This study examined the structural diversity and bioactivity of peptaibol compounds produced by species from the phylogenetically separated Longibrachiatum Clade of the filamentous fungal genus *Trichoderma*, which contains several biotechnologically, agriculturally and clinically important species. HPLC-ESI-MS investigations of crude extracts from 17 species of the Longibrachiatum Clade (*T. aethiopicum*, *T. andinense*, *T. capillare*, *T. citrinoviride*, *T. effusum*, *T. flagellatum*, *T. ghanense*, *T. konilangbra*, *T. longibrachiatum*, *T. novae-zelandiae*, *T. pinnatum*, *T. parareesei*, *T. pseudokoningii*, *T. reesei*, *T. saturnisporum*, *T. sinensis*, and *T. orientale*) revealed several new and recurrent 20-residue peptaibols related to trichobrachins, paracelsins, suzukacillins, saturnisporins, trichoaureocins, trichocellins, longibrachins, hyporientalins, trichokonins, trilongins, metanicins, trichosporins, gliodeliquescins, alamethicins and hypophellins, as well as eight 19-residue sequences from a new subfamily of peptaibols named brevicelsins. Non-ribosomal peptide synthetase genes were mined from the available genome sequences of the Longibrachiatum Clade. Their annotation and product prediction were performed *in silico* and revealed full agreement in 11 out of 20 positions regarding the amino acids predicted based on the signature sequences and the detected amino acids incorporated. Molecular dynamics simulations were performed for structural characterization of four selected peptaibol sequences: paracelsins B, H and their 19-residue counterparts brevicelsins I and IV. Loss of position R6 in brevicelsins resulted in smaller helical structures with higher atomic fluctuation for every residue than the structures formed by paracelsins. We observed the formation of highly bent, almost hairpin-like, helical structures throughout the trajectory, along with linear conformation. Bioactivity tests were performed on the purified peptaibol extract of *T. reesei* on clinically and phytopathologically important filamentous fungi, mammalian cells, and...
Arabidopsis thaliana seedlings. Porcine kidney cells and boar spermatozoa proved to be sensitive to the purified peptaibol extract. Peptaibol concentrations ≥0.3 mg ml⁻¹ deterred the growth of A. thaliana. However, negative effects to plants were not detected at concentrations below 0.1 mg ml⁻¹, which could still inhibit plant pathogenic filamentous fungi, suggesting that those peptaibols reported here may have applications for plant protection.

**Keywords:** *Trichoderma*, Longibrachiatum, peptaibol, brevicelsin, mass spectrometry, antifungal activity, Arabidopsis, mammalian cells

**INTRODUCTION**

At present, more than 300 species of the genus *Trichoderma* (Ascomycota, Hypocreales, Hypocreaceae) have been described (Bissett et al., 2015; Zhang and Zhuang, 2018). The majority of these species were described after the year 2000, as only a few species were initially included in the genus (Bisby, 1939; Rifai, 1969). Section Longibrachiatum of the genus was one of the five *Trichoderma* sections according to Bissett (1984, 1991a,b,c). It forms a monophyletic group phylogenetically separated from the other four *Trichoderma* sections (Kuhls et al., 1997; Samuels et al., 1998) and is designated recently as the Longibrachiatum Clade (Samuels et al., 2012). It is one of the youngest clades of the genus (Kubicek et al., 2011) and has the largest number of available whole-genome sequence data. This clade is ecologically highly versatile as it contains prominent clinically relevant and ecologically restricted species. *Trichoderma longibrachiatum*, *T. orientale*, and *T. citrinoviride* are opportunistic human pathogens causing infections, mainly in immunocompromised patients (Kuhls et al., 1999; Kredics et al., 2003; Hatvani et al., 2013). *T. longibrachiatum* or its transformants have also been suggested for use as biocontrol agents against plant pathogens like *Pythium ultimum* or members of the *Fusarium solani* species complex (Mighelli et al., 1998; Rojo et al., 2007). *T. longibrachiatum* and *T. orientale* are sympatric species but have different reproductive strategies, the former being strictly clonal, whereas the latter recombines sexually (*T. pinnatum* and *T. aethiopicum* are rare and restricted species (Druzhinina et al., 2010). *T. longibrachiatum* and *T. orientale* are cosmopolitan, the related *T. pinnatum* and *T. aethiopicum* are rare and restricted species (Druzhinina et al., 2010). Numerous other species, including *T. reesei*, *T. reesei*, *T. pseudokoningii*, *T. sinense*, *T. effusum*, *T. koningbra*, *T. andinense*, or *T. novae-zelandiae* are also geographically restricted (Druzhinina et al., 2012).

Several secondary metabolites are produced by *Trichoderma* species from the Longibrachiatum Clade. Probably the best known species is *T. reesei*, which produces hydrolytic enzymes degrading cellulose or hemicellulose (Harman and Kubicek, 1998; Kubicek et al., 2009). Peptaibols are membrane-active compounds with the ability to aggregate and form ion channels in lipid bilayer membranes. They are usually short peptides of 8–20 residues with non-proteinogenic amino acids and are biosynthesised by non-ribosomal peptide synthetases (NRPSs) (Marahiel, 1997; Marahiel et al., 1997; May et al., 2002; Degenkolb et al., 2003, 2007; Bushley and Turgeon, 2010; Marik et al., 2017b). In the case of NRPSs, a single large protein is responsible for the activation, incorporation and elongation of the peptides. NRPSs can also incorporate non-proteinogenic residues, thus increasing the chemical diversity of the products. The lack of specificity of the recognition sites and the three-dimensional structure of the enzyme lead to the acceptance of closely related residues (such as Vxx vs. Lxx). Consequently, the number of positionally isomeric and homologous peptaibols biosynthesised by a single NRPS can be large. The repair mechanisms, which usually operate during biosynthesis, are also absent in NRPS pathways, thus further increasing the variability of the products. Characteristic residues of peptaibols include α-aminoisobutyric acid (Aib) and isovaline (Iva), as well as 1,2-amino alcohols such as Leuol, Valol, Pheol, Tyrol, Ileol, Alaol, and Prool at the C-terminus (Degenkolb et al., 2008; Stoppacher et al., 2013). Peptaibols usually form short, linear helical structures, several of which aggregate to form ion channels and may damage lipid membranes. Investigation of the structural and dynamic properties of peptaibol molecules is important for the understanding of their biological activities. Computational molecular dynamics-based simulation is a popular technique for investigating a molecule's dynamic behavior and predicting its three-dimensional structure. Peptaibols like trichobrachins (Násztor et al., 2013), harzianins (Putzu et al., 2017), alamethicin (Leitgeb et al., 2007; Kredics et al., 2013), tripleurin (Tyagi et al., 2019), and others have been investigated using such techniques. Knowledge about the structure of peptaibols might also facilitate the design of bioactive peptides for future applications. The characteristic non-proteinogenic amino acid residues of peptaibols (Aib and C-terminal alcohols) can be parameterised quantum-mechanically, and the effects of their presence can be evaluated. In general, long molecular time scales are required to effectively simulate peptide folding processes. An all-atom enhanced sampling technique known as accelerated molecular dynamics (aMD) can be used, which provides a non-negative boost to the potential energy and speeds up the process of peptide folding.

*Trichoderma* species are widely used against various plant pathogenic fungi as biocontrol agents because of their fast growth and reproduction, their mycoparasitism and their production of secondary metabolites (Chaverri et al., 2015; Degenkolb et al., 2015; Waghunde et al., 2016). Species like *T. viride*, *T. virens*, *T.*
atroviride, T. asperellum, and T. harzianum are frequently studied due to their production of enzymes and antibiotics valuable in agriculture (Schuster and Schmoll, 2010; Contreras-Cornejo et al., 2016) and their antagonist effects against pathogenic fungi such as Botrytis cinerea, Alternaria solani and Rhizoctonia solani (Harman et al., 2004). Incubation of a “T. harzianum” strain later re-identified as T. atroviride (Röhrich et al., 2014) with B. cinerea cell walls resulted in the secretion of cell wall hydrolytic enzymes and antibiotic fractions of peptaibols, which inhibited B. cinerea spore germination, causing a fungicidal effect. Peptaibols and hydrolytic enzymes were found to work synergistically in this antagonistic interaction (Schirmböck et al., 1994).

Trichoderma species also interact with plants through secondary metabolites. Although several studies reported positive effects of Trichoderma species on the physiological and biochemical responses of plants (Contreras-Cornejo et al., 2016), inhibition of plant growth and primary root development have also been described (Rippa et al., 2010; Shi et al., 2016). The most thoroughly investigated model plant, Arabidopsis thaliana, is frequently used to test the bioactivity of the secondary metabolites of Trichoderma species (Kottb et al., 2015). Peptaibols can induce auxin production and disruption of the auxin response gradient in root tips (Shi et al., 2016). The most thoroughly studied peptaibol, alamethicin, was shown to induce resistance in plants (Leitgeb et al., 2007; Kredics et al., 2013) but can also be toxic, causing lesions on Arabidopsis leaves (Rippa et al., 2010). However, it should also be considered that the commercially available alamethicin mixture (Sigma-Aldrich A4665) may also contain the trichothecone-type mycotoxin harzianum A produced by the strain T. brevicompactum used for alamethicin fermentations (Degenkolb et al., 2006).

This study aimed at revealing the genomic background, structural diversity and bioactivity of peptaibol compounds produced by different species from the ecologically diverse Longibrachiatum Clade of the genus Trichoderma.

MATERIALS AND METHODS

Strains and Culture Conditions
Twenty-two strains from 17 Trichoderma species belonging to the Longibrachiatum Clade of the genus were selected from the TU Collection of Industrially Important Microorganisms, Vienna, Austria (TUCIM, www.vt.tuwien.ac.at/tucim/) and the Szeged Microbiology Collection, Szeged, Hungary (SzMC; www.szmc.hu) for investigation of their peptaibol production (Table 1). For testing the antifungal activity of peptaibol extracts, filamentous fungal strains of clinical relevance (Aspergillus fumigatus SzMC 23245, Fusarium falciforme SzMC 11407 and Fusarium keratoplasticum SzMC 11414 from human keratmycosis, India) or phytopathological relevance (Alternaria alternata SzMC 16085, F. solani species complex SzMC 11467 and Phoma cucurbitacearum SzMC 16088) were selected.

| TABLE 1 | Trichoderma strains from the Longibrachiatum Clade involved in the study. |
|----------|-----------------|-----------------|-----------------|-----------------|
| SzMC identifier | Other identifier | Subclade* | Species | Origin | References |
| 1773 | CECT 2412 | Longibrachiatum/Oriental | T. longibrachiatum | Mushroom compost, Wales | Druzhinina et al., 2008 |
| 1775 | CECT 2937 | Longibrachiatum/Oriental | T. longibrachiatum | Antarctica | Kuhls et al., 1997 |
| 1776 | CECT 20105 | Longibrachiatum/Oriental | T. longibrachiatum | Biocontrol strain, Spain | Antal et al., 2005 |
| 12546 | UAMH 7966 | Longibrachiatum/Oriental | T. longibrachiatum | Bone marrow transplant recipient | Richter et al., 1999 |
| 12556 | UAMH 9573 | Longibrachiatum/Oriental | T. orientale | Peritoneal catheter tip, Canada | Kredics et al., 2003 |
| 22602 | TUCIM 1817 | Longibrachiatum/Oriental | T. aethiopicum | Coffee arabica rhizosphere; Jimma, Ethiopia | Druzhinina et al., 2008 |
| 22603 | TUCIM 3421 | Longibrachiatum/Oriental | T. pinnatum | Sri Lanka | Samuels et al., 2011 |
| 22614 | TUCIM 917, QM6a | Parareesei/Reesei | T. reesei | canvas of US army; Solomon Islands | Reese et al., 1950 |
| 22616 | OM9414 | Parareesei/Reesei | T. reesei | Mutant of QM9123 (which is mutant of QM6a) | Kuhls et al., 1996 |
| 22617 | OM9414 G2.Aee1 | Parareesei/Reesei | T. reesei | lae1 null mutant (Aae1) of T. reesei QM9414 | Seiboth et al., 2012 |
| 22615 | TUCIM 661 | Parareesei/Reesei | T. parareesei | Subtropical rain forest; Igazu Falls, Argentina | Atanasova et al., 2010 |
| 22606 | TUCIM 1267 | Saturnisporum | T. saturnisporum | Italy | Samuels et al., 2012 |
| 22607 | TUCIM 132 | Koniangbra/Sinensis | T. koniangbra | Uganda | Samuels et al., 1998 |
| 22608 | TUCIM 3350 | Koniangbra/Sinensis | T. flagellatum | Coffee arabica rhizosphere; Ethiopia | Belayneh Mulaw et al., 2010 |
| 22609 | TUCIM 527 | Koniangbra/Sinensis | T. sinensis | Taiwan | Bissett et al., 2003 |
| 22618 | SJ40 | Citrinovireide/Pseudokoningi | T. citrinovireide | Office bookshelf, settled dust, Espoo, Finland | Castagnoli et al., 2018 |
| 22613 | TUCIM 1277 | Citrinovireide/Pseudokoningi | T. pseudokoningi | the bark of Batschmiadia tawa | Samuels et al., 1998 |
| 22612 | TUCIM 4158 | Novae-zelandiae/Saturinisporosis | T. novae-zelandiae | Native Notophagus forest, New Zealand | Samuels et al., 1998 |
| 22604 | TUCIM 2057 | | T. ghanense** | Agaricus compost; Hungary | Hatvari et al., 2007 |
| 22605 | TUCIM 2883 | | T. capillare** | Wall of a mushroom growing cellar; Hungary | Hatvari et al., 2007 |
| 22610 | TUCIM 1291 | | T. andinense** | Venezuela, high elevation | Samuels et al., 1998 |
| 22611 | TUCIM 254 | | T. effusum** | Soil isolation; Himalaya, India | Bissett et al., 2003 |

*Subclades were defined based on Samuels et al. (2012). **Considered as lone lineages.
The strains were maintained and cultured as described by Marik et al. (2017a).

Peptaibol Extraction

Peptaibols were extracted according to Marik et al. (2017a). For large quantity peptaibol production and purification, *T. reesei* QM9414 (SzMC 22616) was cultured according to Marik et al. (2018). The samples were purified on a Flash chromatograph (Combiflash EZ Prep UV-VIS Teledyne Isco). The cartridge (Combiflash EZ Prep) was filled with 60 cm² silica (30–40 µm), and 1.5 g of crude peptaibols extract was applied above the septum. The flow rate was set to 35 ml min⁻¹ and the wavelength of the UV detector to 270/320 nm. Solvents A and B were chloroform and methanol, respectively (gradient solvent B: 0%, 0 min; 0%, 5 min; 100%, 15 min; 100%, 18 min). Fractions were automatically collected into collector tubes (18 × 180 mm, 30 ml) based on the slope of the UV signal. Fractions were evaporated, dissolved in methanol (100 mg ml⁻¹) and stored at −20°C. The purity of the samples was checked by HPLC-MS as described by Van Bohemen et al. (2016). For this analysis, the appearing yy-ion fragments were quantified and compared to alamethicin (Sigma-Aldrich A-4665, Hungary) dissolved in methanol (VWR, Hungary).

Analytical Procedures and Data Analysis

Crude peptaibol extracts were subjected to HPLC-ESI-MS using a Varian 500 MS equipment with the parameters described previously (Marik et al., 2018). The excitation storage level (m/z/excitation amplitude (V)) and the gradient program for Solvent B were 10%, 0%, 5 min; 100%, 15 min; 100%, 18 min. Fractions were automatically collected into collector tubes (18 × 180 mm, 30 ml) based on the slope of the UV signal. Fractions were evaporated, dissolved in methanol (100 mg ml⁻¹) and stored at −20°C. The purity of the samples was checked by HPLC-MS as described by Van Bohemen et al. (2016). For this analysis, the appearing yy-ion fragments were quantified and compared to alamethicin (Sigma-Aldrich A-4665, Hungary) dissolved in methanol (VWR, Hungary).

