Lxx residue in position 6 of compound **64** is exchanged by Vxx in compound **66**, which carries a C-terminal AMAE residue. The presence of *m*/*z* 1118 indicates a C-terminal AMAE residue for compound **67**. Interestingly, MS^3 of the MS^2 ions *m*/*z* 536 and 621 proved that *m*/*z* 465 and 266 are formed. Isomerism is therefore located in the lipoamino acid residue, as also observed for compound **68**. Assuming structural homology with the previously described compounds **64**−**66**, sequences containing a novel desmethyl-AHMOD residue (*m*/*z* 199) are proposed for compounds **67** and **68**. The Ala residue in position 7 of compound **64** is exchanged by Gly in compound **69**, which carries AHMOD in position 3 and a C-terminal AMAE residue. Compounds with a strongly basic secondary or tertiary amine such as AAE or AMAE, respectively, which is bound to a lipophilic backbone, give a positive reaction in the two-phase vertical stacking assay (*43*). This diagnostic feature, which has previously been described for helio- and roseoferins (*41*, *42*), was also observed for extracts from *T.* cf. *brevicompactum* NRRL 3199. The positive reaction of helioferin in the two-phase vertical stacking assay is correlated with a strong ionophoric activity of this antibiotic (*44*). Therefore, these seven novel compounds from ***Trichoderma*** cf. *brevicompactum* NRRL 3199, which promote the trans**fer** of water-soluble helianthin to a toluene layer, were named **trichoferins** (TFR) **A**−**F**. Their fragmentation patterns and structures are illustrated in **Tables 9** and **10**. *T.* cf. *brevicompactum* strains ATCC 90237 and IBT 40863 also produced compound **64**, trichoferin A, as proven by MS/MS and CID-MS experiments. Minor amounts of an ion *m*/*z* 1207 that could be identical with compound **64** were also found in *T. brevicompactum* CBS 109720, whereas only trace amounts of *m*/*z* 1207 are present in *T. brevicompactum* CBS 112445, IBT 40840, and IBT 40839. It should be mentioned that compounds **65**−**69** (trichoferins B−F) have been detected only in *T.* cf. *brevicompactum* NRRL 3199.

Table 9. Diagnostic Fragment Ions (*m*/*z*) of Trichoferins Produced by Members of the *T. brevicompactum* Complex[a]

ion	**64** A	**65** B	**66** C	**67** D	**68** E	**69** F
$[M + H]^+$	1207	1193	1193	1193	1193	1193
$[M + Na]^+$	1229	1215	1215	1215	1215	1215
$[M - H_2O]^+$	1189	1175	1175	1175	nd	nd
$[M - C_4H_7O]^+$	1135	1120	1120	1120	1120	1120
$[M - C_3H_8NO]^+$	1132	nd	1060	1060	1118	nd
$[M - C_2H_6NO]^+$	nd	1132	nd	nd	nd	nd
b_1	nd	nd	nd	nd	nd	nd
b_2	266	266	266	266	266	266
$b_3 - CO$	nd	nd	nd	nd	419	nd
$b_3 - H_2O$	nd	nd	nd	nd	447	nd
b_3	479	479	479	465	465	nd
$b_4 - H_2O$	nd	nd	nd	nd	518	nd
b_4	550	550	550	536	536	550
b_5 - CO	nd	nd	nd	nd	593	nd
$b_5 - H_2O$	nd	nd	nd	nd	603	nd
b_5	635	635	635	621	621	635
b_6	720	720	720	706	706	720
b_7	833	833	819	805	819	833
b_8	904	904	890	890	890	890
b_9	989	989	975	975	975	975
b_{10}	1074	1074	1060	1060	1060	1060
a_2	238	238	238	238	238	238

[a] See **Table 3** footnote.

Furthermore, the strain produces novel sequences of trichocompactins VII, VIIIa, and VIIIb with Gly in position 6 replaced by Ala (compounds **70**−**72**). In addition to that, three serine-containing trichocompactins IX, Xa, and Xb were detected (compounds **73**−**75**). Interestingly, compounds **5**−**10** could not be found. Fragmentations and sequences of all trichocompactins described in this study are listed in **Tables 2** and **3**. Compounds **44**−**62** were detected again and their structures proven by CID-MS and MS^n experiments. In contrast to what was found for *T.* cf. *brevicompactum* ATCC 90237 and IBT 40863, compounds **61** and **62** were the most prominent ions besides the compounds **1**−**4**.

Table 10. Sequences of Trichoferins Produced by Members of the *T. brevicompactum* Complex[a,b]

		residue										
		1	2	3	4	5	6	7	8	9	10	$[M + H]^+$
64	A	MDA	Pro	AHMOD	Ala	Aib	Aib	Lxx	Ala	Aib	Aib	AMAE	1207
65	B	MDA	Pro	AHMOD	Ala	Aib	Aib	Lxx	Ala	Aib	Aib	AAE	1193
66	C	MDA	Pro	AHMOD	Ala	Aib	Aib	Vxx	Ala	Aib	Aib	AMAE	1193
67	D	MDA	Pro	desmethyl-AHMOD	Ala	Aib	Vxx	Aib	Aib	Aib	Aib	AMAE	1193
68	E	MDA	Pro	desmethyl-AHMOD	Ala	Aib	Aib	Lxx	Ala	Aib	Aib	AMAE	1193
69	F	MDA	Pro	AHMOD	Ala	Aib	Aib	Lxx	Gly	Aib	Aib	AMAE	1193

[a] See **Table 4** footnote. [b] Abbreviations: MDA, 2-methyldecanoic acid; AHMOD, 2-amino-4-methyl-6-hydroxy-8-oxodecanoic acid.