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Bioinformatic Analysis of Peptaibol Synthetase Genes

Peptaibol synthetases of *Trichoderma* species from the Longibrachiatum Clade with accessible full genome sequences, *T. reesei*, *T. pararresei*, and *T. citrinoviride* (GenBank Assembly accession numbers GCA_000167675.2, GCA_001050175.1 and GCA_000351151.1, respectively) and two strains of *T. longibrachiatum* (GCA_003025155.1, GCA_000332775.1) were identified using the Secondary Metabolites from InterProScan (SMIPS) online software, and 20 as well as 14 module NRPSs were selected (Wolfe et al., 2016). In the case of *T. longibrachiatum*, *T. citrinoviride*, *T. reesei*, and *T. pararresei*, the extracted sequences were analyzed using the Antibiotics and Secondary Metabolites Analysis Shell (antiSMASH), the PKS-NRPS Analysis Web-site, the NRPS/PKS substrate predictor and the NRPSPredictor3 SVM, as described by Marik et al. (2017a).

Accelerated Molecular Dynamics Simulations of 20- and 19-Residue Peptaibols

Calculation of the partial charges for the non-standard residues Aib and Pheol and the preparation of unfolded conformations of four selected peptaibols in water were carried out as described by Tyagi et al. (2019). The Leu and Val positions in brevicins...
sequences were predicted based on their positionally isomeric 20-residue paracelsin counterparts. For the Paracelsin B system, 3910 water molecules were added with a box size of 55.05 × 46.82 × 62.33 Å and a volume of 160676.0 Å³, whereas 3557 TIP3P water molecules were added with a box size of 55.05 × 42.11 × 63.40 Å and a volume of 147021.35 Å³ to prepare the Paracelsin H system. Similarly, 4725 water molecules were added to the Brevicelisin I system with a box size of 67.57 × 50.93 × 54.97 Å and a volume of 189190.34 Å³, whereas 4536 water molecules were added to the Brevicelisin IV system with a box size of 68.52 × 45.96 × 58.30 Å and a volume of 183623.0 Å³.

The four systems were prepared for aMD simulations used to enhance sampling with a boost to the whole potential energy and an extra boost to torsional energy. The values of coefficients \( a_1 \) and \( a_2 \) were set to 4, whereas \( b_1 \) and \( b_2 \) were set to 0.16, based on previous studies (Pierce et al., 2012).

**Peptaibol Bioactivity Assays**

For inhibition tests with filamentous fungi, malt extract agar medium completed with yeast extract was cast at 25°C, following the method described by Marik et al. (2018). The purified peptaibol extract of *T. reesei* QM9414 was tested in an agar plate well-diffusion assay with methanol as a control, as well as alamethicin (Sigma-Aldrich A-4665, Hungary) and nystatin (Nystatin 2-hydrate BioChemica, AppliChem A3811,0025, Germany) as reference compounds. All solutions were prepared in two-step dilution series from 0.4 mg ml⁻¹ to 0.0036125 mg ml⁻¹. The inhibition zones were measured as the distance between the edge of the fungal colonies and the edge of the holes containing the peptaibol solutions at the time when the edge of the colony reached the edge of the control hole filled with methanol. At the same time, plates were photographed with a Coolpix S2600 digital camera (Nikon). Minimum inhibitory concentration (MIC) values were defined as the lowest concentrations where an inhibition zone could be detected. Experiments were carried out in triplicate.

In order to investigate the biological effects of peptaibols on plants, *A. thaliana* (Col-0 ecotype) seeds were planted on 0.5 × Murashige and Skoog agar (8%) medium (Horváth et al., 2015) with the addition of 0.5% sucrose (w/v) (pH adjusted to 5.5 with NaOH) in plastic Petri dishes (90 × 17 mm) five seeds per Petri dish in one line. Seeds were surface sterilized with 70% ethanol for 1 min, treated with 4% hypochlorite for 15 min and washed with sterile distilled water. After vernalisation at 4°C for 24 h, seeds were sown onto the agar plates. *Arabidopsis* plants were placed in a greenhouse with a photoperiod of 12 h of light and 12 h of darkness, a light intensity of 300 μmol m⁻² s⁻² and a temperature of 25 ± 1°C. After the third day post germination, plates were placed at an angle of 50° to allow root growth along the agar surface and to promote aerial growth of the hypocotyls. Four 5 mm holes were bored with a sterile cork borer 0.5 cm from the root tips of 5-day-old *Arabidopsis* seedlings (five seedlings per plate) and filled with 40 μl of peptaibol extract. The growth of primary roots was measured every 24 h for 4 days. Photographs of 15-day-old plants were taken using a Coolpix S2600 digital camera (Nikon). The fresh weights of the plants from each plate were measured, and photosynthetic pigments were quantified as described by Lichtenthaler (1987). Statistical analyses were performed using Bonferroni’s multiple comparison tests with the GraphPad Prism software version 6.00 (GraphPad Software, San Diego, CA, USA; www.graphpad.com) using 25 samples.

Bioassays using porcine kidney cells (PK-15) and assays of cell membrane integrity disruption in boar sperm cells were carried out as described previously (Bencsik et al., 2014; Marik et al., 2017b).

**RESULTS**

**Identification of Peptaibols Produced by *Trichoderma* Species From the Longibrachiatum Clade**

Peptaibols produced by species from the Longibrachiatum Clade of genus *Trichoderma* were identified using the strategy described by Marik et al. (2013, 2017a). Extracted ion chromatograms (EIC) resulting from full scan measurements of crude extracts from the examined *Trichoderma* strains are shown in Supplementary Figures 1–22. Singly-charged pseudomolecular ions, such as [M+Na]⁺ or [M+H]⁺, were scarcely detectable in the spectra, whereas doubly charged ([M+2Na]²⁺) ions were present and could be used for identification. Full scan MS spectra contained the series of the fragment ions from the N-terminal part (b₁—b₈ and b₉—b₁₃, Supplementary Figure 23) except for b₇, where the stable Gln-Aib bond is present in the compounds (Krause et al., 2006a). The C-terminal γ₇ fragment was consistently observed and provided a good reference for the quantification of the peptides in the mixture. The first 13 amino acid residues could be identified from the full scan MS spectra, but MS² experiments were performed for the identification of residues at the C-terminus. The last four residues could be identified directly from the MS² spectra (Supplementary Figure 24). The γ₇-AA(19-15) ions were not shown on these spectra, therefore another MS² fragmentation was performed on an Orbitrap-MS system from the γ₇ ions, which proved Vxx and Aib in positions 15 and 16, respectively (Supplementary Figure 25). All the detected peaks could also be reidentified at high resolution on the HPLC-Orbitrap-MS system, except for γ₇-H₂O (Supplementary Tables 1–6). Instead of [M+Na]⁺ and [M+2Na]²⁺ ions, [M+H]⁺ could be observed on these spectra.

The peptaibol sequences could be categorized into three groups, designated as A (Table 2; Supplementary Tables 1, 4), B (Table 3; Supplementary Tables 2, 5) and C (Table 4; Supplementary Tables 3, 6). Groups A and B contain 20-residue peptaibols, whereas group C sequences had lost a residue in position R6. The novelty of the sequences was validated according to the “Comprehensive Peptaibiotics Database” (Stoppacher et al., 2013) as well as the last, offline version of the “Peptaibiotics Database.” The former online resource (Neumann et al., 2015) is unavailable since the autumn of 2017, therefore PubMed searches of publications since
| Peptide | M     | [M+Na]+ (min) | [M+2Na]+ (min) | rt-GK (min) | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 | R12 | R13 | R14 | R15 | R16 | R17 | R18 | R19 | R20 | Peptide identical or positionally isomeric with | References |
|---------|-------|---------------|----------------|--------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|-----------------------------|------------|
| Pept-A-1a | 1906 | 1959 | 991 | 1149 | 788 | 38.17 | Ac | Aib | Aib | Aib | Aib | Aib | Aib | Aib | Vxx | Aib | Gly | Lxx | Aib | Pro | Vxx | Aib | Aib | Aib | Gln | Gin | Gin | Trichoaureocin 1d | Brückner et al., 2002 |
| Pept-A-1b | 1906 | 1959 | 991 | 1149 | 788 | 38.17 | Ac | Aib | Aib | Aib | Aib | Aib | Aib | Aib | Vxx | Aib | Gly | Lxx | Aib | Pro | Vxx | Aib | Aib | Aib | Aib | Aib | Gin | Gin | Trichoaureocin 1d | Brückner et al., 2002 |
| Pept-A-2a | 1906 | 1959 | 991 | 1149 | 788 | 38.17 | Ac | Aib | Aib | Aib | Aib | Aib | Aib | Aib | Vxx | Aib | Gly | Lxx | Aib | Pro | Vxx | Aib | Aib | Aib | Aib | Aib | Gin | Gin | Trichoaureocin 1d | Brückner et al., 2002 |
| Pept-A-2b | 1906 | 1959 | 991 | 1149 | 788 | 38.17 | Ac | Aib | Aib | Aib | Aib | Aib | Aib | Aib | Vxx | Aib | Gly | Lxx | Aib | Pro | Vxx | Aib | Aib | Aib | Aib | Aib | Gin | Gin | Trichoaureocin 1d | Brückner et al., 2002 |

(Continued)
| Peptide | M | [M+Na]+ | [M+2Na]+ | b13 | rt-GK (min) | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 | R12 | R13 | R14 | R15 | R16 | R17 | R18 | R19 | R20 | Compound identical or positionally isomorphic with | References |
|---------|---|---------|----------|-----|------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|--------------------------------|-------------|
| Pept-A-IIIc | 1906 | 1959 | 991 | 1149 | 788 | 39.89 | Ac | Alb | Ala | Alb | Alb | Alb | Alb | Gln | Alb | Vxx | Ala | Gly | Vxx | Alb | Pro | Vxx | Alb | Vxx | Gin | Gin | Pehol | New: Peptaureocin 1d: [Aib]17 → [Ala]17 | Brückner et al., 2002 |
| Pept-A-Iva | 1906 | 1959 | 991 | 1163 | 774 | 40.21 | Ac | Alb | Ala | Alb | Alb | Alb | Alb | Gln | Alb | Vxx | Alb | Gly | Lxx | Alb | Pro | Vxx | Alb | Vxx | Gin | Gin | Pehol | New: Peptaureocin 1d: [Aib]17 → [Ala]17 | Brückner et al., 2002 |
| Pept-A-IVb | 1906 | 1959 | 991 | 1163 | 774 | 40.18 | Ac | Alb | Ala | Alb | Alb | Alb | Alb | Gln | Alb | Lxx | Ala | Gly | Lxx | Alb | Pro | Vxx | Alb | Gln | Gin | Gin | Pehol | New: Peptaureocin 1d: [Aib]17 → [Ala]17 | Brückner et al., 2002 |
| Pept-A-Va | 1950 | 1973 | 998 | 1177 | 774 | 40.73 | Ac | Alb | Alb | Alb | Alb | Alb | Alb | Gln | Alb | Lxx | Ala | Gly | Lxx | Alb | Pro | Vxx | Alb | Gln | Gin | Gin | Pehol | New: Peptaureocin 1d: [Aib]17 → [Ala]17 | Brückner et al., 2002 |
| Peptide | M | [M+Na]⁺ | [M+2Na]²⁺ | b₁₃ | y₁ | rt-GK (min) | R | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 | R12 | R13 | R14 | R15 | R16 | R17 | R18 | R19 | R20 | Compound identical or positionally isomeric with | References |
|---------|---|--------|----------|-----|---|-----------|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|-----------------------------------------------|------------|
| Pept-A-Vb | 1950 | 1973 | 998 | 1177 774 | 41.40 | Ac | Alb | Ala | Alb | Ala | Ala | Gin | Alb | Lxx | Alb | Gly | Lxx | Alb | Pro | Vox | Alb | Alb | Gin | Gin | Pheol | Trichobrachin IIb C | Krause et al., 2007 |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Trichobrachin VIII | Huang et al., 2016 |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Trilongin BII | Mikkola et al., 2012 |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Metanicin C | Kimonyo and Brückner, 2013 |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Hypophellin 5 | Rührich et al., 2013 |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Longibrachin A III. | Tamandegani et al., 2016 |
| Pept-A-Vla | 1907 | 1960 | 991.5 | 1163 775 | 41.46 | Ac | Alb | Ala | Alb | Ala | Ala | Gin | Alb | Lxx | Alb | Gly | Lxx | Alb | Pro | Vox | Alb | Alb | Gin | Gin | Pheol | Suzukacin A 11a, A 09 | Krause et al., 2006b |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Trichocellin TC-A-V, TC-A-VII | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Longibrachin B II | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Trilongin CI | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Hypophellin 2 | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Longibrachin B II, Trilongin CI. | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | New: Longibrachin B II: | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | [Val]⁹ → [Lxx]⁹ and [Ala]¹⁰ → [Ala]¹⁰ | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | New: Trilongin CI: [Vox]⁹ → [Lxx]⁹ and [Ala]¹⁰ → [Ala]¹⁰ | Mikkola et al., 2012 |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | New: Hypophellin 2: | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | [Val]⁹ → [Lxx]⁹ and [Ala]¹⁰ → [Ala]¹⁰ | Rührich et al., 2013 |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | New: Longibrachin B II, Trilongin CI.: | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | [Vox]⁹ → [Lxx]⁹ and [Ala]¹⁰ → [Ala]¹⁰ | Tamandegani et al., 2016 |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | New: Trichocellin TC-B-I: | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | [Ala]¹⁰ → [Ala]¹⁰ and [Aib]¹² → [Lxx]¹² | Wada et al., 1994 |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | New: Trichocellin TC-A-V; -VII: | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | [Vox]³ → [Val]³ and [Aib]¹⁰ → [Ala]¹⁰ | Wada et al., 1994 |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | New: Suzukacin A 11a, 09: | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | [Ala]³ → [Vox]³ and [Ala]¹⁰ → [Ala]¹⁰ | Krause et al., 2006b |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | New: Trichocellin TC-A-V; -VII: | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | [Val]⁹ → [Lxx]⁹ and [Ala]¹⁰ → [Ala]¹⁰ | Plückthun et al., 2002 |

(Continued)
| Peptide | M | [M+Na]^+ | [M+2Na]^2+ | b_{13} | Yγ | rt-GK (min) | R | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 | R12 | R13 | R14 | R15 | R16 | R17 | R18 | R19 | R20 | Compound identical or positionally isomeric with | References |
|---------|---|----------|------------|-------|---|-------------|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|-----------------------------------------------|------------|
| Pept-A-VIIb | 1950 | 1973 | 998 | 1177 774 | 42.46 | Ac | Aib | Ala | Vxx | Ala | Aib | Ala | Glu | Aib | Vxx | Ala | Gly | Lxx | Aib | Pro | Vxx | Aib | Glu | Glu | Pheol | New: Trichoaureocin 1d: [Ala]^3 → [Vxx]^3 and [Val]^9 → [Lxx]^9 | (Brückner et al., 2002) |
|        |     |         |     |           |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | New: Longibrachin A I: [Ala]^3 → [Vxx]^3 | Lecerc et al., 1998 |
|        |     |         |     |           |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | New: Trichoaureocin 3: [Ala]^3 → [Vxx]^3 | Brückner et al., 2002 |
|        |     |         |     |           |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | New: Trichobrachin II 03: [Ala]^3 → [Vxx]^3 | Krause et al., 2007 |
|        |     |         |     |           |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | New: Trichobrachin II 05, 06 Ib A: [Ala]^3 → [Vxx]^3 | Krause et al., 2007 |
|        |     |         |     |           |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | New: Trichokin IIc: [Ala]^3 → [Vxx]^3 | Huang et al., 1996 |
|        |     |         |     |           |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | New: Trichokin III: [Ala]^3 → [Vxx]^3 | Huang et al., 1994 |
|        |     |         |     |           |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | New: Trichokin IIc: [Ala]^3 → [Vxx]^3 | Mikkola et al., 2012 |
|        |     |         |     |           |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | New: Metanicin A: [Ala]^3 → [Vxx]^3 | Kimonyo and Brückner, 2013 |
|        |     |         |     |           |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | New: Gliodeliquescin A: [Ala]^3 → [Vxx]^3 | Brückner and Przybylski, 1984 |
|        |     |         |     |           |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | New: Hypophellin 1: [Ala]^3 → [Vxx]^3 | Röhrich et al., 2013 |
| Pept-A-VIIIa | 1950 | 1973 | 998 | 1163 786 | 42.76 | Ac | Aib | Ala | Aib | Ala | Aib | Aib | Ala | Glu | Aib | Vxx | Ala | Gly | Lxx | Aib | Pro | Vxx | Aib | Glu | Glu | Pheol | New: Trichobrachin II 07, 08, 09, IIb B: [Ala]^3 → [Vxx]^3 | Leclerc et al., 1998 |
|        |     |         |     |           |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | Longibrachin A I (Position isomer of Pept-A-VIIIa and Pept-A-XVa) | Leclerc et al., 1998 |
|        |     |         |     |           |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | Suzukacin II A 10a | Krause et al., 2006b |
|        |     |         |     |           |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | Trichoaureocin 4 | Brückner et al., 2002 |
|        |     |         |     |           |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | Trichobrachin II 07, 08, 09, IIb B | Krause et al., 2007 |
|        |     |         |     |           |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | Trichokin II: [Ala]^3 → [Vxx]^3 | Huang et al., 1996 |
|        |     |         |     |           |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | Trilongin B: [Ala]^3 → [Vxx]^3 | Mikkola et al., 2012 |
|        |     |         |     |           |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | Metanicin B | Kimonyo and Brückner, 2013 |
| Pept-A-VIIIb | 1950 | 1973 | 998 | 1163 786 | 42.84 | Ac | Aib | Ala | Aib | Ala | Aib | Aib | Ala | Glu | Aib | Vxx | Ala | Gly | Lxx | Aib | Pro | Vxx | Aib | Glu | Glu | Pheol | New: Suzukacin II A 10b, 11b, 13: [Ala]^10 → [Ala]^10 | Krause et al., 2006b |
|        |     |         |     |           |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | (Continued) | |
| Peptide M | M+Na+ | M+2Na2+ | b13 | y7 | rt-GK (min) | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 | R12 | R13 | R14 | R15 | R16 | R17 | R18 | R19 | R20 | Compound identical or positionally isomeric with | References |
|----------|--------|---------|-----|----|-------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|---------------------------------|-----------|
| Pept-A-Xa | 1964 | 1987 | 1005 | 1177 | 788 | 43.28 | Ac | Aib | Ala | Aib | Ala | Aib | Ab | Gin | Aib | Vxx | Aib | Gly | Lxx | Aib | Pro | Vxx | Aib | Vxx | Gin | Pheol | New: Trichocellin TC-A-VI, TC-A-VIII: [Aib] \[10\] → [Ala] \[10\] | Wada et al., 1994 |
|          |       |        |      |     |    |       |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Trichoaureocin 6 | Brückner et al., 2002 |
|          |       |        |      |     |    |       |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Trichobrachin II 10, Ibb D | Krause et al., 2007 |
|          |       |        |      |     |    |       |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Trichobrachin IX | Huang et al., 1995 |
|          |       |        |      |     |    |       |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Trilongin BIV | Middelstaedt et al., 2012 |
|          |       |        |      |     |    |       |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Melanicin D | Kimono and Brückner, 2013 |
|          |       |        |      |     |    |       |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Hypophellin 7 | Röhrich et al., 2013 |
| Pept-A-Xb | 1964 | 1987 | 1005 | 1177 | 788 | 43.89 | Ac | Aib | Ala | Aib | Ala | Aib | Aib | Ala | Gln | Aib | Lxx | Aib | Gly | Lxx | Aib | Pro | Vxx | Aib | Vxx | Gin | Pheol | New: Trichocellin TC-A-VI, TC-A-VIII: [Aib] \[10\] → [Ala] \[10\] | Wada et al., 1994 |
|          |       |        |      |     |    |       |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Trichoaureocin 6 | Brückner et al., 2002 |
|          |       |        |      |     |    |       |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Trichobrachin II 10, Ibb D | Krause et al., 2007 |
|          |       |        |      |     |    |       |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Trichobrachin IX | Huang et al., 1995 |
|          |       |        |      |     |    |       |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Trilongin BIV | Middelstaedt et al., 2012 |
|          |       |        |      |     |    |       |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Melanicin D | Kimono and Brückner, 2013 |
|          |       |        |      |     |    |       |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Hypophellin 7 | Röhrich et al., 2013 |
| Pept-A-Xla | 1951 | 1974 | 998.5 | 1177 | 775 | 43.60 | Ac | Aib | Ala | Aib | Aib | Ala | Aib | Gln | Aib | Vxx | Aib | Gly | Lxx | Aib | Pro | Vxx | Aib | Vxx | Gin | Pheol | New: Longibrachin B II: [Aib] \[3\] → [Vxx] \[3\] | Leclerc et al., 1998 |
|          |       |        |      |     |    |       |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Trichocellin TC-A-VI, TC-A-VIII | Wada et al., 1994 |
|          |       |        |      |     |    |       |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Trichocellin TC-A-VI, TC-A-VIII | Wada et al., 1994 |
|          |       |        |      |     |    |       |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Trichocellin TC-A-VI, TC-A-VIII | Wada et al., 1994 |
|          |       |        |      |     |    |       |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Trichocellin TC-A-VI, TC-A-VIII | Wada et al., 1994 |
| Pept-A-Xlb | 1951 | 1974 | 998.5 | 1177 | 775 | 43.60 | Ac | Aib | Ala | Aib | Aib | Ala | Aib | Gln | Aib | Vxx | Aib | Gly | Lxx | Aib | Pro | Vxx | Aib | Vxx | Gin | Pheol | New: Hypophellin 2: [Aib] \[3\] → [Vxx] \[3\] | Röhrich et al., 2013 |
|          |       |        |      |     |    |       |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Trichocellin TC-A-VI, TC-A-VIII | Wada et al., 1994 |
|          |       |        |      |     |    |       |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Trichocellin TC-A-VI, TC-A-VIII | Wada et al., 1994 |
|          |       |        |      |     |    |       |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Trichocellin TC-A-VI, TC-A-VIII | Wada et al., 1994 |
|          |       |        |      |     |    |       |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Trichocellin TC-A-VI, TC-A-VIII | Wada et al., 1994 |
| Pept-A-Xlc | 1951 | 1974 | 998.5 | 1177 | 775 | 43.62 | Ac | Aib | Ala | Aib | Vxx | Aib | Aib | Gln | Aib | Lxx | Aib | Gly | Lxx | Aib | Pro | Vxx | Aib | Vxx | Gin | Pheol | New: Longibrachin B II: [Aib] \[3\] → [Vxx] \[3\], [Vxx] \[9\] → [Lxx] \[9\], and [Aib] \[10\] → [Ala] \[10\] | Tamandegani et al., 2016 |
|          |       |        |      |     |    |       |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Trichocellin TC-A-VI, TC-A-VIII | Wada et al., 1994 |
|          |       |        |      |     |    |       |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Trichocellin TC-A-VI, TC-A-VIII | Wada et al., 1994 |
|          |       |        |      |     |    |       |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Trichocellin TC-A-VI, TC-A-VIII | Wada et al., 1994 |
|          |       |        |      |     |    |       |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Trichocellin TC-A-VI, TC-A-VIII | Wada et al., 1994 |

(Continued)
# Table 2

| Peptide | M   | [M+Na]+ | [M+2Na]+ | rt-GK (min) | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 | R12 | R13 | R14 | R15 | R16 | R17 | R18 | R19 | R20 | Compound identical or positionally isomeric with | References |
|---------|-----|---------|----------|-------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|-----------------------------------------------|-----------|
| Pept-A-XII | 1907 | 1960 | 991.5 | 1163 | 775 | 42.81 | Ac | Alp | Alp | Alp | Alp | Alp | Alp | Gin | Alp | Lxx | Gin | Lxx | Alp | Pro | Vxx | Alp | Gin | Pheol | New; Trichocellin TC-B-II: [Ala]3 → [Vxx]3 and [Ala]10 → [Ala]10, and [Ala]12 → [Lxx]12 | (Positional isomer of Pept-A-Vb) | Wada et al., 1994 |
| Pept-A-XIIa | 1951 | 1974 | 998.5 | 1163 | 789 | 44.14 | Ac | Alp | Alp | Alp | Alp | Alp | Alp | Gin | Alp | Lxx | Gin | Lxx | Alp | Pro | Vxx | Alp | Gin | Pheol | Trilongin CII | Mikkola et al., 2012 |
| Pept-A-XIIb | 1951 | 1974 | 998.5 | 1163 | 789 | 44.16 | Ac | Alp | Alp | Alp | Alp | Alp | Alp | Gin | Alp | Lxx | Gin | Lxx | Alp | Pro | Vxx | Alp | Gin | Pheol | New; Longibrachin B III: [Ala]9 → [Lxx]9 and [Ala]10 → [Ala]10 | Leclerc et al., 1998 |
| Pept-A-XIVa | 1965 | 1988 | 1005.5 | 1177 | 789 | 44.22 | Ac | Alp | Alp | Alp | Alp | Alp | Alp | Alp | Gin | Alp | Lxx | Alp | Lxx | Alp | Pro | Vxx | Alp | Gin | Pheol | Pept-1966-d | Tamandegani et al., 2016 |
| Pept-A-XIVb | 1964 | 1987 | 1005 | 1177 | 788 | 44.13 | Ac | Alp | Alp | Alp | Alp | Alp | Alp | Alp | Gin | Alp | Lxx | Alp | Lxx | Alp | Pro | Vxx | Alp | Gin | Pheol | Pept-1965-c-1, c-2 (Position isomer of Pept-A-XIVa) | Tamandegani et al., 2016 |
| Pept-A-XVa | 1950 | 1973 | 998 | 1163 | 788 | 45.00 | Ac | Alp | Alp | Alp | Alp | Alp | Alp | Alp | Gin | Alp | Vxx | Alp | Vxx | Alp | Gin | Lxx | Pro | Vxx | Alp | Gin | Pheol | New; Trichosporin TS-B-Va: [Ala]10 → [Ala]10 | Iida et al., 1994 |
| Pept-A-XVb | 1964 | 1987 | 1005 | 1177 | 788 | 44.74 | Ac | Alp | Alp | Alp | Alp | Alp | Alp | Alp | Gin | Alp | Lxx | Alp | Lxx | Alp | Pro | Vxx | Alp | Gin | Pheol | Pept-1965-c-1, c-2 (Position isomer of Pept-A-XVb) | Tamandegani et al., 2016 |
| Pept-A-XVIIa | 1950 | 1973 | 998 | 1177 | 744 | 45.21 | Ac | Alp | Alp | Alp | Alp | Alp | Alp | Alp | Gin | Vxx | Alp | Vxx | Alp | Gin | Lxx | Alp | Pro | Vxx | Alp | Gin | Pheol | New; Trichosporin TS-B-Va: [Ala]10 → [Ala]10 | Iida et al., 1990 |
| Pept-A-XVIIb | 1950 | 1973 | 998 | 1177 | 744 | 45.33 | Ac | Alp | Alp | Alp | Alp | Alp | Alp | Alp | Gin | Lxx | Alp | Lxx | Alp | Pro | Vxx | Alp | Gin | Pheol | New; Trichosporin TS-B-Va: [Ala]10 → [Ala]10 | Iida et al., 1990 |

(Continued)
TABLE 2 | Continued

| Peptide M | [M+Na]+ | [M+2Na]+ | \(b_{13}\) | Y7 | rt-GK (min) | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 | R12 | R13 | R14 | R15 | R16 | R17 | R18 | R19 | R20 | Compound identical or positionally isomeric with | References |
|----------|---------|---------|---------|---|--------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pept-A- XVIIIa | 1964 1987 | 1005 | 1177 786 | 46.21 | Ac | Alb | Ala | Alb | Alb | Alb | Alb | Gln | Alb | Vxx | Alb | Gly | Lxx | Alb | Pro | Vxx | Alb | Vxx | Gln | Gln | Pheol | New: Trichoderma clavrnoviride sequence 7; [Ala]10 \(\rightarrow\) [Ala]10 | Maddau et al., 2009 |
| Pept-A- XVIIb | 1950 1973 | 998 | 1163 786 | 46.18 | Ac | Alb | Ala | Alb | Alb | Alb | Alb | Gln | Alb | Vxx | Alb | Gly | Lxx | Alb | Pro | Vxx | Alb | Vxx | Gln | Gln | Pheol | New: Pept-1965-c-1, c-2; [Vxx]17 \(\rightarrow\) [Ala]17 (Position isomer of Pept-A-Xa, Pept-A-XIVb, Pept-A-XVIIIb, and Pept-XX(Va)) | Tamandegani et al., 2016 |
| Pept-A- XVII | 1978 2001 | 1012 | 1191 786 | 46.36 | Ac | Alb | Alb | Vxx | Alb | Alb | Alb | Gln | Alb | Vxx | Alb | Gly | Lxx | Alb | Pro | Vxx | Alb | Vxx | Gln | Gln | Pheol | New: Pept-A-XVIIb (Position isomer of Pept-A-XIIa and Pept-A-Ka) | Pept-A-Ka |
| Pept-A- XIXa | 1951 1974 | 998.5 | 1177 775 | 46.67 | Ac | Alb | Ala | Alb | Alb | Alb | Alb | Alb | Gln | Alb | Vxx | Alb | Gly | Lxx | Alb | Pro | Vxx | Alb | Gln | Glu | Pheol | New: Trichosporin TS-B-V: [Ala]10 \(\rightarrow\) [Ala]10 and [Gln]18 \(\rightarrow\) [Glu]18 | Iida et al., 1990 |
| Pept-A- XIXb | 1951 1974 | 998.5 | 1177 775 | 46.86 | Ac | Alb | Alb | Alb | Alb | Alb | Alb | Glu | Alb | Vxx | Alb | Gly | Lxx | Alb | Alp | Pro | Vxx | Alb | Gln | Glu | Pheol | New: Hypophellin 7; [Alb]3 \(\rightarrow\) [Vxx]3 (Positional isomer of Pept-A-XIXb) | Iida et al., 1990 |
| Pept-A- XX | 1964 1987 | 1005 | 1191 774 | 47.30 | Ac | Alb | Ala | Vxx | Alb | Alb | Alb | Gln | Alb | Vxx | Alb | Gly | Lxx | Alb | Pro | Vxx | Alb | Gln | Glu | Pheol | New: Trichosporin TS-B-Vla: [Alb]10 \(\rightarrow\) [Ala]10 and [Gln]18 \(\rightarrow\) [Glu]18 and Trichosporin TS-B-Vca: [Alb]3 \(\rightarrow\) [Vxx]3 (Position isomer of Pept-A-XIIa) | Maddau et al., 2009 |
| | | | | | | | | | | | | | | | | | | | | | | | | | | New: Trichosporin TS-B-Vla: [Alb]10 \(\rightarrow\) [Ala]10 and [Gln]18 \(\rightarrow\) [Glu]18 | Tamandegani et al., 2016 |
| | | | | | | | | | | | | | | | | | | | | | | | | | | New: Pept-1965-c-1, c-2; [Vxx]17 \(\rightarrow\) [Ala]17 (Position isomer of Pept-A-Xa, Pept-A-XIVb, Pept-A-XVIIIb, and Pept-XX(Va)) | Tamandegani et al., 2016 |
| | | | | | | | | | | | | | | | | | | | | | | | | | | New: Pept-1965-c-1, c-2; [Vxx]17 \(\rightarrow\) [Ala]17 and [Gln]18 \(\rightarrow\) [Glu]18 | Iida et al., 1990 |
| | | | | | | | | | | | | | | | | | | | | | | | | | | New: Trichosporin TS-B-Vca: [Alb]3 \(\rightarrow\) [Vxx]3 (Position isomer of Pept-A-XIIa) | Iida et al., 1990 |