DISCUSSION

Screening of recently described species of *Trichoderma* greatly enhances the possibility to find new peptaibiotics. Remarkably, 69 of the 75 peptides (93%) analyzed in this study represent new sequences. The strains produced 14 12-residue trichocryptins B, 12 11-residue trichocryptins A, 19 11-residue trichobrevins A and B, 6 10-residue trichoferins, and 17 8-residue trichocompactins. The number of new compounds described in this study clearly illustrates the impressive potential of a peptaibiomic approach.

Obviously, there are structural homologies of the new 11- and 12-residue compounds with previously reported peptaibiotics, such as harzianins (*33*, *36*, *38*), antiamoebins (*22*), hypomurocins (*37*), and bergofungins (*35*). Thus, comparable biological activities could be expected, although the decrease in chain length may lead to a reduction in efficacy.

As alamethicins are present in every strain investigated, they should considerably contribute to the biological activity against the causal agents of Eutypa dieback and Esca disease of grapevine. The exceptional antimicrobial activity of alamethicins can be explained by the dipole flip-flop gating model of Boheim and Jung (*45*). Alamethicins, as long-chain, 20-residue peptaibols, may form larger and more stable pores than shorter chain peptaibiotics, thus remarkably lowering the minimal inhibitory concentration (MIC) to microorganisms (for a review see ref *31*).

Structural homologies of trichoferins with the protonophoric roseo- and helioferins and the positive reaction of trichoferin-containing extracts in the two-phase vertical stacking assay indicate an ionophoric activity that may amplify the biocontrol potential of the trichoferin-producing strains. However, the importance of trichocompactins for the bioactivity of the producing strains remains doubtful.

Generally, a decrease in chain length is correlated with a loss of bioactivity as exemplified in the case of the 19-residue chrysospermins (*46*) and the 5-residue peptaibolin (*47*) from *Sepedonium chrysospermum* (teleomorph: *Hypomyces chrysospermus*). Chrysospermins may form nongated membrane channels (*48*), thus exhibiting strong antimicrobial activity against Gram-positive bacteria, yeasts, and fungi. They also accelerate cytodifferentiation of the coelomycete *Phoma destructiva* and cause neuroleptic activity in mice (*49*). For peptaibolin, however, no significant bioactivities have been reported.

Notably, the distribution of peptaibiotics among taxonomic groups/species clusters of *Trichoderma* is currently under investigation in order to explain and correlate their antagonistic properties (*50*).

According to our data, the alamethicins are restricted to the *T. brevicompactum* group, being the most abundant peptaibiotic metabolites of *T. brevicompactum* (*ss*).

When grown on PDA at 25 °C, *T. brevicompactum* also biosynthesized diterpene mycotoxins of the trichothecene group: strains CBS 109720, IBT 40839, and IBT 40840 produced trichodermin, whereas harzianum A was detected in strain CBS 112445 as well. However, phylogenetic analyses suggested the classification of all of these strains as *T. brevicompactum* (*ss*). In contrast, *T.* cf. *brevicompactum* ATCC 90237, IBT 40863, and NRRL 3199 mainly biosynthesized harzianum A. Notably, 17 strains belonging to the *T. brevicompactum* complex consistently produced trichothecenes on all media tested. In contrast to that, formation of trichothecenes has not been observed for any other of the more than 250 *Trichoderma* strains screened (*3*, *27*).

This leads to the conclusion that the pattern of characteristic nonpeptidic mycotoxins (trichothecenes) and peptaibiotics (alamethicins and trichocompactins) might be used in addition to morphological and molecular data to separate the *brevicompactum* complex from other taxa of the genus *Trichoderma*. Morphological, molecular, and chemical data of strain NRRL 3199 support its affiliation with *T.* cf. *brevicompactum* rather than *T. viride* (*3*). Taken together, the differential patterns of alamethicin production as well as the production of two different trichothecene-type mycotoxins clearly support DNA sequencing results. Both molecular and chemotaxonomic approaches clearly indicate the existence of two phylogenetic species within what has been called *T. brevicompactum*, so far. Both trichocryptins and trichobrevins are more widespread in the genus *Trichoderma*, illustrating the limitations of chemotaxonomic conclusions focused exclusively on the pattern of peptaibiotics. Nevertheless, the taxonomy of *T. brevicompactum* remains a rather complex topic and is the subject of an ongoing study.

Summarizing the sequences presented in this paper, it can be concluded that peptaibiotics still seem to be of questionable chemotaxonomic importance. Literature data clearly support this opinion, because fungi belonging to divergent taxonomic groups may produce closely related sequences of peptaibiotics.

The biosynthesis of lipoaminopeptides, for example, has been described for strains of the fungicolous species *Paecilomyces lilacinus* and *Paecilomyces marquandii*, but was also observed in cultures of *Trichoderma polysporum* isolated from infested fruit bodies of *Lentinula edodes* and in the mycoparasite *M. rosea* (reviewed in ref *18*).

To continue, 16-residue peptaibols, antiamoebins, were obtained from *Emericellopsis synnematicola*, *Emericellopsis poonensis*, *Verticillium epiphytum* (syn. *Cephalosporium pimprina*), and *Stilbella fimetaria* CBS 548.84 and ATCC 28144 (syn. *Stilbella erythrocephala*), but have also been isolated from *Clonostachys rosea* f. *catenulata* (syn. *Gliocladium catenulatum*) CBS 511.66 (*21*, *22*).

Consequently, the formation of peptaibiotics should rather be defined as an adaptation to highly specialized modes of life of the producers, mostly being facultative or obligate plant

pathogens or fungicolous fungi occupying some particular ecological niches.

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