(Continued)
### Table 2 Continued

| Peptide | M | [M+Na]+ | [M+2Na]2+ | b13 | y7 | rt-GK (min) | R | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 | R12 | R13 | R14 | R15 | R16 | R17 | R18 | R19 | R20 | Compound identical or positionally isomeric with | References |
|---------|---|---------|-----------|-----|----|------------|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|---|
| Pept-A-XXla | 1964 | 1987 | 1005 | 1177 | 788 | 47.85 | Ac | I | Aib | Ala | Aib | Aib | Aib | Aib | Gln | Aib | Vxx | Ala | Pro | Vxx | Aib | Vxx | Gin | Gin | Peheol | Trichoderma citrinoviride sequence 7 |
| Pept-A-XXlb | 1964 | 1987 | 1005 | 1177 | 788 | 47.75 | Ac | I | Aib | Ala | Aib | Aib | Aib | Aib | Gln | Aib | Lxx | Ala | Gly | Lxx | Aib | Pro | Vxx | Aib | Vxx | Gin | Gin | Peheol | Trichoderma citrinoviride sequence 7 |
| Pept-A-XXllla | 1964 | 1987 | 1005 | 1191 | 774 | 48.93 | Ac | I | Aib | Ala | Vxx | Ala | Aib | Aib | Gln | Aib | Vxx | Aib | Pro | Vxx | Aib | Vxx | Gin | Gin | Peheol | Trichoderma citrinoviride sequence 7 |
| Pept-A-XXlllb | 1964 | 1987 | 1005 | 1191 | 774 | 48.79 | Ac | I | Aib | Ala | Ala | Aib | Aib | Aib | Gln | Aib | Lxx | Ala | Gly | Lxx | Aib | Pro | Vxx | Aib | Vxx | Gin | Gin | Peheol | Trichoderma citrinoviride sequence 7 |
| Pept-A-XXlll | 1965 | 1988 | 1005.5 | 1177 | 789 | 49.13 | Ac | I | Aib | Ala | Aib | Aib | Aib | Aib | Gln | Aib | Vxx | Ala | Pro | Vxx | Aib | Vxx | Gin | Gin | Peheol | Trichoderma citrinoviride sequence 7 |
| Pept-A-XXIV | 1978 | 2001 | 1012 | 1191 | 788 | 49.89 | Ac | I | Aib | Ala | Vxx | Ala | Aib | Aib | Gln | Aib | Vxx | Aib | Pro | Vxx | Aib | Vxx | Gin | Gin | Peheol | Trichoderma citrinoviride sequence 7 |
| Pept-A-XXVa | 1964 | 1987 | 1005 | 1177 | 786 | 49.65 | Ac | I | Aib | Ala | Aib | Aib | Aib | Aib | Gln | Aib | Vxx | Aib | Pro | Vxx | Aib | Vxx | Gin | Gin | Peheol | Trichoderma citrinoviride sequence 7 |
| Pept-A-XXVfa | 1978 | 2001 | 1012 | 1191 | 786 | 51.29 | Ac | I | Aib | Ala | Aib | Aib | Aib | Aib | Gln | Aib | Vxx | Aib | Pro | Vxx | Aib | Vxx | Gin | Gin | Peheol | Trichoderma citrinoviride sequence 7 |
| Pept-A-XXVib | 1978 | 2001 | 1012 | 1191 | 786 | 50.85 | Ac | I | Aib | Ala | Aib | Aib | Aib | Aib | Gln | Aib | Vxx | Aib | Pro | Vxx | Aib | Vxx | Gin | Gin | Peheol | Trichoderma citrinoviride sequence 7 |
| Pept-A-XXVla | 1965 | 1988 | 1005.5 | 1177 | 789 | 51.44 | Ac | I | Aib | Ala | Aib | Aib | Aib | Aib | Gln | Aib | Vxx | Ala | Pro | Vxx | Aib | Vxx | Gin | Gin | Peheol | Trichoderma citrinoviride sequence 7 |
| Pept-A-XXVlb | 1978 | 2001 | 1012 | 1191 | 786 | 51.59 | Ac | I | Aib | Ala | Aib | Aib | Aib | Aib | Gln | Aib | Vxx | Aib | Pro | Vxx | Aib | Vxx | Gin | Gin | Peheol | Trichoderma citrinoviride sequence 7 |

Variable residues are UNDERLINED in the table header; minor sequence variants are UNDERLINED in the sequences. Amino acid exchanges in new compounds are set in italic.
### TABLE 3 | Sequences of the newly identified group B peptaibol compounds from *Trichoderma* species of the Longibrachiatum Clade and their similarities to known peptaibols available in the “Comprehensive Peptaibiotics Database.”

| Peptide | M | [M+Na]+ | [M+2Na]2+ | b13 | y_7 | r1-GK (min) | R | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 | R12 | R13 | R14 | R15 | R16 | R17 | R18 | R19 | R20 | Compound identical or positionally isomer with | References |
|---------|---|---------|-----------|-----|-----|------------|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pept-B-I | 1908 | 1931 | 977 | 1135 | 774 | 22.59 | Ac | Ala | Ala | Ala | Ala | Ala | Ala | Gin | Ala | Lox | Ala | Gly | Ala | Ab | Pro | Vxx | Ala | Ab | Gin | Gin | Pheol | New:  | Pept-B-II, III, and V  |
| Pept-B-II | 1908 | 1931 | 977 | 1135 | 774 | 24.79 | Ac | Ala | Ala | Ala | Ala | Ala | Ala | Gin | Ala | Lox | Ala | Gly | Ala | Ab | Pro | Vxx | Ala | Ab | Gin | Gin | Pheol | New: Paracelsin B:  | [Ala]3 → [Ala]3  |
| Pept-B-III | 1908 | 1931 | 977 | 1135 | 774 | 25.62 | Ac | Ala | Ala | Ala | Ala | Ala | Ala | Gin | Ala | Lox | Ala | Gly | Ala | Ab | Pro | Vxx | Ala | Ab | Gin | Gin | Pheol | New:  | Saturnisporin SA I:  |
| Pept-B-IV | 1922 | 1945 | 984 | 1135 | 788 | 25.72 | Ac | Ala | Ala | Ala | Ala | Ala | Ala | Gin | Ala | Lox | Ala | Gly | Ala | Ab | Pro | Vxx | Ala | Ab | Gin | Gin | Pheol | New:  | Saturnisporin SA II:  |
| Pept-B-V | 1908 | 1931 | 977 | 1135 | 774 | 26.35 | Ac | Ala | Ala | Ala | Ala | Ala | Ala | Gin | Ala | Lox | Ala | Gly | Ala | Ab | Pro | Vxx | Ala | Ab | Gin | Gin | Pheol | New:  | Suzukacillin A 02, A 06:  |
| Pept-B-VI | 1922 | 1945 | 984 | 1149 | 774 | 27.22 | Ac | Ala | Ala | Ala | Ala | Ala | Ala | Gin | Ala | Lox | Ala | Gly | Ala | Ab | Pro | Vxx | Ala | Ab | Gin | Gin | Pheol | New:  | Trichocellin TC-A-I, TC-A-III:  |
| Pept-B-VII | 1922 | 1945 | 984 | 1135 | 788 | 27.80 | Ac | Ala | Ala | Ala | Ala | Ala | Ala | Gin | Ala | Lox | Ala | Gly | Ala | Ab | Pro | Vxx | Ala | Ab | Gin | Gin | Pheol | New:  | Saturnisporin SA II:  |
| Pept-B-VIII | 1908 | 1931 | 977 | 1135 | 774 | 27.80 | Ac | Ala | Ala | Ala | Ala | Ala | Ala | Gin | Ala | Lox | Ala | Gly | Ala | Ab | Pro | Vxx | Ala | Ab | Gin | Gin | Pheol | New:  | Saturnisporin SA II:  |

(Continued)
| Peptide | M  | [M+Na]⁺ | [M+2Na]²⁺ | b₁₃ | Y₇ | r-t-GK (min) | R  | R₁  | R₂  | R₃  | R₄  | R₅  | R₆  | R₇  | R₈  | R₉  | R₁₀ | R₁₁ | R₁₂ | R₁₃ | R₁₄ | R₁₅ | R₁₆ | R₁₇ | R₁₈ | R₁₉ | R₂₀ | Compounds identical or positionally isomorphic with | References |
|---------|----|---------|------------|-----|----|--------------|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----------|-------------|
| Pept-B-IXa | 1908 | 1931 | 977 | 1135 | 774 | 28.44 | Ac | Aib | Ala | Aib | Ala | Aib | Aib | Gln | Aib | Vxx | Aib | Gly | Aib | Pro | Vxx | Aib | Aib | Gln | Gln | Pept-B-XI | References |
| Pept-B-Xb | 1908 | 1931 | 977 | 1135 | 774 | 28.38 | Ac | Aib | Ala | Aib | Aib | Aib | Ala | Gln | Aib | Vxx | Aib | Lxx | Aib | Gly | Aib | Pro | Vxx | Aib | Aib | Gln | Gln | Pept-B-XII | References |
| Pept-B-X | 1922 | 1945 | 984 | 1135 | 788 | 28.77 | Ac | Aib | Ala | Aib | Aib | Aib | Aib | Gln | Aib | Vxx | Aib | Gly | Aib | Pro | Vxx | Aib | Aib | Gln | Gln | Pept-B-XIII | References |
| Pept-B-XI | 1922 | 1945 | 984 | 1135 | 788 | 29.25 | Ac | Aib | Ala | Aib | Aib | Aib | Aib | Gln | Aib | Vxx | Aib | Gly | Aib | Pro | Vxx | Aib | Aib | Gln | Gln | Pept-B-XIV | References |
| Pept-B-XII | 1922 | 1945 | 984 | 1149 | 774 | 29.90 | Ac | Aib | Ala | Aib | Aib | Aib | Aib | Gln | Aib | Lxx | Aib | Gly | Aib | Pro | Vxx | Aib | Aib | Gln | Gln | Pept-B-XV | References |
| Pept-B-XIII | 1922 | 1946 | 984.5 | 1149 | 775 | 30.28 | Ac | Aib | Ala | Aib | Aib | Aib | Aib | Gln | Aib | Lxx | Aib | Gly | Aib | Pro | Vxx | Aib | Aib | Gln | Gln | Pept-B-XIV | References |
| Pept-B-XIVa | 1923 | 1946 | 984.5 | 1149 | 775 | 31.36 | Ac | Aib | Ala | Aib | Aib | Aib | Aib | Gln | Aib | Vxx | Aib | Gly | Aib | Pro | Vxx | Aib | Aib | Gln | Gln | Pept-B-XIVb | References |
| Pept-B-XIVb | 1923 | 1946 | 984.5 | 1149 | 775 | 31.40 | Ac | Aib | Ala | Aib | Aib | Aib | Aib | Gln | Aib | Vxx | Aib | Gly | Aib | Pro | Vxx | Aib | Aib | Gln | Gln | Pept-B-XV | References |
| Pept-B-V | 1922 | 1945 | 984 | 1135 | 788 | 31.48 | Ac | Aib | Ala | Aib | Aib | Aib | Aib | Gln | Aib | Vxx | Aib | Gly | Aib | Pro | Vxx | Aib | Aib | Gln | Gln | Pept-B-VI | References |
| Pept-B-Vb | 1922 | 1945 | 984 | 1135 | 788 | 31.53 | Ac | Aib | Ala | Aib | Aib | Aib | Aib | Gln | Aib | Vxx | Aib | Gly | Aib | Pro | Vxx | Aib | Aib | Gln | Gln | Pept-B-VII | References |
| Pept-B-VI | 1922 | 1945 | 984 | 1149 | 774 | 31.98 | Ac | Aib | Ala | Aib | Aib | Aib | Aib | Gln | Aib | Vxx | Aib | Gly | Aib | Pro | Vxx | Aib | Aib | Gln | Gln | Pept-B-VIII | References |
| Pept-B-VII | 1936 | 1959 | 991 | 1149 | 774 | 32.67 | Ac | Aib | Ala | Aib | Aib | Aib | Aib | Gln | Aib | Vxx | Aib | Gly | Aib | Pro | Vxx | Aib | Aib | Gln | Gln | Pept-B-IX | References |
| Pept-B-VIII | 1936 | 1959 | 991 | 1149 | 774 | 33.49 | Ac | Aib | Ala | Aib | Aib | Aib | Aib | Gln | Aib | Lxx | Aib | Gly | Aib | Pro | Vxx | Aib | Aib | Gln | Gln | Pept-B-X | References |
| Pept-B-IX | 1936 | 1959 | 991 | 1163 | 774 | 33.55 | Ac | Aib | Ala | Aib | Aib | Aib | Aib | Gln | Aib | Lxx | Aib | Gly | Aib | Pro | Vxx | Aib | Aib | Gln | Gln | Pept-B-Xb | References |
| Pept-B-Xb | 1936 | 1959 | 991 | 1163 | 774 | 34.41 | Ac | Aib | Ala | Aib | Aib | Aib | Aib | Gln | Aib | Lxx | Aib | Gly | Aib | Pro | Vxx | Aib | Aib | Gln | Gln | Pept-B-XII | References |

Table 3 Continued...
| Peptide | M  | [M+Na]+ | [M+2Na]+ | b13 | y7 | r-tGK (min) | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 | R12 | R13 | R14 | R15 | R16 | R17 | R18 | R19 | R20 | Compound identical or positionally isomeric with | References |
|---------|----|----------|----------|-----|----|-------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|-----------------------------------------------|------------|
| Pept-B-XX | 1907 | 1960 | 991.5 | 1149 | 789 | 34.15 | Ac | Ala | Ala | Ala | Ala | Ala | Gln | Ala | Lxx | Ala | Gly | Ala | Ala | Pro | Vxx | Ala | Vxx | Gln | Gln | Psychocellin TC-B-II | Wada et al., 1994 |
| Pept-B-XXII | 1906 | 1959 | 991 | 1149 | 788 | 34.59 | Ac | Ala | Ala | Ala | Ala | Ala | Ala | Gln | Ala | Lxx | Ala | Gly | Ala | Ala | Pro | Vxx | Ala | Vxx | Gln | Gln | Pept-B-VIII | Pept-B-XXII |
| Pept-B-XXIII | 1922 | 1945 | 984 | 1149 | 774 | 35.25 | Ac | Ala | Ala | Ala | Ala | Ala | Ala | Ala | Gln | Ala | Lxx | Ala | Gly | Ala | Ala | Pro | Vxx | Ala | Vxx | Gln | Gln | Saturnisporin SA IV | Pept-B-XXIII |
| Pept-B-XXIV | 1950 | 1973 | 998 | 1163 | 788 | 35.97 | Ac | Ala | Ala | Ala | Ala | Ala | Ala | Ala | Ala | Gln | Ala | Lxx | Ala | Gly | Ala | Ala | Pro | Vxx | Ala | Vxx | Gln | Gln | New: | Pept-B-XXIV |
| Pept-B-XXV | 1907 | 1960 | 991.5 | 1163 | 775 | 35.97 | Ac | Ala | Ala | Ala | Ala | Ala | Ala | Ala | Ala | Ala | Gln | Ala | Lxx | Ala | Gly | Ala | Ala | Pro | Vxx | Ala | Vxx | Gln | Gln | New: | Pept-B-XXV |
| Pept-B-XXVI | 1950 | 1973 | 998 | 1177 | 774 | 36.65 | Ac | Ala | Ala | Vxx | Ala | Ala | Ala | Ala | Gln | Ala | Lxx | Ala | Gly | Ala | Ala | Pro | Vxx | Ala | Vxx | Gln | Gln | New: | Pept-B-XXVI |
| Pept-B-XXVII | 1950 | 1973 | 998 | 1163 | 788 | 37.31 | Ac | Ala | Ala | Ala | Ala | Ala | Ala | Ala | Ala | Ala | Ala | Gln | Ala | Lxx | Ala | Gly | Ala | Ala | Pro | Vxx | Ala | Vxx | Gln | Gln | New: | Pept-B-XXVII |
| Pept-B-XXVIII | 1950 | 1973 | 998 | 1177 | 774 | 38.30 | Ac | Ala | Ala | Ala | Ala | Ala | Ala | Ala | Ala | Ala | Ala | Ala | Gln | Ala | Lxx | Ala | Gly | Ala | Ala | Pro | Vxx | Ala | Vxx | Gln | Gln | New: | Pept-B-XXVIII |

(Continued)
| Peptide | M | [M+Na]^+ | [M+2Na]^2+ | b13 | Y7 | rt-GK (min) | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 | R12 | R13 | R14 | R15 | R16 | R17 | R18 | R19 | R20 | Compound identical or positionally isomeric with | References |
|---------|---|----------|------------|-----|-----|------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|--------------------------|----------|
| Pept-B-XXIXb | 1906 | 1959 | 991 | 1149 | 788 | 37.80 | Ac | Alb | Ala | Alb | Alb | Alb | Alb | Gin | Alb | Lxx | Alb | Gly | Alb | Alb | Pro | Vxx | Alb | Vxx | Gin | Gin | Pept-B-VII, VIII, XXIV, and XXV | Maddau et al., 2009 |
| Pept-B-XXX | 1950 | 1973 | 998 | 1177 | 774 | 38.51 | Ac | Alb | Alb | Alb | Alb | Alb | Gin | Gin | Lxx | Alb | Gly | Vxx | Alb | Pro | Vxx | Alb | Gin | Gin | Pept-B-VII, VIII, XXIV, and XXV | Trichoderma citrinoviride sequence 4 (Positional isomer of Pept-B-XXXb, XLIXa, XLIXb, and LII) |
| Pept-B-XXIXl | 1951 | 1974 | 998.5 | 1163 | 789 | 39.13 | Ac | Alb | Alb | Alb | Alb | Gin | Lxx | Alb | Lxx | Alb | Gly | Vxx | Alb | Pro | Vxx | Alb | Gin | Gin | Pept-B-XXIXb and LII | New: Trichotecin TC-B-II: [Ala]6 → [Aib]6 (Positional isomer of Pept-B-XXXIVb and LII) |
| Pept-B-XXXIIa | 1950 | 1973 | 998 | 1163 | 788 | 39.15 | Ac | Alb | Alb | Alb | Alb | Alb | Gin | Lxx | Alb | Lxx | Alb | Gly | Vxx | Alb | Pro | Vxx | Alb | Gin | Gin | Pept-B-XXIV | New: Paracelsin H: [Aib]6 → [Vxx]6 (Positional isomer of Pept-B-XLIIb) |
| Pept-B-XXXIIb | 1964 | 1987 | 1005 | 1177 | 788 | 39.20 | Ac | Alb | Alb | Alb | Alb | Vxx | Gin | Lxx | Alb | Lxx | Alb | Gly | Vxx | Alb | Pro | Vxx | Alb | Gin | Gin | Pept-B-XXIV | New: Saturnisporin SA II: [Ala]6 → [Vxx]6 |
| Pept-B-XXXIIla | 1906 | 1959 | 991 | 1163 | 774 | 38.98 | Ac | Alb | Alb | Alb | Alb | Alb | Gin | Alb | Lxx | Alb | Gly | Alb | Alb | Pro | Vxx | Alb | Gin | Gin | Pept-B-XXIXb and LII | New: Paracelsin H: [Ala]6 → [Vxx]6 (Positional isomer of Pept-B-XXXIIa) |
| Pept-B-XXXIIlb | 1906 | 1959 | 991 | 1163 | 774 | 39.25 | Ac | Alb | Alb | Alb | Alb | Alb | Gin | Alb | Lxx | Alb | Gly | Vxx | Alb | Pro | Vxx | Alb | Gin | Gin | Pept-B-XXIXb and LII | New: Saturnisporin SA II: [Ala]6 → [Vxx]6 |
| Pept-B-XXXIIlc | 1950 | 1973 | 998 | 1177 | 774 | 39.20 | Ac | Alb | Alb | Alb | Alb | Gin | Gin | Lxx | Alb | Lxx | Alb | Gly | Vxx | Alb | Pro | Vxx | Alb | Gin | Gin | Pept-B-XXIIIb | New: Paracelsin H: [Aib]6 → [Vxx]6 (Positional isomer of Pept-B-XXXIIa) |
| Pept-B-XXXIIIa | 1950 | 1973 | 998 | 1163 | 788 | 39.15 | Ac | Alb | Alb | Alb | Alb | Gin | Lxx | Alb | Lxx | Alb | Gly | Vxx | Alb | Pro | Vxx | Alb | Gin | Gin | Pept-B-XXIIIb | New: Saturnisporin SA II: [Ala]6 → [Vxx]6 (Positional isomer of Pept-B-XXXIIIa) |
| Pept-B-XXXIIIb | 1951 | 1974 | 998.5 | 1163 | 789 | 39.13 | Ac | Alb | Alb | Alb | Gin | Lxx | Alb | Lxx | Alb | Gly | Vxx | Alb | Pro | Vxx | Alb | Gin | Gin | Pept-B-XXIIIb and LII | New: Saturnisporin SA II: [Ala]6 → [Vxx]6 (Positional isomer of Pept-B-XXXIIIb) |
| Peptide     | M [M+Na]+ | M [M+2Na]2+ | b13 | y7  | rt-GK(min) | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 | R12 | R13 | R14 | R15 | R16 | R17 | R18 | R19 | R20 | Compound identical or positionally isomeric with | References |
|------------|-----------|-------------|-----|-----|-----------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|---------------------------------|-----------|
| Pept-B-XXVa | 1907 1960 | 991.5       | 1149| 789 | 39.59     | Ac | Ab | Ala | Ab | Ab | Lxx | Ala | Gly | Ab | Ab | Pro | Vxx | Ab | Vxx | Gln | Gln | Pheol | New: Suzukacillin A 05: [Aib]3 → [Vxx]3 and [Aib]5 → [Glu]5 | Krause et al., 2006b |
| Pept-B-XXVb | 1951 1974 | 998.5       | 1163| 789 | 39.13     | Ac | Ab | Ala | Ab | Ab | Lxx | Ab | Gly | Ab | Ab | Pro | Vxx | Ab | Vxx | Gln | Pheol | New: Pept-1966-d: [Lxx]12 → [Aib]12 | Krause et al., 2006b |
| Pept-B-XXVIA | 1906 1959 | 991         | 1163| 774 | 39.17     | Ac | Ab | Ala | Ab | Ab | Lxx | Ala | Gly | Ab | Ab | Pro | Vxx | Ab | Vxx | Gln | Pheol | New: (Positional isomer of Pept-B-XXI and LII) | Krause et al., 2006b |
| Pept-B-XXVIB | 1906 1959 | 991         | 1163| 774 | 39.78     | Ac | Ab | Ala | Ab | Ab | Ala | Lxx | Ala | Gly | Ab | Ab | Pro | Vxx | Ab | Vxx | Gln | Pheol | New: Pept-B-XXXI | Krause et al., 2006b |
| Pept-B-XXXVI | 1964 1987 | 1005        | 1191| 774 | 39.85     | Ac | Ab | Ala | Vxx | Ab | Vxx | Lxx | Ala | Gly | Ab | Ab | Pro | Vxx | Ab | Vxx | Gln | Gln | Pheol | New: (Positional isomer of Pept-B-XXVII) | Krause et al., 2006b |
| Pept-B-XXVII | 1907 1960 | 991.5       | 1163| 775 | 40.54     | Ac | Ab | Ala | Ab | Ab | Ab | Lxx | Ab | Gly | Ab | Ab | Pro | Vxx | Ab | Gln | Gln | Pheol | New: Trichoderma citrinoviride sequence 5: [Aib]6 → [Aib]6 | Maddau et al., 2009 |
| Pept-B-XXVIII | 1950 1973 | 998         | 1163| 788 | 40.13     | Ac | Ab | Ala | Ab | Ab | Ab | Lxx | Ab | Gly | Ab | Ab | Pro | Vxx | Ab | Vxx | Gln | Pheol | New: Saturnisporin SA IV: [Aib]5 → [Vxx]5 | Maddau et al., 2009 |
| Pept-B-XXXIX | 1964 1987 | 1005        | 1177| 788 | 39.57     | Ac | Ab | Ala | Ab | Vxx | Ab | Lxx | Ab | Gly | Ab | Ab | Vxx | Pro | Vxx | Gln | Pheol | New: Trichoderma citrinoviride sequence 8: [Aib]6 → [Aib]6 | Maddau et al., 2009 |
| Pept-B-XIX | 1950 1973 | 998         | 1163| 788 | 40.55     | Ac | Ab | Ala | Ab | Ala | Ab | Lxx | Ab | Gly | Ab | Vxx | Pro | Vxx | Gln | Pheol | New: Trichoderma citrinoviride sequence 6: [Aib]6 → [Aib]6 | Maddau et al., 2009 |
| Pept-B-XLIa | 1964 1987 | 1005        | 1177| 788 | 40.98     | Ac | Ab | Ala | Ab | Ab | Ab | Lxx | Ab | Gly | Ab | Vxx | Pro | Vxx | Gln | Pheol | New: Trichoderma citrinoviride sequence 6: [Aib]6 → [Aib]6 | Maddau et al., 2009 |

(Continued)
TABLE 3 | Continued

| Peptide   | M     | [M+Na]+ (min) | [M+2Na]+ | b13 | Y7 | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 | R12 | R13 | R14 | R15 | R16 | R17 | R18 | R19 | R20 | References | References |
|-----------|-------|---------------|-----------|-----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|---|---|
| Pept-B- XLII | 1964 1987 | 1005 | 1177 | 788 | 40.82 | Ac | Ala | Ala | Ala | Ala | Ala | Vxx | Gin | Ala | Lxx | Ala | Gly | Ala | Ala | Pro | Vxx | Ala | Vxx | Gin | Gin | Pept-B- XXXIIb | Trichoderma citrinoviride sequence B |
| Pept-B- XLIIa | 1950 1973 | 998 | 1177 | 774 | 41.54 | Ac | Ala | Ala | Ala | Ala | Ala | Gin | Lxx | Ala | Gly | Vxx | Pro | Vxx | Ala | Gin | Pept-B- XXXIIb | Trichoderma citrinoviride sequence B |
| Pept-B- XLIIb | 1906 1959 | 991 | 1163 | 774 | 41.64 | Ac | Ala | Ala | Ala | Ala | Ala | Gin | Lxx | Ala | Gly | Vxx | Pro | Vxx | Ala | Gin | Pept-B- XXXIIb | Trichoderma citrinoviride sequence B |
| Pept-B- XLIIc | 1964 1987 | 1005 | 1191 | 774 | 41.26 | Ac | Ala | Ala | Ala | Ala | Vxx | Vxx | Gin | Ala | Lxx | Ala | Gly | Vxx | Pro | Vxx | Ala | Gin | Pept-B- XXXIIb | Trichoderma citrinoviride sequence B |
| Pept-B- XLIIIa | 1965 1988 | 1015.5 | 1191 | 775 | 41.65 | Ac | Ala | Ala | Ala | Vxx | Vxx | Gin | Ala | Lxx | Ala | Gly | Vxx | Pro | Vxx | Ala | Gin | Pept-B- XXXIIb | Trichoderma citrinoviride sequence B |
| Pept-B- XLIIb | 1950 1973 | 998 | 1163 | 788 | 41.92 | Ac | Ala | Ala | Ala | Ala | Ala | Gin | Lxx | Ala | Gly | Vxx | Pro | Vxx | Ala | Gin | Pept-B- XXXIIb | Trichoderma citrinoviride sequence B |
| Pept-B- XLIIc | 1978 2001 | 1012 | 1191 | 788 | 42.47 | Ac | Ala | Vxx | Ala | Vxx | Gin | Ala | Lxx | Ala | Gly | Vxx | Pro | Vxx | Ala | Gin | Pept-B- XXXIIb | Trichoderma citrinoviride sequence B |

(Continued)
### TABLE 3 | Continued

| Peptide     | M   | [M+Na]⁺ | [M+2Na]²⁺ | b₁₁ | y₇ | nh-GK (min) | R₁ | R₂ | R₃ | R₄ | R₅ | R₆ | R₇ | R₈ | R₉ | R₁₀ | R₁₁ | R₁₂ | R₁₃ | R₁₄ | R₁₅ | R₁₆ | R₁₇ | R₁₈ | R₁₉ | R₂₀ | Compound identical or positionally isomeric with References |
|-------------|-----|---------|-----------|-----|----|-------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|---
| Peptide | M    | $\text{[M+Na]}^+$ | $\text{[M+2Na]}^{2+}$ | $b_{13}$ | $y_7$ | t-GK (min) | R   | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 | R12 | R13 | R14 | R15 | R16 | R17 | R18 | R19 | R20 | Compound identical or positionally isomorphic with | References |
|---------|------|--------------------|------------------------|---------|------|------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|---------------------------------------------|------------|
| Pept-B-| 1964 | 1987               | 1005                   | 1177    | 788  | 44.44      | Ac  | Alb | Ala | Ala | Ala | Gln | Alb | Gly | Alb | Alb | Pro | Vxx | Alb | Vxx | Glu | Gln | Gln | Peptol | New: Suzukacillin A 02, A 06: [Aib]$^3 \rightarrow [Vxx]^3$, [Ala]$^6 \rightarrow [Vxx]^6$ | Krause et al., 2006b |
| B-LVII |      |                    |                        |         |      |            |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | Pept-B-XXXI and XXXIVb | New: Suzukacillin A 05: [Aib]$^3 \rightarrow [Vxx]^3$, [Ala]$^6 \rightarrow [Vxx]^6$ | Krause et al., 2006b |
| Pept-B-| 1950 | 1973               | 998                    | 1177    | 774  | 43.99      | Ac  | Alb | Ala | Ala | Ala | Gln | Alb | Gly | Vxx | Alb | Pro | Vxx | Alb | Gln | Gln | Peptol | New: Paracelsin H: [Aib]$^5 \rightarrow [Vxx]^5$ and [Ala]$^6 \rightarrow [Vxx]^6$ | Pócsfalvi et al., 1997 |
| B-LIII |      |                    |                        |         |      |            |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | Pept-B-XXX | Pept-B-XXXI | New: Saturnisporin SA II: [Ala]$^5 \rightarrow [Vxx]^5$ and [Aib]$^6 \rightarrow [Vxx]^6$ | Rebuffat et al., 1993 |
| Pept-B-| 1978 | 2001               | 1012                   | 1191    | 788  | 44.00      | Ac  | Alb | Ala | Ala | Ala | Vxx | Vxx | Glu | Gln | Alb | Alb | Gln | Gln | Peptol | New: Paracelsin H: [Aib]$^5 \rightarrow [Vxx]^5$ and [Ala]$^6 \rightarrow [Vxx]^6$ | Pócsfalvi et al., 1997 |
| B-LIV  |      |                    |                        |         |      |            |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | Pept-B-XLIa | Pept-B-XXXI | New: Saturnisporin SA N: [Ala]$^5 \rightarrow [Vxx]^5$ and [Aib]$^6 \rightarrow [Vxx]^6$ | Rebuffat et al., 1993 |
| Pept-B-| 1964 | 1987               | 1005                   | 1177    | 788  | 44.44      | Ac  | Alb | Ala | Ala | Ala | Gln | Alb | Gly | Vxx | Alb | Pro | Vxx | Alb | Vxx | Glu | Gln | Gln | Peptol | New: Saturnisporin SA IV: [Ala]$^5 \rightarrow [Vxx]^5$ and [Aib]$^6 \rightarrow [Vxx]^6$ | Krause et al., 2006b |
| B-LVII |      |                    |                        |         |      |            |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | Pept-B-XXXI and XXXIVb | New: Suzukacillin A 04, 08: [Aib]$^5 \rightarrow [Vxx]^5$ and [Ala]$^6 \rightarrow [Vxx]^6$ | Krause et al., 2006b |
| Pept-B-| 1950 | 1973               | 998                    | 1177    | 774  | 43.99      | Ac  | Alb | Ala | Ala | Ala | Alg | Alb | Gln | Alb | Gly | Vxx | Alb | Pro | Vxx | Alb | Gln | Gln | Peptol | New: Saturnisporin SA IV: [Ala]$^5 \rightarrow [Vxx]^5$ and [Aib]$^6 \rightarrow [Vxx]^6$ | Krause et al., 2006b |
| B-LVII |      |                    |                        |         |      |            |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | Pept-B-XXXI and XXXIVb | New: Trichocellin TC-B-I, II: [Ala]$^5 \rightarrow [Vxx]^5$ and [Aib]$^6 \rightarrow [Vxx]^6$ | Wada et al., 1994 |
| Pept-B-| 1936 | 1959               | 991                    | 1163    | 774  | 45.81      | Ac  | Alb | Ala | Ala | Ala | Gln | Alb | Gly | Alb | Alb | Pro | Vxx | Alb | Gln | Gln | Peptol | New: Trichocellin TC-B-I, II: [Ala]$^5 \rightarrow [Vxx]^5$ and [Aib]$^6 \rightarrow [Vxx]^6$ | Wada et al., 1994 |

(Continued)
### TABLE 3 | Continued

| Peptide | M | [M+Na]+ | [M+2Na]+ | b13 | Y7 | rt-GK (min) | R | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 | R12 | R13 | R14 | R15 | R16 | R17 | R18 | R19 | R20 | References |
|---------|---|---------|---------|-----|----|----------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|------------------|
| Pept-B-LIX | 1992 | 2015 | 1019 | 1205 | 788 | 45.74 | Ac | Ala | Ala | Lox | Ala | Gly | Ala | Pro | Ala | Vxx | Gln | Gln | Pheol | New: Peptacin H: [Aib]3 → [Vxx]3, [Aib]5 → [Vxx]5, and [Aib]6 → [Vxx]6  | Pócsfalvi et al., 1997 |
| Pept-B-LXa | 1964 | 1987 | 1005 | 1177 | 788 | 46.50 | Ac | Ala | Ala | Ala | Ala | Ala | Ala | Gln | Ala | Vxx | Ala | Gly | Lox | Ala | Pro | Vxx | Ala | Vxx | Gln | Gln | Pheol | New: Suzukacillin A04, B: [Aib]5 → [Vxx]5, [Aib]7 → [Vxx]7, and [Aib]8 → [Vxx]8 | Krause et al., 2006b |
| Pept-B-LXb | 1964 | 1987 | 1005 | 1177 | 788 | 46.29 | Ac | Ala | Ala | Ala | Ala | Ala | Ala | Gln | Ala | Lox | Ala | Gly | Vxx | Ala | Pro | Vxx | Ala | Vxx | Gln | Gln | Pheol | New: Suzukacillin A 07: [Aib]3 → [Vxx]3, [Aib]5 → [Vxx]5, and [Aib]6 → [Vxx]6 | Krause et al., 2006b |
| Pept-B-LXI | 1964 | 1987 | 1005 | 1177 | 788 | 48.35 | Ac | Ala | Ala | Ala | Ala | Ala | Ala | Gln | Lox | Ala | Gly | Vxx | Ala | Pro | Vxx | Ala | Vxx | Gln | Gln | Pheol | New: Trichocellin TC-A-III, A-IV: [Aib]3 → [Vxx]3, [Aib]5 → [Vxx]5, and [Aib]6 → [Vxx]6 | Wada et al., 1994 |

Variable residues are UNDERLINED in the table header; minor sequence variants are UNDERLINED in the sequences. Amino acid exchanges in new compounds are set in italic.
### TABLE 4 | Sequences of the newly identified brevicelsins (group C) from Trichoderma species of the Longibrachiatum Clade and their similarities to known peptaibols available in the “Comprehensive Peptaibiotics Database.”

| Peptide     | M | [M+Na]+ | [M+2Na]+ | b13 | Rt-GK (min) | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 | R12 | R13 | R14 | R15 | R16 | R17 | R18 | R19 | R20 | Compound identical or positionally isomeric with | References |
|-------------|---|---------|----------|-----|------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|-----------------------------------------------|------------|
| Brevicelsin-| 1851| 1874 | 948.5 | 1078 774 | 28.72 | Ac | Alb | Ala | Alb | Alb | Alb | - | Gin | Alb | Lxx | Alb | Gly | Alb | Pro | Vxx | Alb | Alb | Alb | Gin | Gin | Pheol | New: Hypophellin 18, 35, 39: Lxx\(^{1}\) → [Aib]\(^{1}\) | Röhrich et al., 2013 |
|             |    |         |       |       |        |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |                               |            |
|             |    |         |       |       |        |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |                               |            |
| Brevicelsin-| 1855| 1888 | 955.5 | 1092 774 | 29.98 | Ac | Alb | Ala | Vxx | Ala | Alb | - | Gin | Alb | Lxx | Alb | Gly | Alb | Alb | Pro | Vxx | Alb | Alb | Alb | Gin | Gin | Pheol | New: Hypophellin 17, 34: [Aib]\(^{3}\) → [Vxx]\(^{3}\), Lxx\(^{1}\) → [Aib]\(^{1}\) | Röhrich et al., 2013 |
|             |    |         |       |       |        |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |                               |            |
|             |    |         |       |       |        |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |                               |            |
|             |    |         |       |       |        |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |                               |            |
| Peptide             | M   | [M+Na]+ | [M+2Na]+ | b_{13} | y_{7} | rt-GK (min) | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 | R12 | R13 | R14 | R15 | R16 | R17 | R18 | R19 | R20 | Compound identical or positionally isomeric with | References                  |
|---------------------|-----|---------|----------|--------|------|-------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|------------------------------------------------|-----------------------------|
| Brevicelsin-III     | 1852| 1875    | 949      | 1078   | 775  | 30.56       | Ac | Alb| Ala| Alb| Ala| Alb| -  | Gin| Alb| Lxx| Alb| Gly| Alb| Alb| Pro| Vxx| Alb| Alb| Glu| Gln| Pheol| New: Saturnisporin SA I; SA III: [Alb]^{3} → [Vxx]^{3}, [Ala]^{6} | Rebuffat et al., 1993       |
|                     | 1865| 1888    | 955.5    | 1078   | 788  | 31.82       | Ac | Alb| Ala| Alb| Ala| Alb| -  | Gin| Alb| Lxx| Alb| Gly| Alb| Alb| Pro| Vxx| Alb| Vxx| Glu| Gln| Pheol| New: Hypophellin 20, 40: [Lxx]^{11} → [Alb]^{11} | Röhrich et al., 2013        |
|                     | 1879| 1902    | 962.5    | 1092   | 788  | 32.82       | Ac | Alb| Ala| Alb| Ala| Vxx| -  | Gin| Alb| Lxx| Alb| Gly| Alb| Alb| Pro| Vxx| Alb| Vxx| Glu| Gln| Pheol| New: Hypophellin 22, 45: [Lxx]^{11} → [Alb]^{11} (Positional isomer of Brevicelsin VIII) | Röhrich et al., 2013        |

(Continued)
| Peptide | M | [M+Na]+ | [M+2Na]+ b13 | y7 | rt-GK (min) | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 | R12 | R13 | R14 | R15 | R16 | R17 | R18 | R19 | R20 | Compound identical or positionally isomeric with | References |
|---------|---|---------|-------------|----|-----------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Brevicelsin-VI | 1865 | 1888 | 955.5 | 1092 774 | 33.20 | Ac | Aib | Ala | Aib | Ala | Vxx | - | Gin | Aib | Lxx | Aib | Gly | Aib | Aib | Pro | Vxx | Aib | Aib | Gin | Gin | Pheol | New: Suzukacillin A 04, A 08, 07: [Aib]5 → [Vxx]5, [Ala]6, [Aib]6 | Krause et al., 2006b |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | New: Trichocellin TC-A-II, IV: [Aib]3 → [Vxx]5, [Ala]6 | Wada et al., 1994 |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | New: Hypophellin 4: [Lxx]11 → [Aib]11 | Röhrich et al., 2013 |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | New: Paracelsin B, D: [Aib]2 → [Vxx]5, [Ala]6, [Aib]6 | Pócsfalvi et al., 1997 |
| Brevicelsin-VII | 1866 | 1889 | 956 | 1078 789 | 33.65 | Ac | Aib | Ala | Aib | Ala | Aib | - | Gin | Aib | Lxx | Aib | Gly | Aib | Aib | Pro | Vxx | Aib | Vxx | Gin | Gin | Pheol | New: Saturnisporin SA-I, SA-III: [Aib]3 → [Vxx]5, [Ala]6, [Aib]6 | Rebuffat et al., 1993 |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | New: Suzukacillin A 02, A 06, A 05: [Aib]3 → [Vxx]5, [Ala]6, [Aib]6 | Krause et al., 2006b |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | New: Trichocellin TC-A-I, III: [Aib]3 → [Vxx]5, [Ala]6 | Wada et al., 1994 |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | New: Hypophellin 21, 43: [Lxx]11 → [Aib]11 | Röhrich et al., 2013 |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | New: Hypocitrinin-7: [Lxx]11 → [Aib]11 | Röhrich et al., 2014 |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | New: (Positional isomer of Brevicelsin V) | → Brevicelsin V |

Variable residues are UNDERLINED in the table header; minor sequence variants are UNDERLINED in the sequences. Amino acid exchanges in new compounds are set in italic.
2017 were performed with the keyword “peptaibol.” Several sequences proved to be homologous or positionally isomeric to the peptaibol subfamilies of trichobrachins, paracelsins, suzukacillins, saturnisporins, trichoaureocins, trichocellins, longibrachins, hyporentials, trichokonins, trilongins, metanicsins, trichosporins, glidoeliquescins, alamethicins, and hypophenyls. Some sequences had amino acid exchanges in comparison with previously described compounds from the peptaibol groups listed above.

Of the 49 sequences from group A consisting exclusively of 20-residue peptaibols, 27 have been previously described in the literature, and 22 were new, differing by 1–3 amino acids from known sequences (Table 2). Group B also comprises 20-residue sequences (Table 3). The main difference between group B and group A peptaibols is located at the R12 position, where Aib instead of Lxx is present in most of the group B sequences. Another major difference from group A is that the R5 position is not conserved due to a high percentage of Vxx instead of Aib. Of the 86 group B sequences, 37 were identified as new. An entirely new compound, Pept-B-LIX, with a mass of 1992 Da was detected in the crude extracts of three strains (T. konilangbra SzMC 22607, T. flagellatum SzMC 22608 and T. sinensis SzMC 22609). All sequences of group C produced by three strains (T. flagellatum SzMC 22608, T. sinensis SzMC 22609 and T. parareesei SzMC 22615) proved to belong to a new group of peptaibols, which was named brevicelsins, as they are similar to, but one amino acid shorter than paracelsins (Brückner and Graf, 1983; Pócsfalvi et al., 1997) (Table 4).

Qualitative and Semi-quantitative Peptaibol Profiles of the Strains

After investigation of all strains producing peptaibols from group A, “a” and “b” versions of their peptaibol compounds were apparent. Pept-A-XI has a “c” version of the compound, and a few others are represented by only a single sequence (Supplementary Table 7). Compounds such as Pept-A-IV-a and -b were produced constantly in high quantities by all strains. Both Pept-A-IX-a and -b were produced in high quantities by all strains except T. aethiopicum SzMC 22602, T. pinnatum SzMC 22603 and T. longibrachiatum SzMC 1775. Similarly, Pept-A-XVI-a and -b were produced by all strains. In this group, seven mainly produced peptaibol varieties appeared on the spectra, Pept-A-IV-a and -b, Pept-A-VI-a and -b, Pept-A-IX-a and -b, Pept-A-XV-a and -b, Pept-A-XVI-a and -b, Pept-A-XIXa as well as Pept-A-XXI-a and -b.

The analysis of the four T. longibrachiatum strains (SzMC 1773, 1775, 1776, and 12546) revealed similar, but still different, profiles (Supplementary Table 8). Environmental isolates of T. longibrachiatum produced more similar profiles, whereas the peptaibol profile of the clinical isolate was different from those of the three environmental strains. Pept-B-XX and Pept-B-XXVII were produced by all of the strains examined, whereas the other compounds were produced only by certain strains. Five peptaibol compounds (Pept-B-VII, Pept-B-XVII, Pept-B-XX, Pept-B-XXVII, and Pept-XLIV-a and b) were produced at high levels. Certain strains could also produce other compounds, such as Pept-B-XXVIII, Pept-B-XXIX-a and b, Pept-B-XXXIIa, Pept-A-IVb, Pept-XLIIb, Pept-XLIII, Pept-B-XLIVa, Pept-B-LI, Pept-B-LIV, and Pept-B-LIVb, at high levels. The most diverse peptaibol profile was observed in T. reesei QM6a (SzMC 22614), which produced 41 different peptaibol compounds, whereas the least diverse profiles were that of T. effusum SzMC 22611 and T. konilangbra SzMC 22607, which produced 11 and 12 sequences, respectively. Some species producing mostly group B peptaibols, T. reesei QM6a (SzMC 22614), T. saturnisporum SzMC 22606 and T. konilangbra SzMC 22607 could also produce peptaibols from group A. Interestingly, group A sequences could not be detected from the two mutant strains of T. reesei SzMC 22614 (T. reesei SzMC 22616 and SzMC 22617). Brevicelsins from group C were only produced by three species, T. sinensis, T. flagellatum and, to a lesser extent, T. parareesei. Brevicelsin I and Brevicelsin IV were produced by the examined strains (T. flagellatum SzMC 22608, T. sinensis SzMC 22609 and T. parareesei SzMC 22615) of all three species, but T. parareesei produced only these two compounds of group C in addition to the group B sequences.

We carried out a cluster analysis of the peptaibol diversity profiles in different Trichoderma species of the Longibrachiatum Clade based on the production levels of different peptaibols by various fungal producers (Supplementary Tables 7, 8). According to their peptaibol profiles, members of the Longibrachiatum Clade were divided into two main clusters (Figure 1). The first cluster involves species producing exclusively group A peptaibols. Among them, T. novae-zelandiae is characterized with a relatively poor, but sharply distinct, profile of abundantly produced peptaibol compounds from group A, like Pept-A-XXIb, XVb, XII, Vb, Ib, and IIIc. Further species in this cluster include members of the phylogenetic subclades Longibrachiatum/orientale and Citrinoviride/Pseudokoningii, along with the lone lineages T. ghanense and T. capillare (Table 1). This cluster is consisting of three subclusters, the first one containing the closely related species T. aethiopicum and T. pinnatum and the second one involving T. longibrachiatum and T. orientale—all belonging to the phylogenetic subclade Longibrachiatum/orientale—while the third subcluster is corresponding with the subclade Citrinoviride/Pseudokoningii (Table 1; Figure 1). The second main cluster is comprised of species producing mainly group B peptaibols and includes 2 subclusters, with the first containing the phylogenetic subclades Parareesei/Reesei, Saturnisporum and the lone lineages T. andinense and T. effusum, while the second harboring the three examined species from subclade Konilangbra/Sinensis (Table 1; Figure 1). All three examined members of this subclade produced the entirely new compound Pept-B-LIX (1992 Da).

Annotation of NRPS Domains From the Genomes of T. longibrachiatum, T. citrinoviride, T. reesei, and T. parareesei

The NRPS gene sequences from T. longibrachiatum (https://genome.jgi.doe.gov/Trilo1/Trilo1.home.html, Xie et al., 2014), T. citrinoviride (https://genome.jgi.doe.gov/Trici4/Trici4.home.html), T. reesei (https://genome.jgi.doe.gov/Trire2/Trire2.home.html, Martinez et al., 2008) and T. parareesei (NCBI Bioproject
FIGURE 1 | Heatmap showing the correlation between the production of peptaibols and the phylogenetic relationship between the strains. Monophyletic species are indicated by the bottom bar of the same color, species attributed to single phylogenetic lineages are marked with a star, while mutant strains are indicated with a filled pentagon. The color scale denotes production level increasing from zero (deep blue) to high (deep red).
Id: PRJNA287603, Yang et al., 2015) predicted by the SMIPS software were analyzed using the fungiSMASH software pipeline (Blin et al., 2017), which was designed to identify gene clusters of secondary metabolite biosynthesis from nucleotide sequences and to predict the products of the clusters identified. The *T. longibrachiatum*, *T. citrinoviride*, *T. reesei*, and *T. pararosei* genome sequences contain genes encoding 20-module NRPSs of 69,505, 68,508, 69,516, and 69,516 bp, as well as 14-module NRPSs of 43,422, 44,196, 49,386, and 52,395 bp with adenylation, condensation, thiolation, single acyl transferase and thioesterase domains. Figure 2 shows the schematic structure of the 20-mer NRPS gene cluster and the encoded modular enzyme from *T. longibrachiatum*. The 5’ ends of 20-module synthetase sequences contain a ketide synthase, whereas a Phe-specific permease-like and an aldo/keto reductase-like gene can be found downstream from the NRPS gene cluster. These two genes were also identified in the region downstream of the 18-module peptaibol synthetase gene clusters of the mushroom green mold agents *T. aggressivum* and *T. pleuroti* (Mark et al., 2017a). The identification of the presence of Pro in the peptaibol sequences and the close proximity of a Pro-specific permease gene to the NRPS gene cluster in these six *Trichoderma* species suggests that the permease may have a role in the secretion of these secondary metabolites.

Table 5 shows the incorporated amino acids predicted by the NRPS/PKS substrate predictor and NRPSPredictor3, based on the annotated adenylation domains and the eight amino acid residue signature sequences. The four 20-module NRPSs from the Longibrachiatum Clade were identical in positions R15 and R16 according to the signature sequences and the incorporated amino acids, respectively. Two positions (R6 and R9) were different only in *T. longibrachiatum*, whereas position R17 showed identity between *T. longibrachiatum*/*T. citrinoviride*, and *T. reesei*/*T. pararosei*. The most variable position was predicted to be R12, in which all signature sequences differed, and the incorporated amino acid was different in the case of *T. citrinoviride*.

Comparison of the amino acids predicted by the NRPS/PKS substrate predictor and the ones detected showed agreement at 11 positions in all four species. In positions R6, R11, and R18, the prediction did not match with the detected Ala, Gly and Glu, respectively. Position R11 of the four species showed identity with position R10 of *T. aggressivum* (Mark et al., 2017a) in its signature sequence (DVGYLIAV), but the amino acid prediction in these positions was incorrect in all cases. At the last position, the predictor software identified the signature sequence of adenylation domains, but the amino acid prediction failed. These unsuccessful predictions suggest that these signature sequences are missing from the database. Based on the signature sequences, the highest variability is in position R12, where the amino acids detected are also variable.

**Structural Characterization of 20- and 19-Residue Peptaibols**

Two previously described sequences, Paracelsin B (AcAib-Ala-Ala-Ala-Gln-Leu-Aib-Gly-Ala-Pro-Val-Alb-Ala-Ala-Gln-Gln-Pheol) and Paracelsin H (AcAib-Ala-Ala-Ala-Gln-Leu-Aib-Gly-Ala-Pro-Val-Alb-Gln-Gln-Pheol), together with their 19-residue counterparts Brevicelin 1 (AcAib-Ala-Ala-Ala-Gln-Leu-Aib-Gly-Ala-Pro-Val-Alb-Gln-Gln-Pheol) and Brevicelin IV (AcAib-Ala-Ala-Ala-Gln-Leu-Aib-Gly-Ala-Pro-Val-Alb-Gln-Gln-Pheol) were selected for structural characterization. Based on their sequences, Paracelsin B and H appear to correspond with Pept-B-XII and Pept-B-XVIII, respectively, both of which were produced by six examined species (*T. reesei*, *T. saturnisporum*, *T. andinense*, *T. effusum*, *T. pararosei*, and *T. flagellatum*). Our aim was to observe structural differences resulting from the loss of Ala at the R6 position.

All peptides show a strong tendency to form right-handed helical structures with a slight bend at the Aib-Pro position (Figure 3). Cluster analysis of the simulation trajectories of all four peptaibols revealed different energetically stable conformations that occur during folding, and the representative structures of the most populated cluster are provided for each peptaibol. All peptides fold into an energetically favored, highly bent helical conformation along with a linear helical conformation. Based on the reweighted potential of mean force (PMF) values calculated for end-to-end distance (distance in Å from the N-terminus to the C-terminus), it can be speculated that a highly curved conformation for all peptaibols, except for Paracelsin H, lies in the energy minimum and requires an
| Amino acid position in peptaibols | Sequence of amino acid binding pocket in NRPS module | Possible amino acids predicted NRPS/PKS substrate predictor/NRPSPredictor3 SVM | Amino acids detected in peptaibol sequences | Sequence of amino acid binding pocket in NRPS module | Possible amino acids predicted NRPS/PKS substrate predictor/NRPSPredictor3 SVM | Amino acids detected in peptaibol sequences | Sequence of amino acid binding pocket in NRPS module | Possible amino acids predicted NRPS/PKS substrate predictor/NRPSPredictor3 SVM | Amino acids detected in peptaibol sequences | Sequence of amino acid binding pocket in NRPS module | Possible amino acids predicted NRPS/PKS substrate predictor/NRPSPredictor3 SVM | Amino acids detected in peptaibol sequences |
|----------------------------------|---------------------------------------------------|--------------------------------------------------------------------------|-----------------------------------------------|---------------------------------------------------|--------------------------------------------------------------------------|-----------------------------------------------|---------------------------------------------------|--------------------------------------------------------------------------|-----------------------------------------------|---------------------------------------------------|--------------------------------------------------------------------------|-----------------------------------------------|
| 1 | D L G Y L A G V | Ab, Iva | Ab | D L G Y L A G V | Ab, Iva | Ab | D L G Y L A G V | Ab, Iva | Ab | D L G Y L A G V | Ab, Iva | Ab | D L G Y L A G V | Ab, Iva |
| 2 | D I L F N G L I | Ala, Ala | Ala | D I L F N G L I | Ala, Ala | Ala | D I L F N G L I | Ala, Ala | Ala | D I L F N G L I | Ala, Ala | Ala | D I L F N G L I | Ala, Ala |
| 3 | D V G F L A G V | Ab, Iva | Ab, Ala | D V G F L A G V | Ab, Iva | Ab, Ala | D V G F L A G V | Ab, Iva | Ab, Ala | D V G F L A G V | Ab, Iva | Ab, Ala | D V G F L A G V | Ab, Iva |
| 4 | D V G C I E G V | Ab, Iva | Ab, Ala | D V G C I E G V | Ab, Iva | Ab, Ala | D V G C I E G V | Ab, Iva | Ab, Ala | D V G C I E G V | Ab, Iva | Ab, Ala | D V G C I E G V | Ab, Iva |
| 5 | D G M V G G N | Gln, Gln | Gln | D G M V G G N | Gln, Gln | Gln | D G M V G G N | Gln, Gln | Gln | D G M V G G N | Gln, Gln | Gln | D G M V G G N | Gln, Gln |
| 6 | D L G Y L A G V | Ab, Iva | Ab | D L G Y L A G V | Ab, Iva | Ab | D L G Y L A G V | Ab, Iva | Ab | D L G Y L A G V | Ab, Iva | Ab | D L G Y L A G V | Ab, Iva |
| 7 | D I L F N G L I | Ala, Ala | Ala | D I L F N G L I | Ala, Ala | Ala | D I L F N G L I | Ala, Ala | Ala | D I L F N G L I | Ala, Ala | Ala | D I L F N G L I | Ala, Ala |
| 8 | D L G Y L A G V | Ab, Iva | Ab | D L G Y L A G V | Ab, Iva | Ab | D L G Y L A G V | Ab, Iva | Ab | D L G Y L A G V | Ab, Iva | Ab | D L G Y L A G V | Ab, Iva |
| 9 | D A F L I G V | Iva, Iva | Iva | D A F L I G V | Iva, Iva | Iva | D A F L I G V | Iva, Iva | Iva | D A F L I G V | Iva, Iva | Iva | D A F L I G V | Iva, Iva |
| 10 | D L G Y L A G V | Ab, Iva | Ab | D L G Y L A G V | Ab, Iva | Ab | D L G Y L A G V | Ab, Iva | Ab | D L G Y L A G V | Ab, Iva | Ab | D L G Y L A G V | Ab, Iva |
| 11 | D V G Y L A V | Ab, Iva | Ab | D V G Y L A V | Ab, Iva | Ab | D V G Y L A V | Ab, Iva | Ab | D V G Y L A V | Ab, Iva | Ab | D V G Y L A V | Ab, Iva |
| 12 | D L G Y L A V | Ab, Iva | Ab | D L G Y L A V | Ab, Iva | Ab | D L G Y L A V | Ab, Iva | Ab | D L G Y L A V | Ab, Iva | Ab | D L G Y L A V | Ab, Iva |
| 13 | D L G F L A G V | Ab, Iva | Ab | D L G F L A G V | Ab, Iva | Ab | D L G F L A G V | Ab, Iva | Ab | D L G F L A G V | Ab, Iva | Ab | D L G F L A G V | Ab, Iva |
| 14 | D V L F C G L I | Pro, Pro | Pro | D V L F C G L I | Pro, Pro | Pro | D V L F C G L I | Pro, Pro | Pro | D V L F C G L I | Pro, Pro | Pro | D V L F C G L I | Pro, Pro |
| 15 | D A G M I I G V | Iva, Iva | Iva | D A G M I I G V | Iva, Iva | Iva | D A G M I I G V | Iva, Iva | Iva | D A G M I I G V | Iva, Iva | Iva | D A G M I I G V | Iva, Iva |
| 16 | D L G F L A G V | Ab, Iva | Ab | D L G F L A G V | Ab, Iva | Ab | D L G F L A G V | Ab, Iva | Ab | D L G F L A G V | Ab, Iva | Ab | D L G F L A G V | Ab, Iva |
| 17 | D V L F W F A G | Ab, Iva | Ab, Iva | D V L F W F A G | Ab, Iva | Ab, Iva | D V L F W F A G | Ab, Iva | Ab, Iva | D V L F W F A G | Ab, Iva | Ab, Iva | D V L F W F A G | Ab, Iva |
| 18 | D G M V G G N | Gln, Gln | Gln | D G M V G G N | Gln, Gln | Gln | D G M V G G N | Gln, Gln | Gln | D G M V G G N | Gln, Gln | Gln | D G M V G G N | Gln, Gln |
| 19 | D G M V G G N | Gln, Gln | Gln | D G M V G G N | Gln, Gln | Gln | D G M V G G N | Gln, Gln | Gln | D G M V G G N | Gln, Gln | Gln | D G M V G G N | Gln, Gln |
| 20 | D A A F I M G V | -- | -- | D A A F I M G V | -- | -- | D A A F I M G V | -- | -- | D A A F I M G V | -- | -- | D A A F I M G V | -- |
energy “jump” of <1 kcal mol$^{-1}$ to attain the linear backbone conformation (Figure 4A). Overall, the end-to-end distance values as low as 5 to 27 Å, that lie close to the energy minima, show that all conformations starting from a hairpin-like helix structure to a straight backbone with just a slight bend are easily accessible. The PMF values increase rapidly beyond these two points for all four peptaibols, as shown in the inset image focusing only on PMF values up to 2 kcal mol$^{-1}$. However, the sequences Paracelsin B and Brevicelsin I, with an Aib residue in position R17, have higher PMF values for higher end-to-end distance values; the energy cost for attaining linearity of the helical backbone is slightly higher than in Paracelsin H and Brevicelsin IV, where a Val residue replaces Aib in the R17 position. The energy minimum for Paracelsin H lies at an end-to-end distance of 22 Å, whereas Brevicelsin IV exhibits a slight fall at this point, even though its energy minimum also lies at 10 Å. The presence of Aib residue in position R17 (in Paracelsin B and Brevicelsin I) results in a highly dynamic folding process, which means that many conformations were visited during the trajectory, whereas Val in the same position (in Paracelsin H and Brevicelsin IV) led to fewer energetically stable conformers. The root-mean-square-atomic fluctuation (RMSF) graph (Figure 4B) shows higher fluctuation in the N- and C-terminal regions of all peptides in comparison with their central regions. However, the most significant observation is that there is considerably higher atomic fluctuation of the 19-residue peptaibols Brevicelsin I and IV in comparison to the 20-residue peptaibols Paracelsin B and H. It seems that the loss of one residue, resulting in a shorter sequence, results in higher atomic fluctuations, whereas longer peptaibols are comparatively more stable. In all four sequences, a small but sharp spike in the RMSF value of Gln at R6 of the 19-residue peptaibols and R7 of the 20-residue peptaibols reinforces the importance of glutamines in channel formation and stabilization (Whitmore and Wallace, 2004). Aib17 has higher average atomic fluctuation than Val17, due to its tendency to oscillate between right- and left-handed helical forms, whereas Val17 takes a rigid conformation.

Antifungal Effects of T. reesei Peptaibols on Filamentous Fungi

The purified peptaibol extracts of T. reesei QM9414 were tested on human and plant pathogenic filamentous fungi, furthermore, the producer strain itself, as well as its $\Delta$lae1 mutant (Table 6). Treatment with 0.4 and 0.2 mg ml$^{-1}$ purified peptaibol solution resulted in growth inhibition of all strains, whereas a weaker,
but still notable, inhibition was detected after treatment with the purified extract at a concentration of 0.1 mg ml\(^{-1}\). The peptaibol extract from *T. reesei* QM9414 exhibited an inhibition profile highly similar to that of alamethicin.

### Bioactivities of *T. reesei* Peptaibols on Arabidopsis thaliana Plants

In order to evaluate the value of peptaibols as antifungal agents for plant protection, the purified (98%) peptaibol extract of *T. reesei* QM9414 was investigated for toxicity in the model plant *A. thaliana*. The extract was diluted to 50, 10, 5, 1, 0.5, 0.3, 0.1, and 0.05 mg ml\(^{-1}\). All of the treated plants were inhibited after treatment with the peptaibol extract at concentrations of 50, 10, and 5 mg ml\(^{-1}\). Root growth was observed only at concentrations ≤1 mg ml\(^{-1}\); however, inhibited growth could be observed down to concentrations of 0.1 mg/ml (Figure 5). Treatment with 1 mg ml\(^{-1}\) peptaibol solution resulted in a hook formation of the primary roots. Chlorophyll-a, -b and carotenoid levels decreased after treatment with extracts of ≥0.3 mg ml\(^{-1}\) (Figure 6). Treatment with a peptaibol solution of 0.1 mg ml\(^{-1}\) resulted in a similar rate of production of photosynthetic pigments but an increased anthocyanin level in 15-day-old plants. The root growth of these plants was suppressed in 6- to 9-day-old plants, although the plants showed normal biomass and could probably eventually survive this minimal toxicity because of the increased levels of anthocyanin (Figure 7).

### DISCUSSION

In this study, the structural diversity and bioactivity of peptaibol compounds produced by *Trichoderma* species belonging to the Longibrachiatum Clade were investigated and compared. The Longibrachiatum Clade is ecologically highly versatile as it contains both environmental and opportunistically pathogenic species, some of which can be found worldwide, whereas others are ecologically restricted. In total, 143 20-residue peptaibols could be identified from the 17 species examined, including 59 new and 76 recurrent compounds, as well as eight new 19-residue sequences. The peptaibols can be categorized into groups A, B and C, based on their primary structure, where groups A and B consist of 20-residue peptaibols, whereas group C is comprised exclusively of 19-residue sequences. The main difference between peptaibols of group A in relation to group B is in the R12 position. Sequence analysis identified several conserved regions along with some variable positions (R3, R5, R6, R10, R12, and R17), which have also been reported in a previous study (Pócsfalvi et al., 1997). Vxx was usually found instead of Ala and Aib at certain variable positions like R3, R5 and R6, which has never been observed among similar peptaibols. Although all of these amino acids have helix-forming properties, a substitution by Val would render a more linear and less fluctuating helical conformation owing to its bulkier sidechain. The highly curved backbone conformation is not energetically favored with increasing number of Val in peptaibol sequences. It has been hypothesized that the equilibrium between the bent (closed form) and linear conformations (open amphipathic form) may act as a “conformational switch” of voltage gating in ion channels across bilayers (North et al., 1995). Clearly, such substitutions have an important functional relevance, especially at the terminal positions like R3 and R17.

### Bioactivities of *T. reesei* Peptaibols on Mammalian Cells

The endpoint of toxic concentration—the last dilution step of the purified peptaibol solution which is toxic to mammalian cells—was determined for the peptaibol extract of *T. reesei* QM9414 (Table 7). After 20 min incubation at 37°C or 24 h at room temperature, the boar sperm motility inhibition end point was detected after treatment with 3 µg ml\(^{-1}\) peptaibol solution. The acrosome of the exposed sperm cells reacted at the same concentration, which inhibited motility, indicating that the toxic effect involves the plasma membrane. The inhibition end point of proliferation in porcine kidney PK-15 cells was observed at a concentration of 8 µg ml\(^{-1}\) peptaibol solution.

| Tested filamentous fungal strain | MIC of purified peptaibol extract (mg ml\(^{-1}\)) | MIC of alamethicin\(^ \dagger \) standard (mg ml\(^{-1}\)) | MIC of nystatin standard (mg ml\(^{-1}\)) |
|----------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| *Alternaria alternata* SzMC 16085 | 0.1                                           | 0.05                                          | 0.003125                                      |
| *Aspergillus fumigatus* SzMC 23245 | 0.1                                           | 0.1                                           | 0.0125                                       |
| *Fusarium falciforme* SzMC 11407 | 0.05                                          | 0.05                                          | 0.025                                        |
| *Fusarium keratothricum* SzMC 11414 | 0.1                                           | 0.1                                           | 0.05                                         |
| *Fusarium solani* SC SzMC 11467 | 0.1                                           | 0.1                                           | 0.05                                         |
| *Phoma cucurbitacearum* SZMC 16088 | 0.05                                          | 0.05                                          | 0.1                                          |
| *Trichoderma reesei* QM9414 | 0.1                                           | 0.05                                          | 0.00625                                      |
| *Trichoderma reesei* QM9414 G2,Δiae1 | 0.05                                          | 0.05                                          | 0.00625                                      |

\(^ \dagger \) Harzianum A contamination could not be detected in the alamethicin standard based on the exact mass of its deprotonated molecular ion ([M-H]\(^-\)), m/z = 399.1808.
Marik et al. Peptaibols From the Longibrachiatum Clade of Trichoderma

FIGURE 5 | Primary root growth of 6 (A), 7 (B), 8 (C), and 9 (D) days old Arabidopsis thaliana plants after treatment with peptaibol extract from Trichoderma reesei QM9414. Methanol was used for the control plants as all peptaibol extracts were prepared in this solvent. Significance is assessed based on P-values: *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001 and ****P ≤ 0.0001.

of their full-length precursors—like it is the case for the 16-residue brevikindiins deriving from 18-residue trichokindin-like peptaibols (Degenkolb et al., 2016) – but differ from group B sequences by the internal deletion of position 6. This position is critical, since the following Gln plays an important role in the formation of ion channels (Wilson et al., 2011). Brevicelsins could be found only in three species: T. flagellatum, T. sinense and T. parareesei. A full genome sequence is available for T. parareesei. Analysis of this sequence, however, revealed no extra 19-module NRPS synthetases but only a 20-module enzyme. The 19-residue peptaibols could be produced by the same, 20-module NRPS via the interaction of non-neighboring modules known as internal module skipping. The mechanisms of this phenomenon resulting in additional classes of 10-, 13-, 18-, and 19-residue peptaibols were proposed by Degenkolb et al. (2012). R6 is also skipped in T. phellincola peptaibols (Röhrich et al., 2013), which does, however, contain Lxx in position R12, similar to group A peptaibols and unlike brevicelsins with Aib in this position.

The unique group A peptaibol profile of T. novae-zelandiae (Figure 1) may be related to the geographical origin of this species, which is endemic to New Zealand, and to its occupying a basal position in the Longibrachiatum Clade (Samuels et al., 2012). This species has tuberculate conidia, a trait also found in the Viride Clade (Jaklitsch et al., 2006), and it may be an ancestral trait of the Longibrachiatum Clade. Our results suggest that the production of group A peptaibols may be another ancestral trait of the Longibrachiatum Clade, while the switch to the production of group B peptaibols might have occurred multiple times and seems therefore to be the result of convergent evolution. This switch from group A to group B has not fully completed in certain species: wild-type T. reesei as well as T. saturnisporum and T. konilangbra are also producing some group A compounds in addition to group B peptaibols. Except from T. reesei, which was separated from its closest relative T. parareesei, the clustering based on peptaibol profiles reflected the close relationships within phylogenetic subclades in most of the cases (e.g., within subclades Longibrachiatum/Orientale, Citrinoviride/Pseudokoningii, or Konilangbra/Sinensis). For example, the species from the Konilangbra/Sinensis subclade are phylogenetically close to each other and are only known from the Paleotropical/Asian areas including Ethiopia (T. flagellatum), Uganda (T. konilangbra) and Taiwan (T. sinensis) (Samuels et al., 2012). The very close relationship of T. sinensis and T. flagellatum is also reflected by their ability to produce group C peptaibols in addition to group B sequences. The phylogenetic relationships between the subclades are less reflected by the clustering based on peptaibol profiles. Distantly related subclades (e.g., Longibrachiatum/Orientale and Citrinoviride/Pseudokoningii) may share similar profiles, while closely related subclades may exhibit substantially different ones—e.g., members of subclade Citrinoviride/Pseudokoningii may produce group A peptaibols, while group B compounds are produced by their close relative T. effusum. This could be explained by multiple events of switching.
from the production of group A to group B during the evolution of the Longibrachiatum Clade.

Based on molecular dynamics simulations, 20-residue peptaibols result in higher linearity of helices than their 19-residue counterparts and are also relatively stable in terms of the atomic fluctuations of each residue. Paracelsins B, H and their 19-residue deletion sequences Brevicelsin I and IV all fold into right-handed helical structures with a slight bend at the Aib-Pro bond, except for Brevicelsin IV where the bend occurs at the Aib11-Aib12 bond. The Aib-Pro bond at R13-R14 in the case of 20-residue sequences is important for the secondary structure of the bent molecule. An important observation was made with respect to Val substitution instead of Aib at R17 which seems to hinder the formation of a bent backbone in close proximity to

| Purified peptaibol extract | Sperm motility inhibition | Acrosome reaction | Inhibition of proliferation of Porcine kidney cells PK-15 |
|-----------------------------|--------------------------|-------------------|---------------------------------------------------------|
| T. reesei QM9414            | 3                        | 3                 | 3                                                        |
|                             | 8                        |                   |                                                          |
| REFERENCE SUBSTANCE         |                          |                   |                                                          |
| Alamethicin                 | 5                        | 0.2               | 0.2                                                      |

The values are the median of three measurements, represented by four microscopic fields. The variation between measurements was one dilution step.
the N-terminal side-chains, because it is a chiral, hydrophobic amino acid with a bulkier side-chain than that of the achiral Aib.

Frequent occurrence of Aib could be detected at the termini of the sequences, which are very important for the determination of the formation of helical structures including α- or 3π-helices (De Zotti et al., 2010; Gessmann et al., 2012a,b). The other promotor of the helical structure, D-Iva, is most often found close to the N-terminus, prior to the Gln-Aib bond in position R6, based on different previously described peptaibols such as boletusin 1, chrysospermins, peptaivirins, trichorzinins TA and TB, or the TA1938, 1924, 1910 and 1909a compounds (El-Hajji et al., 1987; Rebuffat et al., 1989; Dornberger et al., 1995; Lee et al., 1999; Yun et al., 2000; Panizel et al., 2013).

The growth of filamentous fungi pathogenic to plants or humans could be inhibited by the purified peptaibol extract of *T. reesei* QM9414. A stronger inhibition was observed in the case of the ΔΔΔΔ mutant of *T. reesei* than in the case of the other strains, suggesting that the mutation in the methyl transferase gene, which is known as a global epigenetic regulator of gene expression, may also affect tolerance to these metabolites. A previous study (Mark et al., 2018), in which crude peptaibol extracts were tested on several bacterial, yeast and filamentous fungal strains showed similar results. The inhibitory effects of peptaibols to bacteria and filamentous fungi have previously been reviewed (Szekeres et al., 2005; Daniel and Rodrigues Filho, 2007). It has also been demonstrated that purified trichokin VI triggers a change of fungal membrane permeability and disintegration of subcellular structures, has an effect on mitochondrial membrane permeabilisation and intracellular ROS production, induces phosphatidylserine exposure and eventually triggers metacaspase-independent apoptosis in *F. oxysporum* (Shi et al., 2012).

Alamethicin, the most studied peptaibol was shown to induce resistance in plants (Leitgeb et al., 2007; Kredics et al., 2013), although it can also be toxic, causing lesions on *Arabidopsis* leaves (Rippa et al., 2010). At higher concentration, it induces rRNA cleavage-associated rapid death (Rippa et al., 2007). Alamethicin could permeabilise mainly the apical meristem and epidermis cells of the root tips, but not the basal meristem cells, cortex cells or the root cap of *A. thaliana* (Dotson et al., 2018). If the root was pretreated with cellulase, permeabilisation could not be observed. This study proved cellulose-induced resistance and cell-specificalamethicin permeabilisation of *A. thaliana* roots. Engelberth et al. (2001) successfully demonstrated the high biological activity of alamethicin that caused emission of volatile compounds from lima beans (*Phaseolus lunatus*) placed under low concentration of the peptaibol solution. When it was applied to *Bryonia dioica* tendrils at the same concentration, it elicited jasmonate-induced tendril coiling. Therefore, peptaibols may be used as potential elicitors of plant defense responses. Recently, antiviral activity of trichorzin was also reported on cowpea plants against *Cucumber mosaic virus* (Kai et al., 2018). In this recent study, bioactivity tests with the selected, purified peptaibol extract of *T. reesei* QM9414 demonstrated toxicity to *A. thaliana* plants at higher concentrations. An interesting effect of the peptaibol extract was the induction of hook formation in the root tips. A previous study revealed similar results, where the inoculation of *A. thaliana* with *T. atroviride* resulted in shortened primary root growth of the plants and ended in a hook formation, although the lateral root numbers were increased (Pelagio-Flores et al., 2017). An inhibitory effect on primary root growth in *A. thaliana* was also observed after interaction with *T. longibrachiatum* SMF2, and its peptaibols induced auxin production and disruption of the auxin response gradients in root tips (Shi et al., 2016).

Boar sperm cells are frequently used for the detection of toxins, which affect plasma membranes (Vicente-Carrillo, 2018; Castagnoli et al., 2018). Due to the high sensitivity of boar sperm cells to toxins, many studies have concluded that these tests are appropriate for toxin detection (Peltola et al., 2004; Andersson et al., 2009, 2010). Similar measurements of peptaibol extracts produced by *T. longibrachiatum* Thb have been reported, and a mixture of trilongins proved to be a strong inhibitor of motility than trilongins alone, or any of the crude extracts (Mikkola et al., 2012). Single ion channels remained in an open state for a longer time when exposed to a combination of the long peptaibols (trilongins BI–BIV) with the short ones (trilongin AI), than for the long peptaibols alone. Furthermore, peptaibols (trichokin VI) could inhibit HepG2 cancer cells by inducing autophagy and apoptosis through an influx of Ca²⁺, which triggered the activation of μ-calpain and proceeded to the translocation of Bax to mitochondria and the subsequent promotion of apoptosis (Shi et al., 2010). Another peptaibol, emericellipsin A, which is a short lipopeptaibol, exhibited selective cytotoxic activity against HepG2 and HeLa cell lines (Rogozhin et al., 2018), similar to culicinin D, another short linear peptaibol which has been described as a potent anticancer compound (He et al., 2006). In the present study, the partially purified peptaibol extract of *T. reesei* QM9414 proved to inhibit boar spermatozoa and porcine kidney PK-15 cells at 0.1 mg ml⁻¹, which rises the question of a possible in vivo toxicity. Degenkolb et al. (2008) discussed this issue in detail and suggested that the toxicity of peptaibols may be well below the threshold of human consequence, and it may require direct contact with cell membranes, like in the case of common amphiphilic detergents. This is supported by previous observations demonstrating the very low toxicity of various peptaibols orally administered to rodents and ruminants (Hou et al., 1972; Nayar et al., 1973; Hino et al., 1994).

In conclusion, negative effects on *Arabidopsis* plants could not be detected below a certain concentration of the purified peptaibol extract from *T. reesei* QM9414, which could still inhibit plant pathogenic filamentous fungi. This observation suggests that purified peptaibol extracts may have potential value for plant protection. *T. reesei* is a well-characterized, widely used cellulase producer in the biotechnological industry, and so its peptaibols could be produced as the main product, or a valuable by-product of fermentation.

**DATA AVAILABILITY**

All datasets generated for this study are included in the manuscript and/or the Supplementary Files.
AUTHOR CONTRIBUTIONS

LK, TM, AS, and CV designed the study and coordinated the draft of the manuscript. TM and DB took part in the extraction, HPLC separation, sequence determination, and antifungal activity testing of the peptaibol compounds. GE, DR, and AS conducted the mass spectrometry measurements. ID performed the sequence alignments and the comparative sequence analysis of peptaibol profiles. PU performed the annotation and bioinformatic analysis of NRPS gene clusters. CT contributed with the molecular dynamics simulations of peptaibols. TM, AS, and LB designed and performed the bioactivity tests on A. thaliana. MA and HS conducted the bioactivity assays on mammalian cells. TM, LK, and CV analyzed the results and designed the figures and tables. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2019.01434/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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